Programa de Doctorado en Recursos Naturales y Gestión Sostenible

# Caracterización funcional de las variedades de la Raza Aviar Utrerana

*Functional characterization of varieties of Utrerana Avian Breed*


Antonio González Ariza


TESIS DOCTORAL


Directores

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Juan Vicente Delgado Bermejo



Departamento de Genética | Córdoba 2021

TITULO: *Functional characterization of varieties of Utrerana Avian Breed*

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**FACULTAD DE VETERINARIA**

DEPARTAMENTO DE GENÉTICA

PROGRAMA DE DOCTORADO EN RECURSOS NATURALES Y GESTIÓN SOSTENIBLE

# CARACTERIZACIÓN FUNCIONAL DE LAS VARIEDADES DE LA RAZA AVIAR UTRERANA

***Functional characterization of varieties of Utrerana Avian Breed***

MEMORIA PARA OPTAR AL GRADO DE DOCTOR PRESENTADA POR

Antonio González Ariza

BAJO LA DIRECCIÓN DE:

María Esperanza Camacho Vallejo

Francisco Javier Navas González

En Córdoba, a 23 de junio de 2021

**TÍTULO DE LA TESIS: CARACTERIZACIÓN FUNCIONAL DE LAS VARIEDADES DE LA RAZA AVIAR UTRERANA**

**DOCTORANDO/A: ANTONIO GONZÁLEZ ARIZA**

## INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

**Doña María Esperanza Camacho Vallejo** y **D. *Francisco Javier Navas González***, Investigadores del Instituto de Formación Agraria y Pesquera de Andalucía, como profesionales con experiencia en la formación de doctorado,

**INFORMAN**

Que el trabajo de tesis presentado por **D. Antonio González Ariza**, titulado ***"Caracterización funcional de las variedades de la raza aviar Utrerana"*** ha sido realizado bajo nuestra dirección y cumple con los artículos 24 y 35 de la norma reguladora de los Estudios de Doctorado de la Universidad de Córdoba para su presentación como compendio de publicaciones, así como para obtener la mención internacional.

La tesis ha generado ocho publicaciones, cinco de ellos ya publicadas en revistas indexadas en primer cuartil (dos en primer decil), y tres sometidas a revistas de igual categoría. Además, la tesis cuenta con una colaboración externa que dio lugar a un artículo en primer cuartil colateral.

Además, la tesis ha dado lugar a dos artículos en revistas internacionales no indexadas y un artículo en una revista nacional no indexada, pero de gran prestigio en el sector. Avances de estos resultados se han presentado igualmente en diversos eventos internacionales en forma de ocho comunicaciones; una ponencia invitada en eventos nacionales de reconocido prestigio, cinco comunicaciones en formato poster y dos comunicaciones orales, presentadas en conferencias internacionales.

Asimismo, el doctorando no ha olvidado la transferencia al sector de sus resultados, llevándolo a cabo con la publicación de un tríptico divulgativo recogiendo los principales resultados derivados de la tesis, la participación en un programa de televisión y artículos de prensa.

Con todo lo expuesto, no nos queda más que reconocer nuestro orgullo por haber dirigido esta gran tesis doctoral y nuestro agradecimiento al candidato, antes de hacer constar nuestro acuerdo unánime sobre la madurez de la tesis y del candidato para proceder a su defensa.

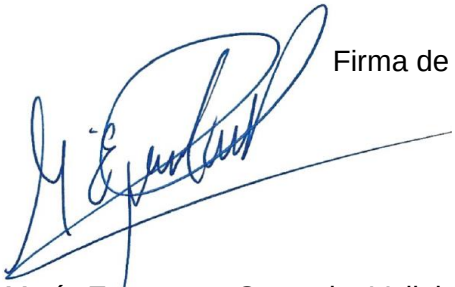
Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 21 de junio de 2021

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 23 de junio de 2021

Firma de los directores



Fdo.: María Esperanza Camacho Vallejo  Fdo.: Francisco Javier Navas González

**TÍTULO DE LA TESIS: CARACTERIZACIÓN FUNCIONAL DE LAS VARIEDADES DE LA RAZA AVIAR UTRERANA.**

**DOCTORANDO/A: ANTONIO GONZÁLEZ ARIZA**

## INFORME RAZONADO DEL TUTOR

**D. *Juan Vicente Delgado Bermejo***, catedrático del Departamento de Genética de la Facultad de Veterinaria de la Universidad de Córdoba, como tutor del doctorando D. Antonio González Ariza

INFORMO:
Que el trabajo de tesis presentado por el doctorando, titulado "Caracterización funcional de las variedades de la Raza Aviar Utrerana" ha sido realizado bajo mi tutorización y la evolución y desarrollo de la tesis doctoral ha cumplido sobradamente con los objetivos inicialmente propuestos, cumpliendo así las expectativas que desde un principio fueron depositadas en el doctorando y en su labor investigadora.

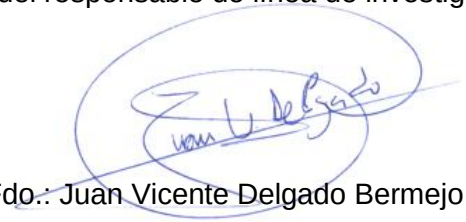
Muestra de esta positiva evolución y del acertado desarrollo de la tesis son 8 publicaciones indexadas en primer cuartil (2 en primer decil) que componen la presente tesis. Además de las anteriormente enumeradas publicaciones, caben destacar otras contribuciones derivadas de la tesis cuyo fin ha sido el transferir los resultados de las investigaciones desarrolladas al sector.

Además, a lo largo del desarrollo de la tesis doctoral, y fruto de dicho desarrollo, también fueron una serie de comunicaciones a conferencias y simposios, entre los cuales destacan los siguientes: XX Simposio Iberoamericano sobre Conservación y Utilización de Recursos Zoogenéticos, en Corumbá (Brasil), del 11 al 14 de noviembre de 2019 y el XXI Simposio Iberoamericano sobre Conservación y Utilización de Recursos Zoogenéticos, en Córdoba (España), del 15 al 16 de diciembre de 2020).

En definitiva, el tutor del doctorando, constatan que la memoria de tesis presentada reúne todos los requisitos que permiten su presentación y defensa para optar al grado de Doctor. Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, a 21 de junio de 2021

Firma del responsable de línea de investigación



Fdo.: Juan Vicente Delgado Bermejo

# *Agradecimientos*

Desde pequeño fui una persona con las ideas claras. Desde que tengo uso de razón, siempre supe que sería veterinario. Las cosas empezaron a cambiar conforme se acercaba mi último año de carrera. Ahí comenzó la incertidumbre sobre qué sería capaz de hacer con mi vida. Sin embargo, se plantó delante de mí mi primera oportunidad laboral, y yo, que no soy muy de decir "no" a las cosas, ahí que me aventuré. Fue entonces cuando el grupo AGR218 me dio una calurosa acogida y poquito a poco se convirtió en mi segunda familia, mi familia laboral, haciendo que estos tres últimos años hayan sido maravillosos, a pesar de lo que nos ha tocado vivir en 2020, y me han permitido experimentar un crecimiento humano, laboral, profesional e intelectual inmenso. Por eso, ahora mismo solo puedo agradecer a todas las personas que en estos últimos años, de una manera u otra, me han ayudado a dar a luz a este trabajo.

Esperanza, muchas gracias por tu apoyo y dedicación constante, por abrirme las puertas de tu casa, por enseñarme que, en investigación, el tesón y la garra, que tanto te caracterizan, son muy importantes y hacerme ver que muchas veces las hormiguitas trabajadoras, con un poquito de humildad, podemos llegar tan lejos como los grandes virtuosos.

Javier, naciste para ser investigador. Siempre he dicho que quiero estar en el mismo equipo que los mejores, y tú lo eres. Cada vez que me siento contigo, es un aprendizaje y un estímulo hacia la perfección constante. Porque siempre estás dispuesto a echar una mano, y a pesar de que hay días en los que te acribillo a llamadas, siempre me recibes con tu mejor sonrisa. Muchas gracias.

Juanvi, muchas gracias por abrirme las puertas del grupo, e incluso de tu despacho y por contar conmigo en la estructura del pedazo de grupo que has creado. Es admirable la capacidad de liderazgo que tienes y como en tan solo dos minutos, haces que la mayor tempestad se convierta en el mejor día primaveral. Porque todo lo que has creado y todo lo que has hecho por los recursos genéticos animales no hay quien lo iguale. Muchas gracias por todo lo que a diario haces por mí.

Ander, sabes de sobra que eres el culpable de que ahora mismo esté escribiendo estas líneas. De tu mano entré en este grupo, y en estos tres años has sido como un hermano mayor para mí. Gracias por los miles de consejos, por tu apoyo

incondicional y por enseñarme a desenvolverme cuando solo era un pajarillo recién salido del nido.

Sergio, muchas gracias porque siempre confiaste en mí, por tus enseñanzas, tanto laborales, como de la vida, pura y dura, en sí. Gracias por meterme en mil y un lío, por contar conmigo para todo, y por tantos momentos de risas, de esas que, aunque lleves trabajando desde bien temprano, hacen olvidar todos los males.

Jose y Joaquín, muchas gracias por vuestro gran apoyo durante toda la tesis, por vuestras palabras de preocupación, por vuestros sabios consejos y también por vuestras regañinas, que son tan necesarias y merecidas a veces, y sobre todo, por vuestra amistad.

Cecilio y Amparo, gracias porque, aunque no hablemos a diario, sé que puedo contar con vosotros para todo. Muchas gracias por vuestro apoyo.

A las doctoras Inês Carolino y Teresa Lupi, muchas gracias por vuestra inestimable ayuda en esta tesis.

A todos los criadores de la Asociación Nacional de Criadores de Gallinas Utreranas, en especial a Daniel y Félix, por esas valiosísimas fotos que ilustran esta tesis.

A la Unión de Criadores de Gallos Combatiente Español, por su confianza y hacerme sentir como en casa desde el minuto 1. En especial, a Mari, Rocío y Viki, que me han disfrutado y sufrido a partes iguales, diariamente.

Gracias a todos los técnicos del Centro Agropecuario de Diputación y del Centro IFAPA Alameda del Obispo, por toda vuestra ayuda y simpatía.

Gracias al Instituto Gran Capitán, en especial a la familia profesional de Hostelería y Turismo, por vuestra profesionalidad y cooperación en esta tesis.

Martina, Amado, Carmen Marín, Carlos, Carmen Entrenas, Alba, Miguel, Gabriela, Pedro, Esther, Pepe y el resto de los compañeros del grupo AGR218, que han compartido conmigo estos maravillosos años. Podría escribir otro libro describiendo todas las virtudes que cada uno tenéis. Está claro que, con gente como vosotros, todo se vuelve mucho más fácil. Sois mucho más que un equipazo y me enorgullece poder compartir mis días con vosotros. Muchísimas gracias.

Al equipo espermatozoide, mi Alberto y mi Carlos. Vuestro apoyo fue crucial al inicio de esta andadura y desde entonces no habéis parado de apoyarme y alentarme. Sois unos amigos cojonudos. Lo que unió la carrera de veterinaria, que no lo separe nadie.

A mis abuelos, tíos, primas, Narciso, Manoli, José Antonio, Silvia y resto de la familia, muchas gracias por todo el cariño y el amor que me demostráis a diario. Aunque dicen que la familia es la que toca, yo os volvería a elegir un millón de veces.

Francisco, la boca se me llena de orgullo cuando hablo de ti. No solo eres mi hermano, también eres mi amigo, mi apoyo, mi confidente, mi consejero y la red con la que me siento seguro. A pesar de que eres el hermano menor, tu madurez y tu sabiduría, a veces, hacen parecer lo contrario. Muchas gracias por todo.

Mamá y papá, está claro que todo lo que consigo es gracias a vosotros. Me habéis educado, me habéis dado unos valores y entre una infinidad de cosas más, me habéis enseñado lo que significan las palabras respeto y humildad. Habéis hecho que esté muy orgulloso de mis raíces, porque lo habéis dado todo por mi hermano y por mí. Creísteis en mí cuando ni yo supe hacerlo y me disteis el impulso para conseguir todas mis metas. ¡Infinitas gracias!

Mari Carmen, mi pequeña. Qué bonita es la vida a tu lado. Tú me diste los primeros consejos en el mundo de la investigación, eres mi mayor motivación, te preocupas, me cuidas y me aguantas, cuando sabes que hay días que no me aguanto ni yo. Eres el ejemplo de trabajo duro, de perfeccionismo y entusiasmo por lo que haces. Eres primavera para todos los que estamos a tu alrededor. Gracias por acompañarme en este camino y dejarme acompañarte en el tuyo, porque este es solo un éxito más de todos los que nos quedan por vivir juntos.

A todas las gallinas y los gallos que han contribuido a esta tesis, pues han sido claves para que podamos seguir disfrutando y conservando un trocito de nuestro patrimonio cultural, la Gallina Utrerana.

*A mis tres estrellas del cielo*

“Equipado con sus cinco sentidos, el hombre explora el universo que lo rodea y a sus aventuras las llama Ciencia.”

*Edwin Powell Hubble*

# *Index*

# *Tesis Doctoral como*

# *Compendio de Publicaciones*

- Primera publicación:
  - Título: *Discriminant Canonical tool for differential Biometric characterization of multivariety endangered hen breeds.*
  - Autores (por orden de firma): ***González Ariza A.***, *A. Arando Arbulu, J. M. León Jurado, F. J. Navas González, J. V. Delgado Bermejo, and M. E. Camacho Vallejo.*
  - Revista (año, volumen, paginación): *Animals*. *Submitted on 16th June, 2021.*
  - Base de Datos Internaticional o Nacional en las que está indexada: *Journal of Citation Reports (JCR), 2019 datos año previo. Datos para 2021 no publicados.*
  - Área temática en la Base de Datos de referencia: *Veterinary Science.*
  - Índice de impacto de la revista en el año de publicación del artículo: *2.323.*
  - Lugar que ocupa/Nº de revistas del Área temática: *14/141 (D1/Q1).*

- Segunda publicación:
  - Título: *Characterisation of biological growth curves of different varieties of an endangered native hen breed kept under free range conditions*.
  - Autores (por orden de firma): ***González Ariza A.***, *S. Nogales Baena, T. M. Lupi, A. Arando Arbulu, F. J. Navas González, J. M. León Jurado, J. V. Delgado Bermejo, and M. E. Camacho Vallejo.*
  - Revista (año, volumen, paginación): *Italian Journal of Animal Science 2021, 20, 806-813.*
  - Base de Datos Internaticional o Nacional en las que está indexada: *Journal of Citation Reports (JCR), 2019 datos año previo. Datos para 2021 no publicados.*
  - Área temática en la Base de Datos de referencia: *Agriculture, Dairy and Animal Science.*
  - Índice de impacto de la revista en el año de publicación del artículo: *1.805.*
  - Lugar que ocupa/Nº de revistas del Área temática: *14/63 (Q1).*

- Tercera publicación:
  - Título: *Mathematical modeling of egg production curve in a multivariety endangered hen breed.*
  - Autores (por orden de firma): ***González Ariza A.***, *A. Arando Arbulu, J. M. León Jurado, F. J. Navas González, S. Nogales Baena, and M. E. Camacho Vallejo.*
  - Revista (año, volumen, paginación): *Research in Veterinary Science. Submitted on 29th April, 2021.*
  - Base de Datos Internaticional o Nacional en las que está indexada: *Journal of Citation Reports (JCR), 2019 datos año previo. Datos para 2021 no publicados.*
  - Área temática en la Base de Datos de referencia: *Veterinary Sciences.*

  - Índice de impacto de la revista en el año de publicación del artículo: *1.892.*
  - Lugar que ocupa/Nº de revistas del Área temática: *31/141 (Q1).*

- Cuarta publicación:
  - Título: *Non-Parametrical Canonical Analysis of Quality-Related Characteristics of Eggs of Different Varieties of Native Hens Compared to Laying Lineage.*
  - Autores (por orden de firma): ***González Ariza A.**, F. J. Navas González, A. Arando Arbulu, J. M. León Jurado, C. J. Barba Capote, and M. E. Camacho Vallejo.*
  - Revista (año, volumen, paginación): *Animals 2019, 9, 153.*
  - Base de Datos Internaticional o Nacional en las que está indexada: *Journal of Citation Reports (JCR), 2019.*
  - Área temática en la Base de Datos de referencia: *Veterinary Science.*
  - Índice de impacto de la revista en el año de publicación del artículo: *2.323.*
  - Lugar que ocupa/Nº de revistas del Área temática: *14/141 (D1/Q1).*

- Quinta publicación:
  - Título: *Egg Quality-related Data Mining based Discriminant Analysis Tool for Native Hen Breed Productive Characterization.*
  - Autores (por orden de firma): ***González Ariza A.**, Arando A., Navas González F. J., León J. M., Delgado J. V., and Camacho M. E.*
  - Revista (año, volumen, paginación): *Poultry Science. Submitted on 8th June, 2021.*
  - Base de Datos Internaticional o Nacional en las que está indexada: *Journal of Citation Reports (JCR), 2019 datos año previo. Datos para 2021 no publicados.*
  - Área temática en la Base de Datos de referencia: *Agriculture, Dairy and Animal Science.*
  - Índice de impacto de la revista en el año de publicación del artículo: *2.659.*
  - Lugar que ocupa/Nº de revistas del Área temática: 7/63 *(Q1).*

- Sexta publicación:
  - Título: *Hen breed and variety factors as a source of variability for the chemical composition of eggs.*
  - Autores (por orden de firma): ***González Ariza A.**, F. J. Navas González, A. Arando Arbulu, J. V. Delgado Bermejo, and M. E. Camacho Vallejo.*
  - Revista (año, volumen, paginación): *Journal of Food Composition and Analysis 2021, 95, 103673.*
  - Base de Datos Internaticional o Nacional en las que está indexada: *Journal of Citation Reports (JCR), 2019 datos año previo. Datos para 2021 no publicados.*

- Área temática en la Base de Datos de referencia: *Food Science and Technology.*
- Índice de impacto de la revista en el año de publicación del artículo: *3.721.*
- Lugar que ocupa/Nº de revistas del Área temática: *30/139 (Q1).*

- Séptima publicación:
  - Título: *Sensory Preference and Professional Profile Affinity Definition of Endangered Native Breed Eggs Compared to Commercial Laying Lineages' Eggs.*
  - Autores (por orden de firma): ***González Ariza A.**, A. Arando Arbulu, F. J. Navas González, J. M. León Jurado, C. J. Barba Capote, and M. E. Camacho Vallejo.*
  - Revista (año, volumen, paginación): *Animals 2019, 9, 920.*
  - Base de Datos Internaticional o Nacional en las que está indexada: *Journal of Citation Reports (JCR), 2019.*
  - Área temática en la Base de Datos de referencia: *Veterinary Science.*
  - Índice de impacto de la revista en el año de publicación del artículo: *2.323.*
  - Lugar que ocupa/Nº de revistas del Área temática: *14/141 (D1/Q1).*

- Octava publicación:
  - Título: *Discriminant Canonical Analysis as a Validation Tool for Multivariety Native Breed Egg Commercial Quality Classification.*
  - Autores (por orden de firma): ***González Ariza A.**, A. Arando Arbulu, F. J. Navas González, J. V. Delgado Bermejo, and M. E. Camacho Vallejo.*
  - Revista (año, volumen, paginación): *Foods 2021, 10, 632.*
  - Base de Datos Internaticional o Nacional en las que está indexada: *Journal of Citation Reports (JCR), 2019 datos año previo. Datos para 2021 no publicados.*
  - Área temática en la Base de Datos de referencia: *Food Science and Technology.*
  - Índice de impacto de la revista en el año de publicación del artículo: *4.092.*
  - Lugar que ocupa/Nº de revistas del Área temática: *27/139 (Q1).*

# *Producción Científica*

Otras aportaciones científicas derivadas directamente de la Tesis Doctoral:

- Otras publicaciones Indexadas en el JCR:
    - Iglesias Pastrana, C., F. J. Navas González, C. Marín Navas, A. Arando Arbulu, **A. González Ariza**, J. M. León Jurado, M. G. Pizarro Inostroza, and M. E. Camacho Vallejo. 2019. Sexual Dimorphism for Copying Styles Complements Traditional Methods for Sex Determination in a Multivariety Endangered Hen Breed. Animals 9:1165.

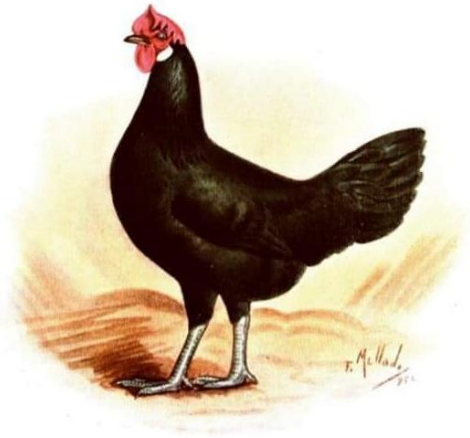
- Trabajos bibliográficos y de divulgación:
    - **González-Ariza, A.**, S. Nogales, F. J. Navas-González, J. V. Delgado, J. M. León, C. J. Barba, A. Arando, and M. E. Camacho. 2019. Preliminary results of the characterization of the growth of the Utrerana avian breed. Actas Iberoamericanas de Conservación Animal 14:14-20.
    - **González-Ariza, A.**, C. J. Barba, J. V. Delgado, J. M. León, A. Arando, F. J. Navas-González, S. Nogales, and M. E. Camacho. 2019. Preliminary results on the reproductive characterization of Utrerana avian breed. Actas Iberoamericanas de Conservación Animal 14:21-26.
    - **González-Ariza, A.**, A. Arando, J. M. León, J. V. Delgado, F. J. Navas-González, M. A. Martínez, S. Nogales, M. Macrì, J. Doctor, C. J. Barba, and M. E. Camacho. 2019. Estrategia de conservación de la gallina Utrerana: valorización de sus productos. FEAGAS 42:108-110.

- Ponencias invitadas:
    - **González Ariza, A.**, and A. Arando Arbulu. Características morfológicas que definen los diferentes troncos raciales en avicultura. En Avicor19, Hinojosa del Duque, Córdoba, España, 4-8 Diciembre, 2019.

- Contribuciones a congresos:
  a. Comunicaciones orales:
     - **González Ariza, A.**, A. Arando Arbulu, F. J. Navas González, F. A. Ruíz Morales, J. M. León Jurado, C. J. Barba Capote, J. V. Delgado Bermejo, and M. E. Camacho Vallejo. Definición del perfil de consumidor con mayor afinidad hacia el huevo de la raza aviar Utrerana. En XXI Simposio Iberoamericano sobre Conservación y Utilización de Recursos Zoogenéticos, 15-16 Diciembre, 2020, On-line.
     - **González Ariza, A.**, F. J. Navas González, A. Arando Arbulu, J. V. Delgado Bermejo, and M. E. Camacho Vallejo. Caracterización del perfil de ácidos grasos de la yema de la raza aviar Utrerana. En XXI Simposio Iberoamericano sobre Conservación y Utilización de Recursos Zoogenéticos, 15-16 Diciembre, 2020, On-line.

  b. Posters:
     - **González-Ariza, A.**, F. J. Navas-González, A. Arando, J. M. León, J. Doctor, C. Marín, M. G. Pizarro, S. Nogales, C. Barba, J. V. Delgado, and M. E. Camacho. Análisis canónico de las características de calidad del huevo en la raza aviar Utrerana. En XX Simpósio Iberoamericano sobre Conservação e Uso de Recursos Zoogenéticos Locais, 11-14 Noviembre, 2019, Corumbá-MS, Brasil.
     - **González-Ariza, A.**, A. Arando, J. M. León, J. Doctor, M. G. Pizarro, M. Gallardo, C. Marín, F. J. Navas-González, J. V. Delgado, and M. E. Camacho. Calidad externa del huevo de la raza aviar Andaluza Azul. En XX Simpósio Iberoamericano sobre Conservação e Uso de Recursos Zoogenéticos Locais, 11-14 Noviembre, 2019, Corumbá-MS, Brasil.
     - **González-Ariza, A.**, A. Arando, J. M. León, J. Doctor, M. Gallardo, M. G. Pizarro, F. J. Navas-González, J. V. Delgado, and M. E. Camacho. Resultados preliminares de caracterización reproductiva de la raza aviar Andaluza Azul. En XX Simpósio Iberoamericano sobre Conservação e Uso de Recursos Zoogenéticos Locais, 11-14 Noviembre, 2019, Corumbá-MS, Brasil.

- Arando, A., **A. González-Ariza**, J. M. León, J. Doctor, F. J. Martín, J. A. Soler, J. V. Delgado, and M. E. Camacho. Investigación, difusión y retroalimentación de atributos culinarios en productos y subproductos de la raza aviar Utrerana. En XX Simpósio Iberoamericano sobre Conservação e Uso de Recursos Zoogenéticos Locais, 11-14 Noviembre, 2019, Corumbá-MS, Brasil.
- Vega-Plá, J., A. Martínez, J. V. Delgado, A. Arando, A. Canales, N. García, M. Gómez-Carpio, **A. González**, C. González-Felgueroso, V. Landi, et al. Genetic characterization of Spanish autochthonous chicken breeds using microsatellites. En 37th International Society for Animal Genetics Conference, 7-12 Julio, 2019, Lleida, Spain.



# *Summary*

Despite the wide biodiversity of Spanish avian species of zootechnical interest, projects aiming at characterizing these genotypes and their products is necessary. The characterization of Utrerana hen production is needed to obtain differentiated products obtained through sustainable production systems. These sustainable avian productions not only have less impact on the environment and human health, but also are developed under the scope of animal welfare.

In the first study, a morphological characterization of two endangered autochthonous breeds (Utrerana and Sureña) and their varieties was developed. A forward stepwise discriminant canonical analysis was used to determine genotype (breed/variety) clustering patterns. White nails, ocular index and back length reported to have the highest discriminant power in female's morphological differentiation and characterization. For males, ocular index and black/corneous and white beak colours reported the greatest discriminant potential. Mahalanobis distances evidences the separation between both breeds and the proximity across their varieties. Despite the capacity to adapt to alternative production systems that has been ascribed to both breeds, Sureña and Utrerana avian breeds morphologically differ, hence their adaption methods may do too.

The aim of the second study was to model the growth patterns of the four varieties of Utrerana avian breed. Brody, Von Bertalanffy, Verhulst, logistic and Gompertz models were fitted. For this purpose, a total of 16235 weight data observations from 2004 animals reared in free range system were collected. Logistic was the best suited model to predict the biological growth curve of the White variety across sexes, while Von Bertalanffy was the best fitting model for the rest of individuals of the breed. Black variety was the heaviest one, with values of 2605.96 and 2032.61 g (for males and females, respectively) for the *a* parameter, while the lowest maturity weight was reported for the White variety ($a$ = 2442.99 and 1874.24 g, for males and females, respectively). Growth characterization is essential for the conservation of the Utrerana hen as a strategy to meet the demands of new market niches and a greater profitability of this differentiated product.

The third study aimed to compare the egg laying performance of the four varieties of Utrerana hen. A flock of 60 Utrerana hens (15 per variety) were individually housed to enable the daily egg traceability. For the study of the biological laying

curves, seven nonlinear regression models were fitted: compartmental, Gamma, linear hyperbolic, logistic curvilinear, McNally, Narushin-Takma and quadratic logarithmic. Best fitting values were reached by the six-parameter model of Narushin-Takma in the white laying curve ($R^2$=0.828), franciscan ($R^2$=0.888) and black varieties ($R^2$=0.899), while quadratic logarithmic resulted the best-fitting model for the laying performance of partridge Utrerana hen ($R^2$=0.917). This study determined which livestock models better adapt to the breed laying patterns and thus, permit to improve the economic profitability which in turn may ensure the conservation of these local genetic resources.

The objectives of the fourth study were to characterize the productive capability of Utrerana breed and to compare the relationships that exist between the parameters that determine the internal and external quality of the egg. To this aim a nonlinear canonical correlation analysis. Two flocks, one comprising 68 Utrerana hens and a control group of Leghorn hens (n = 17), were individually housed to allow individual identification of eggs and for the assessment of egg quality characteristics. Significant differences were reported for almost all variables when both breeds were compared. External quality-related traits are better predictors of internal quality-related traits than vice versa. The methods described enable the implementation of an effective noninvasive method for internal quality determination and egg classification aimed at suiting the consumers' needs.

In the fifth study, external and internal egg quality traits were measured in 819 eggs laid by hens of 10 different genotypes: white, franciscan, black and partridge varieties of Utrerana, Blue Andalusian, Spanish White-Faced, Andalusian Tufted white and black varieties, Araucana; and Leghorn Lohmann LSL-Classic lineage (commercial hybrid line) hen breeds. A forward stepwise discriminant canonical analysis and data mining CHAID decision tree method were applied to determine the egg quality-linked clustering patterns of eggs across hen genotypes. Araucana eggs quality was the most distant from the rest. Clear quality differentiation signs are evidenced for Mediterranean native breeds' eggs when compared to Leghorn's. Consequently, these evidences of egg quality differentiation may favour the standardization of breed and variety linked distinctive products.

In the sixth study, eggshell, white and yolk chemical composition of the egg of Utrerana breed varieties was compared to that of Leghorn Lohmann LSL-Classic lineage's. Eggshell, yolk and white macroelements and microelements, carbohydrates, moisture, ashes, protein, fat (polyunsaturated and saturated), sugars, cholesterol, and α-tocopherol contents were quantified and assessed. Simultaneously, itemized yolk fatty acids composition was evaluated. While calcium contents were higher in Utrerana eggshell (358.53 g/kg vs. 337.01 g/kg) and white (593.75 mg/kg vs. 584.31 mg/kg), protein contents were higher in Utrerana yolk (17.40 % vs. 16.90 %) and white (10.60 % vs 10.30 %). Utrerana yolks reported higher α-tocopherol (102.00 mg vs. 88.00 mg), total polyunsaturated fatty acids (19.80 % vs. 16.60 %), and some monounsaturated fatty acids content (C18:1 n9: 42.68 % vs. 41.31 %; C16:1 n9: 0.60 % vs. 0.50 %). Knowledge on the differential properties of eggs depending on the animals which originated permits to develop a correctly approach of a broader spectrum of market needs.

The seventh study aimed to compare Utrerana native hen eggs' sensory properties to Leghorn Lohmann LSL-Classic lineage's commercial and ecological eggs through free-choice profiling. Affine and non-affine profiles were defined using the information provided by professionally-instructed panelists on six sets using nonlinear canonical correlation analysis. Observers reported a significantly higher appreciation ($p > 0.05$) towards yolk color, odor, flavor, texture, overall score, and whole and on plate broken egg visual value when Utrerana eggs were compared to the rest of egg commercial categories. Professional Profile A (PPA), or egg non-affine profile, consumed less eggs and scored sensory attributes lower than Professional Profile B (PPB), or affine profile. Additionally, PPB accounted for higher knowledge about the Utrerana breed, hence conferred greater importance to the product's ecological and autochthonous nature. PPA was generally characterized by women under 20 years old with no higher studies, while PPB comprised 21-40 years old men with secondary studies.

The objective of the eighth study was to develop a tool to validate multivariety breed egg quality classification depending on quality-related internal and external traits using a discriminant canonical analysis approach and data mining CHAID decision tree. A flock of 60 Utrerana hens and a control group of 10 Leghorn hens were placed

in individual cages to follow the traceability of the eggs and perform an individual internal and external quality assessment. Egg groups were sorted depending on their commercial size (S, M, L, and XL), laying hen breed, and variety. Albumen weight, eggshell weight, and yolk weight accounted for the greatest discriminant ability to determine differences among egg quality categories (Wilks' lambda: 0.335, 0.539, and 0.566 for albumen weight, eggshell weight, and yolk weight, respectively). Shared properties between partridge and franciscan varieties may stem from their eggs presenting heavier yolks and slightly lower weights, while white Utrerana and Leghorn hens' similarities may be ascribed to hybridization reminiscences.

The aforementioned studies seek the obtention of a deeper knowledge on the zoometric, phaneroptic and productive characterization of the Utrerana breed. This lays the basis for strategies aiming at more appropriately responding to a wider scope of market demands and consumers trends, which in turn may ensure the sustainability of conservation policies of this animal genetic resource.

# *Resumen*

A pesar de que existe una amplia biodiversidad de especies aviares españolas de interés zootécnico, es necesario destinar proyectos para caracterizar estos genotipos y sus productos. La caracterización productiva de gallina de Utrerana se torna imprescindible para la obtención de productos diferenciados, obtenidos a través de sistemas productivos sostenibles. Estas producciones avícolas sostenibles no sólo tienen un menor impacto en el medio ambiente y la salud humana, sino que también se desarrollan en el ámbito del bienestar animal.

En el primer estudio se desarrolló una caracterización morfológica de dos razas autóctonas (Utrerana y Sureña) y sus variedades. Se utilizó un análisis discriminante canónico por pasos hacia adelante para determinar los patrones de agrupación de genotipos (raza/variedad). Se reportó que las uñas blancas, el índice ocular y la longitud del dorso poseen mayor poder discriminante en la diferenciación y caracterización morfológica de la hembra. Para los machos, el índice ocular y los colores del pico negro/córneo y blanco tuvieron el mayor potencial discriminatorio. Las distancias de Mahalanobis evidencian la separación entre ambas razas y la proximidad entre sus variedades. A pesar de la capacidad de adaptación a sistemas de producción alternativos que se ha atribuido a ambas razas, las razas aviares Sureña y Utrerana difieren morfológicamente, por lo que sus métodos de adaptación también podrían hacerlo.

El objetivo del segundo estudio fue modelar los patrones de crecimiento de las cuatro variedades de la raza aviar Utrerana. Se utilizaron los modelos Brody, Von Bertalanffy, Verhulst, logistic y Gompertz. Para este propósito, se recogió un total de 16235 pesadas de 2004 animales, criados en sistema campero. El modelo logístico fue el más adecuado para predecir la curva de crecimiento biológico de la variedad blanca en ambos sexos, mientras que Von Bertalanffy fue el modelo que mejor se ajustó para el resto de los individuos de la raza. La variedad negra fue la de mayor peso, con valores de 2605,96 y 2032,61 g (para machos y hembras, respectivamente) para el parámetro *a*, mientras que el menor peso a la edad adulta fue reportado en la variedad blanca (*a* = 2442,99 y 1874,24 g, para machos y hembras, respectivamente). La caracterización del crecimiento es fundamental para la conservación de la gallina Utrerana como estrategia para atender la demanda de

nuevos nichos de mercado y obtener una mayor rentabilidad de este producto diferenciado.

El tercer estudio tuvo como objetivo comparar el comportamiento de puesta de las cuatro variedades de la gallina Utrerana. Se alojó un lote de 60 gallinas Utreranas individualmente (15 por variedad), lo que permitió la trazabilidad diaria de los huevos. Para el estudio de las curvas de puesta se ajustaron siete modelos de regresión no lineal: compartimental, Gamma, lineal hiperbólico, logístico curvilíneo, McNally, Narushin-Takma y cuadrático logarítmico. Los mejores valores de ajuste fueron alcanzados por el modelo de seis parámetros de Narushin-Takma en las curvas de puesta de las variedades blanca ($R^2$ = 0.828), franciscana ($R^2$ = 0.888) y negra ($R^2$ = 0.899), mientras que el modelo cuadrático logarítmico resultó el mejor modelo de ajuste para el rendimiento de puesta de la variedad perdiz ($R^2$ = 0,917). Este estudio determinó que estos modelos ganaderos se adaptan mejor a los patrones de puesta de la raza y, por lo tanto, permiten mejorar la rentabilidad económica, que a su vez puede asegurar la conservación de estos recursos genéticos locales.

Los objetivos del cuarto estudio fueron caracterizar la capacidad productiva de la raza Utrerana y comparar las relaciones existentes entre los parámetros que determinan la calidad interna y externa del huevo. Para ello, se realizó un análisis de correlación canónica no lineal. Dos lotes, uno compuesto por 68 gallinas Utrerana y otro por un grupo de control de gallinas Leghorn (n = 17), se alojaron individualmente para permitir la identificación individual y evaluación de las características de calidad de los huevos. Se reportaron diferencias significativas para casi todas las variables estudiadas cuando se compararon ambas razas. Los rasgos de calidad externos fueron mejores predictores de los rasgos internos que, al contrario. Así, los métodos descritos permiten la implementación de un método no invasivo eficaz para la determinación de la calidad interna y la clasificación de huevos, con el objetivo de satisfacer las necesidades de los consumidores.

En el quinto estudio se midieron los rasgos de calidad interna y externa en 819 huevos producidos por gallinas de 10 genotipos diferentes: variedades blanca, franciscana, negra y perdiz de la raza Utrerana, Azul Andaluza, Española Cara Blanca, Andaluza Moñuda blanca y negra, Araucana, y gallinas del linaje Leghorn

Lohmann LSL-Classic (línea híbrida comercial). Se aplicó un análisis discriminante canónico hacia delante y el método de árbol de decisión CHAID para determinar los patrones de agrupación de huevos vinculados a la calidad de estos genotipos. La calidad de los huevos de Araucana fue la más distante del resto. Además, los huevos de razas autóctonas mediterráneas presentaron claros signos de diferenciación de calidad en comparación con los de Leghorn. En consecuencia, estas evidencias en cuanto a diferenciación de la calidad del huevo pueden favorecer la estandarización de productos distintivos vinculados a la raza y la variedad.

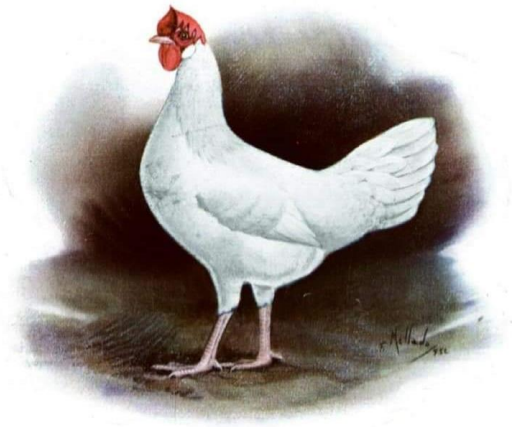
En el sexto estudio, se comparó la composición química del cascarón, la clara y la yema del huevo de las variedades de Utrerana con la del linaje Leghorn Lohmann LSL-Classic. Se cuantificaron y evaluaron los macroelementos y microelementos de cascarón, yema y clara, como carbohidratos, humedad, cenizas, proteínas, grasas (poliinsaturadas y saturadas), azúcares, colesterol y α-tocoferol. Simultáneamente, se evaluó la composición detallada de los ácidos grasos de la yema. Mientras que el contenido de calcio fue mayor en el cascarón (358,53 g/kg frente a 337,01 g/kg) y clara de huevo de Utrerana (593,75 mg/kg frente a 584,31 mg/kg), el contenido de proteína fue mayor en la yema (17,40% frente a 16,90%) y clara de Utrerana (10,60% vs 10,30%). Las yemas de Utrerana tuvieron un mayor contenido de α-tocoferol (102,00 mg frente a 88,00 mg), ácidos grasos poliinsaturados totales (19,80% frente a 16,60%) y algunos ácidos grasos monoinsaturados (C18: 1 n9: 42,68% frente a 41,31%; C16: 1 n9: 0,60% frente a 0,50%). El conocimiento de las propiedades diferenciales de los huevos en función de los animales de origen permite desarrollar correctamente un enfoque más amplio en cuanto a las necesidades del mercado.

El séptimo estudio tuvo como objetivo comparar las propiedades sensoriales de los huevos de gallina Utrerana con los huevos comerciales y ecológicos del linaje Leghorn Lohmann LSL-Classic, a través de perfiles de libre elección. Los perfiles afines y no afines se definieron utilizando la información proporcionada por panelistas profesionales instruidos y utilizando análisis de correlación canónica no lineal. Los observadores reportaron una apreciación significativamente mayor ($p > 0.05$) hacia el color de la yema, el olor, el sabor, la textura, la puntuación general y el valor visual del huevo entero y roto en el plato cuando se compararon los huevos de

Utrerana con el resto de las categorías comerciales de huevos. El perfil profesional A (PPA), o perfil no afín de huevo, se constituyó por personas que consumían menos huevos y puntuaron peor los atributos sensoriales que el perfil profesional B (PPB) o perfil afín. Además, el PPB tenía un mayor conocimiento sobre la raza Utrerana, por lo que otorgó mayor importancia al carácter ecológico y autóctono del producto. El PPA se caracterizó generalmente por mujeres menores de 20 años sin estudios superiores, mientras que el PPB comprendió hombres de 21 a 40 años con estudios secundarios.

El objetivo del octavo estudio fue desarrollar una herramienta para validar la clasificación de la calidad del huevo de esta raza multivariedad, en función de los rasgos internos y externos relacionados con la calidad, utilizando un enfoque de análisis canónico discriminante y un árbol de decisiones CHAID de extracción de datos. Un lote de 60 gallinas Utreranas y un grupo de control de 10 gallinas Leghorn se colocaron en jaulas individuales para seguir la trazabilidad de los huevos y realizar una evaluación individual de la calidad interna y externa. Los grupos de huevos se clasificaron según su tamaño comercial (S, M, L y XL), raza de gallina ponedora y variedad. El peso de la clara, cascarón y yema presentaron la mayor capacidad discriminante para determinar las diferencias entre las categorías de calidad del huevo (Wilks' lambda: 0,335, 0,539 y 0,566 para el peso del albumen, el peso de la cáscara y el peso de la yema, respectivamente). Las propiedades compartidas entre las variedades perdiz y franciscana pueden deberse a que sus huevos presentan yemas más pesadas y pesos totales ligeramente más bajos, mientras que las similitudes de las gallinas Utrerana blanca y Leghorn pueden atribuirse a reminiscencias de hibridación entre ambas.

Los estudios mencionados buscan la obtención de un conocimiento más profundo sobre la caracterización zoométrica, faneróptica y productiva de la raza Utrerana. Esto sienta las bases para estrategias que apunten a responder de manera más adecuada a un espectro más amplio de demandas del mercado y tendencias de los consumidores, lo que, a su vez, puede asegurar la sostenibilidad de las políticas de conservación de este recurso zoogenético.



# *Introduction*

Avian eggs represent an important source of nutrients which contain all the proteins, lipids, minerals, and vitamins that enable the development of the embryo. Some of these constituents, such as enzymes or immune proteins have multibiological functions (Nowaczewski et al., 2013). As a result, egg is present in a great fraction of the human diet across the different cultures in the world. This added value of eggs is supplemented by its wide cuisine applicability as a product. They can be used for breakfast or home meal preparations, baking and as an ingredient in many culinary recipes.

Currently, almost all poultry production comes from commercial hybrid lines, which are characterized by high productive performance and a good feed conversion index (Mottet and Tempio, 2017; Tallentire et al., 2018). These genotypes present homogeneous product characteristics that may no longer fulfil market demands considering the need of customers for new products. Additionally, the obtention and production of these highly productive lines promotes a decrease in the genetic variability of the species and has negative effects on the development of sustainable practices based on local breeds (Hoffmann, 2011).

The emergence of new commercial lines of laying hens with a much higher productive capacity caused the displacement of the autochthonous breed throughout the twentieth century. In many cases, their hybridization with more productive lines relegated autochthonous breeds, including the Utrerana avian breed, to a secondary form of ornamental poultry farming, based on the morphological and phaneroptical selection of breeding animals (Garcia and Cordero, 2006).

In Spain, two types of hens can be found: Atlantic and Mediterranean trunks. Hens of Atlantic trunk are generally semi-heavy birds, with red earlobes and brown-shelled eggs. On the other hand, the Mediterranean population comprises light individuals, with white earlobes and white-shelled eggs (Orozco, 1989). Andalusia (in the south of Spain) is influenced by the Mediterranean climate, with maximum temperatures going above 40 °C in summer, as reported by the State Meteorological Agency (AEMET) from Spain, and very high temperatures from late spring and the whole summer. In this area, Utrerana hen has contributed to poultry production under backyard and extensive systems. The creation of the breed started with the

selection of a heterogeneous population of chickens from the Andalusian countryside during the first half of the 20th century (Orozco, 1989). Formerly, it was productively oriented towards a laying aptitude, with an annual output of 120-180 eggs, white in colour and with an average weight of 62-64 g. Its four varieties, characterize by the colour of their plumage and legs: white, franciscan, black, and partridge (Campo, 2007). Consequently, these widely accessible birds kept in sustainable systems, with low capital investment, and few inputs requirements, have historically been the source of production of high biological value proteins present in their products, such as eggs and meat (Bettridge et al., 2018; Canales et al., 2019).

The present thesis was developed in the context of a project entitled "Conservation strategy of Utrerana hen: valorization of its products", funded by FEDER (Project PP:AVA.AVA201601.16). The need for the characterization of the products of the Utrerana hen breed is largely due to the situation that it faces. This breed is classified as an endangered breed, according to the Royal Decree Law 45/2019, of February 8th, which updates the national program of conservation, breeding, and promotion of livestock breeds, and presented a census of 1525 individuals on 31 December, 2020 (MAPA, 2021).

To face this alarming situation, the implementation of programs for the recovery, conservation and productivity improvement of the breed are necessary. These programs must try to provide it with an easily distinguishable identity and, distinctive productive position able to satisfy the demands of the market. The pivotal element for these strategies to thrive is the assessment of local products. The implementation of new commercial approaches may be key for the conservation of this and other local breeds, for instance, preventing the loss of linkage between local products and their area of production, as side effect of industrial products market colonization (Vaarst et al., 2015).

There is a growing interest among consumers in animal products obtained through sustainable production systems. The purpose of these systems is to obtain quality food, with less impact on the environment and human health, under the scope of animal welfare (Barba et al., 2016). Alternative forms of farming, involving local breeds, are necessary to avoid the loss of biodiversity, the disappearance of animal

genetic resources, to improve the efficiency in the search for economic sustainability and promote the linkage of population to rural areas (Alderson, 2018; Toalombo et al., 2019).

Utrerana breed is adapted to these systems due to its high rusticity and low disease prevalence (Del Castillo, 1951). Additionally, native poultry breeds genome makes them more resistant than commercial hybrid lines to climate change derived conditions occurring at specific geographical area (Mpenda et al., 2019). Even if the dual-purpose nature of some of these native breeds makes them be less competitive than egg or meat specialized breeds (Castellini et al., 2010), they are usually more resistant to pathologies, thermal stress, environmental conditions and have a great ability to search for food in the wild (Palacios et al., 2016; Lordelo et al., 2017). Additionally, the comparatively reduced productivity of these animals may be counteracted by the greater quality of their products, which helps widening the variety of foods offered to the market.

The biometric characterization of local breeds, as well as the relationship among them, can provide an insight to the mechanism and events that contributed to the origin and development of native poultry breeds (Brito et al., 2021). In these regards, breeds standardization could be an important tool for the evaluation of individuals within their flocks and thus be able to configure the most efficient strategies for selection of the best animals (Otecko et al., 2019). Moreover, morphometric and phaneroptic approaches may play a fundamental role in poultry management due to them being faster and rather economically profitable (Dorji and Sunar, 2014).

The birth of approximately 50% of males on each incubation batch has promoted the traditional use of the meat carcass of this breed for self-consumption (Campo, 2007). Growth can be explained through mathematical functions, which could also predict for the age of sexual maturity or the suitable age for commercial slaughter, while they help to monitor general health conditions and nutritional requirements (Kaplan and Gürcan, 2018). Across the life cycle of animals, total growth duration can be divided into three phases: an acceleration phase, a deceleration phase, and a stabilization phase for ripening (Nogales et al., 2017). As a result, growth patterns are typically better fitted by models presenting a sigmoidal structure. The study of

the growth patterns and how certain factors such as variety or sex affect it, is necessary to establish the meat productivity potential of breeds (Sariyel et al., 2017).

Moreover, the modelling for knowledge, analysis and interpretation of the egg production curves over time allows to make behavioral predictions, to know the productive performance at a given moment, to make flock balances and analysis of laying curve shape and of specific parameters such as peak and persistence (Savegnago et al., 2012). Nonlinear regression models are widely used to fit egg production data. Mathematically, the egg production curves can be divided in three phases: the first phase is the increase in laying from the lay of the first egg which occurs at the age of sexual maturity until the hen reaches the maximum point. Afterwards, a linear trend is progressively reached during the second phase, to then, experience decreases to zero during the third phase (Narinç et al., 2014). The laying curve in hen closely resembles the milk production curve of dairy cows and small ruminants, hence multiple functions can be used to model both production systems (Pizarro Inostroza et al., 2020).

The number of eggs and the time of the laying period are related through egg production curves (Yang et al., 1989). It has been shown that mathematical models are suitable to characterize the laying performance of hen flocks. The use of nonlinear regression functions can be considered as an alternative method to evaluate laying patterns and laying curve parameters as potential breeding criteria to be considered in genetic studies of poultry farms. Furthermore, the evaluation of laying curves enables to develop a thorough assessment of in-farm zootechnical management practices, flocks' performance and income forecasting (Miyoshi et al., 1996).

The acceptability of the product by the consumers depends on quality traits that are related with the eggshell, the albumen, and the yolk of eggs (Duman et al., 2016). Depending on the need to break the egg to take quality measurements, quality traits can be classified into external or internal quality ones (Begli et al., 2010) . Previous research reported that quality of hen eggs can be influenced by genetic and non-genetic components, such as the age of the hen, feed intake and environmental and meteorological factors (Sokołowicz et al., 2018; Zheng et al., 2020).

Egg parameters have been reported to affect fertility, embryo development, hatchability, and chicken viability (Abioja et al., 2020). Among the considerable number of characteristics of egg quality that can be measured, external factors such as egg weight is the most important (Dudusola, 2010; Hanusova et al., 2015). Internal egg quality comprises an important set of factors to consider, especially when approaching the marketing opportunities of the product. A dense albumen height is among the most important determinants of the internal quality (Scott and Silversides, 2001; Monira et al., 2003). In addition to these factors, other parameters such as the major and minor diameters of the egg, eggshell, yolk color and the weight and pH of the albumen and yolk offer the opportunity to perform a more complete characterization of egg quality (Islam et al., 2017; Sirri et al., 2018). Contextually, breed genotype can significantly affect most of these features: egg shape, yolk and albumen quality, shell and egg weight and amount of yolk, hence the study of it repercussion is determinant (Zita et al., 2009).

As a way to better respond to the necessities of the market, recent research has focused on scientifically proving the superior organoleptic and sensorial perception reported for native breed eggs when compared to those of a laying lineage by cuisine professionals (Berkhoff et al., 2020). Such attributes may base on the difference in the chemical composition across the eggs produced by different strains. Chemical composition of eggs has been reported to be rather conditioned by the effects of nutrition than genetics. However, there are some relevant pieces of evidence for a hereditary influence in certain traits of egg composition; namely, relative proportion of yolk and white, white quality, qualitative protein polymorphism, total protein content, contents of cholesterol, vitamin A, thiamine, riboflavin, fatty acids, enzymes and deposition of metabolic products in the eggs (Washburn, 1979).

Additionally, the composition of heavy metals in eggs, which usually derives from the fishmeal that is supplied to animals in their diets, has been deemed an important trait to evaluate (Farahani et al., 2015). In this context, feed provided to laying hens may often be contaminated with mercury or arsenic, among others trace elements. However, the ability of these animals to metabolize such substances, and the heritable component of this ability may be determinant, as they may translate into

the reduction of the accumulation of residues in the final products that reach the market, which in turn minimizes the potential harms to humans (Ding et al., 2019).

Egg components are not only quantitatively conditioned by genetic factors but can also be qualitatively affected. Contextually, chemical composition concerning the metabolism and concentration of diverse elements of nutritive interest in laying hens, like fatty acid composition have been reported to be affected by other factors such as age, genotype and environmental (Rizzi and Chiericato, 2010). Furthermore, apart from the influence of genetic factors themselves, an interaction between strain and environmental conditions has been reported in literature and has been suggested to condition the absorption and use of dietary components by laying hens. This genetically-dependent interaction may play a decisive role in the determination of the ability to incorporate some diet nutrients like fatty acids to the products to which they give origin (Rizzi and Chiericato, 2010). Among other important nutritional resources, albumen quality widely reflects the variability in protein polymorphism and has been shown to be a highly heritable traits (Washburn, 1979). This high heritability contributes to the greater possibilities to perform effective selection strategies based on a wider genetic variability among individuals.

Egg quality is directly related to characteristics determining consumer acceptability. For instance, some traits, such as egg weight and dense white height, are highly valued by consumers (Hanusova, et al., 2015). However, there is a number of egg sensory attributes that are more difficult to assess, for which taster panels are used. Food chemical composition is appreciated through the taste sense. Still, some caution should be taken to transform taste sensations into reliable measures, given the existence of interpanelist variability sources which cannot be eliminated even after training (Williams and Arnold, 1985). Panelists have been suggested to be unable to routinely describe the sensory attributes they perceive; hence, profiling methods must be homogenized (same sensory lexicon, among others). To prevent this from occurring, techniques such as hedonic scale measurements are used (Bárcenas et al., 2001). Developing sensory tools to define potential consumers profiles and attitudes towards products has always concerned food scientists. Tests are diverse and range from those analyzing the process of standardization for food evaluation and perception derived from product/consumer interaction to panelists

sensation elaboration and verbalization (Stone et al., 2008). Empirically determining panelists discriminating capacity for organoleptic characteristics commonly involves the implementation of linear or logistic regression models used in preference surveys and social epidemiology (Frie and Janssen, 2009). However, in the case of multivariate analysis, non-linear canonical correlation analysis (OVERALS) more appropriately allows to map a series of explanatory factors that correlate to different sensory attributes and eggs' cuisine applicability (Van der Burg et al., 1994).

In line with this situation, the definition of the breed's genetic background, its productive role and the interconnections between both aspects became compulsory to maximize Utrerana's potential to satisfy current commercial demands. The characterization of Utrerana's egg as the main product of the breed was configured in the context of a set of strategies that sought the obtention of competitive sustainable products in the framework of the recent emerging diseases and climate change, at the same time that the future survival and conservation of the breed is ensured.

# *Aims*

The main objective of this PhD thesis was to perform a productive valorization of products derived from Utrerana avian breed as a generic objective to ensure the conservation of this breed, while offering an application model in other avian populations. For this reason, a series of specific aims was established:

A. First objective: Biometric characterization of Utrerana avian breed:

- *González Ariza A., A. Arando Arbulu, J. M. León Jurado, F. J. Navas González, J. V. Delgado Bermejo, and M. E. Camacho Vallejo. Discriminant Canonical tool for differential Biometric characterization of multivariety endangered hen breeds. Submitted to Animals.*

To develop a morphological characterization of Utrerana hen population and compare it with geographically close genotypes through quantitative zoometric measurements and qualitative attributes with discriminant capacity.

B. Second objective: Characterization of the productive capacity of Utrerana avian breed:

- *González Ariza, A., S. Nogales Baena, T. M. Lupi, A. Arando Arbulu, F. J. Navas González, J. M. León Jurado, J. V. Delgado Bermejo, and M. E. Camacho Vallejo. Characterization of biological growth curves of different varieties of an endangered native hen breed kept under free range conditions. Italian Journal of Animal Science 1:806-813.*
- *González Ariza A., A. Arando Arbulu, J. M. León Jurado, F. J. Navas González, S. Nogales Baena, and M. E. Camacho Vallejo. Mathematical modeling of egg production curve in a multivariety endangered hen breed. Submitted to Research in Veterinary Science.*

To describe the biological growth and laying performance curves of Utrerana avian breed through the use of non-linear regression models.

C. Third objective: Analysis of the egg quality-related characteristics:

- *González Ariza A., F. J. Navas González, A. Arando Arbulu, J. M. León Jurado, C. J. Barba Capote, and M. E. Camacho Vallejo. Non-Parametrical Canonical Analysis of Quality-Related Characteristics of Eggs of Different Varieties of Native Hens Compared to Laying Lineage. Animals 2019, 9, 153.*
- *González Ariza A., Arando A., Navas González F. J., León J. M., Delgado J. V., and Camacho M. E. Egg Quality-related Data Mining based Discriminant Analysis Tool for Native Hen Breed Productive Characterization. Submitted to Poultry Science.*
- *González Ariza A., F. J. Navas González, A. Arando Arbulu, J. V. Delgado Bermejo, and M. E. Camacho Vallejo. Hen breed and variety factors as a*

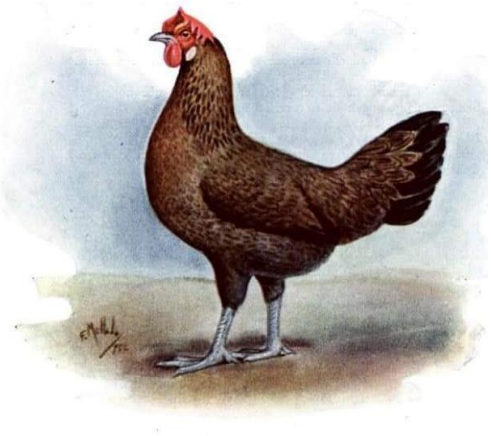
*source of variability for the chemical composition of eggs. Journal of Food Composition and Analysis 2021, 95, 103673.*

To characterize the physical and chemical egg quality-related traits of the varieties of Utrerana hens compared to a globally distributed laying lineage, as a means to identify the benefits of greater genetic diversity on the quality of products derived from sustainable native breeds. To compare the relationships among determining parameters of the internal and external quality of the egg of Utrerana hen through a nonlinear canonical correlation analysis (OVERALS) to develop a predictive tool that may enable indirect scoring of the internal quality of the egg from the set of external quality variables. To determine the differential clustering patterns of egg quality-related traits from the eggs laid by four Spanish native breeds (white-shelled eggs layers) and their varieties in comparison to a foreign native breed outgroup and a control flock of a commercial laying lineage.

D. Fourth objective: Analysis of the potential commercial of Utrerana egg:

- *González Ariza A., A. Arando Arbulu, F. J. Navas González, J. M. León Jurado, C. J. Barba Capote, and M. E. Camacho Vallejo. Sensory Preference and Professional Profile Affinity Definition of Endangered Native Breed Eggs Compared to Commercial Laying Lineages' Eggs. Animals 2019, 9, 920.*
- *González Ariza A., A. Arando Arbulu, F. J. Navas González, J. V. Delgado Bermejo, and M. E. Camacho Vallejo. Discriminant Canonical Analysis as a Validation Tool for Multivariety Native Breed Egg Commercial Quality Classification. Foods 2021, 10, 632.*

To determine the ability of panelists to discriminate organoleptic characteristics across egg type categories basing on hedonic scales. To infer different professional profiles regarding their affinity towards eggs and their sociological context, as a strategy to plan potential marketing strategies to reinforce affine professionals and to attract non-affine ones. to design a statistical tool that permits determining whether specific eggs may correctly fit the features of the different commercial size categories (S, M, L, and XL), which may support the standardization of the Utrerana varieties' eggs as products.



# *Results*

# Chapter 1.

## Biometric characterization of Utrerana Avian Breed.

- *González Ariza A., A. Arando Arbulu, J. M. León Jurado, F. J. Navas González, J. V. Delgado Bermejo, and M. E. Camacho Vallejo.* ***Discriminant Canonical tool for differential Biometric characterization of multivariety endangered hen breeds.*** *Submitted to Animals.*

# Discriminant Canonical tool for differential Biometric characterization of multivariety endangered hen breeds

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**Simple Summary:** The identification and characterization of endangered breeds and their inclusion into catalogues is the main initial pivotal policy that countries must implement to maximize the sustainability of genetic diversity in these populations. In this context, breed undefinition boosts the risk of irreversible breed loss due to its substitution by dominant breeds. Breed loss results detrimental for the fraction of the genetic pool which is linked to the value of livestock as perfectly adapted elements of domestic ecosystems among other desirable features. The present study aims to design a biometric characterization tool in autochthonous avian breeds and their varieties in Andalusia (south of Spain): Utrerana and Sureña hens. For this, different quantitative and qualitative measurements were collected in 473 females and 135 males belonging to these breeds. Despite the fact that both genotypes belong to a common original trunk, discriminant canonical analysis (DCA) revealed clear differences between both breeds and within the varieties that they comprise. Particularly, certain variables such as ocular index and phaneroptical characteristics, which may be inner related to the capacity of the breeds to adapt to the environmental conditions in which they thrive, could allow breeders to develop breeding programs focused on the enhancement productive potential of individuals.

**Abstract:** This study aimed to develop a tool to perform the morphological characterization of two endangered autochthonous breed and their varieties (n =

608; 473 females and 135 males). Kruskal Wallis H test reported evidences of sex dimorphism (P<0.05 at least). After multicollinearity analysis performance (VIF>5 variables were discarded), white nails, ocular index and back length (Wilks' Lambda values of 0.191, 0.357 and 0.429, respectively) reported to have the highest discriminant power in female's morphological characterization. For males, ocular index and black/corneous and white beak colours (Wilks' Lambda values of 0.180, 0.210 and 0.349, respectively) displayed the greatest discriminant potential. The first two functions explained around 90% of intergroup variability. A stepwise discriminant canonical analysis (DCA) was used to determine genotype clustering patterns. Mahalanobis distances revealed the degree of proximity between breeds and varieties. Despite the adaptability capacity that has been ascribed to both breed to alternative production systems, Sureña and Utrerana avian breeds morphologically differ. This breed dimorphism may indeed evidence differential adaptability mechanisms which may be linked to their aptitude (dual purpose and egg production). The present tool may serve as a model for the first stages of breed protection to be applicable in other endangered avian breeds worldwide.

**Keywords**: Local breeds; genetic resources; biometric characteristics; phaneroptics; biodiversity

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## 1. Introduction

In Spain, two hen trunks have historically been differentiated; the Atlantic trunk, generally comprised by larger-format, red ear-lobes, brown-shelled eggs dual purpose birds; and the Mediterranean trunk, consisting of lighter individuals, with white ear-lobes, and of a white-shelled eggs laying morphotype (Orozco, 1989). The aforementioned features have been considered by breeders on a regular basis for breed ascription and animal classification. This segregation of the Atlantic and Mediterranean trunks would later be supported from a molecular perspective through the estimation of genetic distances using microsatellite markers (Dávila et al., 2009).

As a result, natural and human selection derived in a high heterogeneity and variability of morphological characteristics in avian breeds (Sreenivas et al., 2018; Liverpool-Tasie et al., 2019). Such high heterogeneity was promoted when breeding

objectives (meat, eggs or dual-purpose breeds), hence morphological characteristics started to differ and polarize among populations to adapt to environment requirement s at the minimum biological cost. These differentiation process determined breeds turned to base their adaptability strategies on their particular and specific enhanced body features (Hill, 2012).

Andalusia (Southern Spain) is influenced by Mediterranean climate, with maximum temperatures rising above 40 °C in summer, as reported by the State Meteorological Agency (AEMET) from Spain. In this context, very high temperatures are present from late spring on and last for the whole summer. Among the breeds in the area, two laying hen genotypes have traditionally configured poultry production under backyard and extensive systems: Utrerana and Sureña avian breeds (Macrì et al., 2019; Araújo de Carvalho et al., 2020).

Utrerana and Sureña avian breeds share a common geographic location, socioeconomic context, and history. In addition, there are four varieties of plumage color that are present in both breeds: White, Franciscan, Black and Partridge in Utrerana breed; and White, Franciscan, Black, Partridge, Blue and Splash in Sureña Breed. However, Sureña hen have a large format than most Mediterranean hen breeds (Ocaña et al., 2008; González Ariza et al., 2019b).

These widely accessible low capital/input investment birds were historically kept in sustainable systems for decades, thus, became the source of production of high biological value proteins in rural livelihoods until globalization called for the intensification of animal production (Bettridge et al., 2018; Canales et al., 2019).

As a direct consequence, population census of Spanish breeds suffered a regression due to the introduction of selected commercial strains of birds with a higher production during the last half of the 20th century (González Ariza et al., 2019a; Dalle Zotte et al., 2020). In this way, Utrerana avian breed became classified as an endangered breed, according to Royal Decree 45/2019, of February 8$^{th}$, while Sureña avian breed is in the process of being included in the Official Livestock Breeds Catalogue of the Ministry of Agriculture, Fisheries and Environment (MAPA) of Spain.

Consumers interest for quality food products revolved market demands as a conscious response to the drawbacks implied by intensive production. Food

alternatives produced through sustainable production systems became popular provided these systems characterized by a low impact on the environment and human health while they also considered animal welfare (Toalombo et al., 2019). Increased demands soon translated into commercial chains starting to request differentiated products, whose properties significantly differed from products obtained through hybrid commercial strains (Torres et al., 2019).

For local producers to be able to fulfil market demands, products and the elements needed to ensure their constant supply, must be defined through breed characterization either it is zoometrically, genetically or even productively. Contextually, the characterization of local populations, as well as the relationship among already stablished breeds, can provide evidences on the mechanism and events that contributed to the origin and development of native poultry breeds in the south region of Spain, but also of the adaptive mechanisms that may have permitted their survival in time (Brito et al., 2021). Additionally, breeds standardization could be an important tool for the evaluation of birds within their flocks and determine certain measurements for selection of the best animals (Otecko et al., 2019). In these regards, morphometric and phaneroptic approaches may be fundamental in poultry management due to they are fast and economically profitable (Dorji and Sunar, 2014).

This information altogether enables the correct development and implementation of the administrative structures needed to guarantee the stability and future viability of breeds though the development conservation and breeding programs, and the sustainable commercialization of their products once censuses are enough.

In this context, the aim of this study was to determine the contribution of quantitative and qualitative morphological-related traits to the zoometric characterization through the development of a discriminant canonical analysis (DCA), as a tool that permits determining phenotypic variability in the Andalusian avian breeds and within their varieties as a strategy to support the standardization of native breeds and implement conservation strategies that ensuring the consolidation of local genotypes as recognized breeds.

## 2. Materials and Methods

### *2.1. Animals, sample size, and distribution*

Biometric data were collected from 608 adult birds (from 1 to 7 years old), 473 hens (77.80%) and 135 roosters (22.20%), belonging to different varieties of Utrerana and Sureña breeds as described in Figure 1. Sample size must account for at least 20 times as many observations as variables. As this assumption was fulfilled, study sample permitted to obtain reliable estimates of the canonical factor loadings for interpretation and to draw valid conclusions (Stevens, 2012).

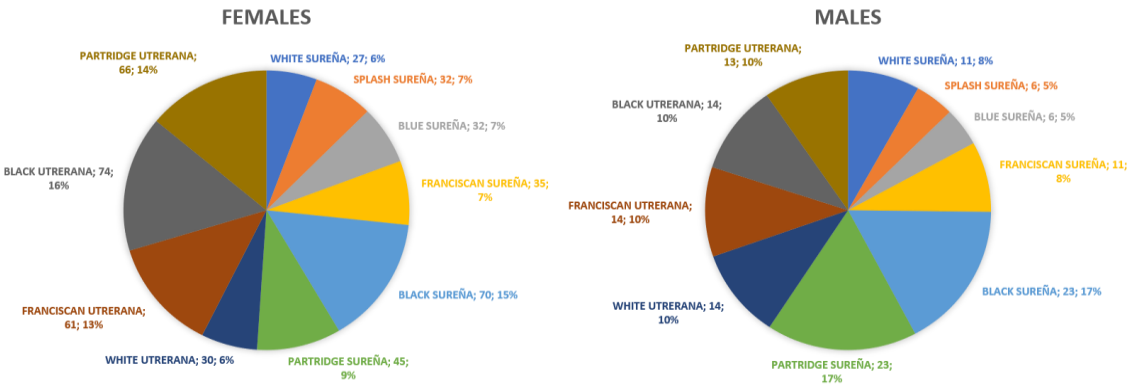


**Figure 1.** Percentage and number of individuals (n) used in each studied genotype.

The sample was collected at 16 farms of each breed across the seven provinces in Andalusia (Cádiz, Córdoba, Granada, Huelva, Jaén, Málaga, and Sevilla). All animals were reared under extensive backyard conditions.

National guidelines for the care and the use of laboratory and farm animals and avian-specific codes for good practices were followed during the data collection. For this, standards consistent with European Union legislation (2010/63/EU, from the September 22nd, 2010) as transposed into Spanish law (Royal Decree Law 53/2013, from the February 1st, 2013). The study protocol was submitted to The Ethics Committee of Animal Experimentation of the University of Córdoba (Spain), and deemed exempt from review.

### *2.2. Biometric measurements collection*

Biometrical analysis was performed in each bird, measuring 27 quantitative and 5 qualitative variables, following the procedure for morphological characterization of native chicken breeds described in previous studies (Francesch et al., 2011; Toalombo Vargas et al., 2020). A summary of the quantitative biometric variables

and how to measure them is shown in Table 1. All corporal measurements were taken on the right side of the animal. Figure 2 shows details of the head measurements taken. A suspended electronic scale (measurement precision = 5 g; Kern CH50K100, Kern & Sohn, Balingen, Germany), a Vernier scale (Electro DH M 60.205, Barcelona, Spain), and a tape measure were used for measurement collection.

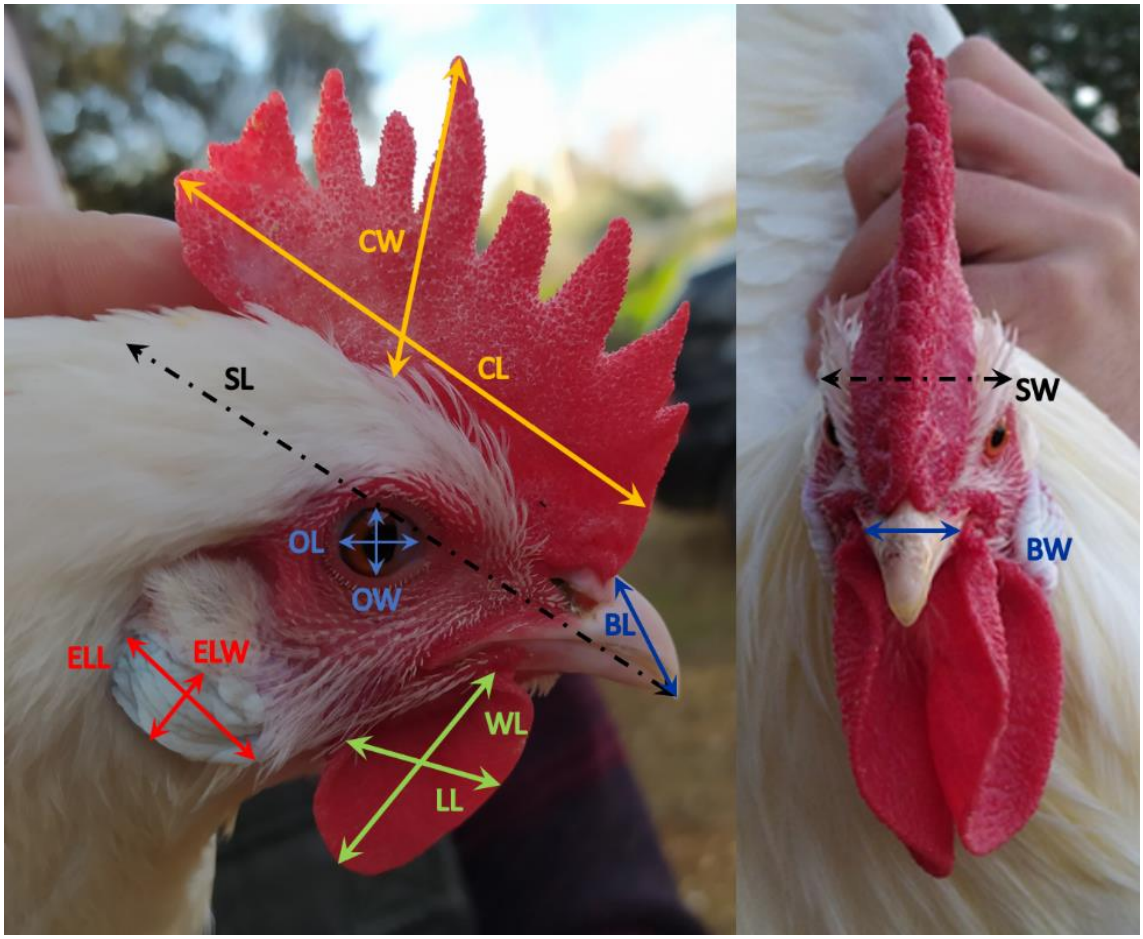


**Figure 2.** Details of a hen and a rooster heads with their corresponding measures. CL: comb length, CW: comb width, OL: ocular length: OW: ocular width, BL: beak length, BW: beak width, ELL: ear lobe length, ELW: ear lobe width, WL: wattle length, WW: wattle width.

The following qualitative traits were evaluated in the present study: eyes color, beak color, presence or absence of spurs, tarsus color, and nails color. Moreover, skull index, ocular index, beak index, and tarsus index were computed, as shown in Table 2.

**Table 1.** Biometric variables and measuring procedure used in the present study.

| Corporal region | Variable | How to measure it |
|---|---|---|
| General characteristics | Body weight | With an electronic scale |
| | Ornitological measurement | Leaning the bird on its back, distance between the tip of the beak and the tip of a central rectrix, in a straight line |
| | Wingspan | Distance between the ends of the longest primaries with outstretched wings |
| Head | Skull length | Taken between the most protruding point of the occipital and the tip of the beak |
| | Skull width | Taken at eyes level |
| | Comb length | Measured between the insertion of the comb in the beak and the end of the comb's lobe |
| | Comb width | Measured from the tip of the central spike until the insertion of the comb in the skull. When number of spikes is even, the highest was chosen |
| | Number of spikes in comb | By manual counting |
| | Ocular length | Measured between eyelids corners |
| | Ocular width | Measured including the folds of the eyelid, perpendicular to ocular length |
| | Beak length | Measured from the tip of the beak until the insertion of the beak in the head |
| | Beak width | Measured at level of insertion of beak in the head |
| | Ear lobes length | Maximum length, keeping the bird's head perpendicular to the neck |
| | Ear lobes width | As in the previous measure, measured the second-largest dimension |
| | Wattles length | Measured from the insertion of wattle in the beak until the end of the wattle, in a straight line |
| | Wattles width | As in the previous measure, measured the second-largest dimension |
| Neck | Neck length | Distance from the base of the neck to the chest |
| Body | Back length | Distance from insertion of the neck into the body to the tail insertion. |
| | Keel of esternum length | Leaning the bird on its back, distance between the two vertices of the sternum |
| | Breast circumference | Measured at the level of the tip of the keel, passing the tape measure through the back of the wing insert |
| | Longitudinal Diameter | Measured from the cranial end of coracoid to the most caudal portion of pubis |
| | Tail length | Distance from the tip of a central rectrix to the insertion of the tail |
| Extremities | Folding wing length | Distance from the carpal joint until the end of the longest primary |
| | Thigh length | Distance from the middle region of the coxal bone to the knee joint |
| | Tarsus length | Distance from the notch of the shinbone-tarsus until the tip of the nail of the middle finger |
| | Anteroposterior tarsus diameter | Diameter of the tarsus in a anteroposterior direction in the middle part of the metatarsus bone |
| | Lateromedial tarsus diameter | Diameter of the tarsus in a lateromedial direction in the middle part of the metatarsus bone |

**Table 2.** Mathematical description of biometric indices.

| Trait | Mathematical expression | |
|---|---|---|
| Skull index | $SI = SL/SW$ | where SI: skull index; SL: skull length; SW: skull width |
| Ocular index | $OI = OL/OW$ | where OI: ocular index; OL: ocular length; OW: ocular width |
| Beak index | $BI = BL/BW$ | where BI: beak index; BL: beak length; BW: beak width |
| Tarsus index | $TI = APTD/LMTD$ | where TI: tarsus index; APTD: anteroposterior tarsus diameter; LMTD: lateromedial tarsus diameter |

*2.3. Normality and Kruskall Wallis tests*

Normality test suggested normality assumption was not met. Hence a non-parametric approach was followed. Kruskall Wallis H test was performed to detect differences in the median across sexes and genotypes. Kruskall Wallis H Test reported medians to significantly differ across all possibilities for sex and breed/variety combinations. Consequently, a separate DCA was performed for males and females.

*2.4. Discriminant Canonical Analysis*

In the present research, 36 explanatory variables were used to perform the DCA: body weight, ornithological measurement, wingspan, skull length, skull width, ocular length, ocular width, beak length, beak width, comb length, comb width, number of spikes in comb, ear lobes length, ear lobes width, wattles length, wattles width, neck length, back sternum length, tail length, thigh length, folding wing length, tarsus length, anteroposterior tarsus diameter, lateromedial tarsus diameter, eyes color, beak color, presence or absence of spurs, tarsus color, nails color, skull index, ocular index, beak index, and tarsus index. In each sex, the breed and variety of the bird was used as a classification criteria in order to measure the variability in morphological traits between and within the used classification groups and establish and outline population clusters (González Ariza et al., 2021a; Marín Navas et al., 2021).

The statistical analysis issues a set of discriminant functions that can be used in as a tool to determine the clustering patterns described by the population sample

through a linear combination of morphological-related traits. Furthermore, this canonical tool was used to plot pairs of canonical variables and graphically depict the group differences into an easily interpretable territorial map. Regularized forward stepwise multinomial logistic regression algorithms were used to perform the variable selection. Priors were regularized in accordance with the group sizes computed from the prior probability option in SPSS v26.0 software instead of considering them to be equal and prevent group with different sample sizes that affects the quality of the classification (Tai and Pan, 2007).

Previous studies have reported DCA to be robust and its outputs to be consistent when sample sizes among groups were highly unequal. Potential distortion effects derived from unequal sample sizing can palliated using at least 20 samples for every 4 or 5 predictors. Additionally, the maximum number of independent variables must be n-2 (where n = simple size). The present design was developed aiming at meeting these requirements sufficiently, to ensure the validity of the conclusions drawn.

Before discriminant analysis, independence of regressors were ensured by multicollinearity analysis. The same variables were chosen by the forward and the backward stepwise selection methods. Hence, progressive selection method was chosen as preferable since it is less time-consuming than the backward selection method.

Discriminant routine of the Classify package of SPSS v26.0 software and discriminant analysis routine of the analyzing data package of XLSTAT 2014 (Pearson Edition) were used to perform the DCA.

2.4.1. Multicollinearity preliminary testing

Redundancies in the variables used were identified after performing the multicollinearity assumption prior to run the DCA. Multicollinearity analysis seeks to avoid the overinflation of the explanatory potential of variance due to the inclusion of an unnecessary large number of variables. As an indicator of multicollinearity, the variance inflation factor was calculated by follow the formula:

$$VIF = 1/(1 - R^2),$$

where $R^2$ is the coefficient of determination of the regression equation.

A recommended maximum VIF value of 5 was used in the study, as suggested by Rogerson (2001). Tolerance (1 - $R^2$) is the amount of variability in a certain independent variable that is not explained by the rest (Nanda et al., 2018). When tolerance values are lower than 0 and simultaneously, VIF values ≥10, multicollinearity must be considered a troublesome. VIF was computed using the discriminant analysis routine of the analyzing data package of XLSTAT 2014 (Pearson Edition).

2.4.2. Canonical Correlation Dimension Determination

Pearson's ρ was used to interpret canonical correlations. The maximum number of canonical correlations between two sets of variables is the number of variables in the smaller set. Although most of the relationship between different sets are explained by the first canonical correlation, all canonical correlations must be considered. Dimensions with canonical correlation values of ≥0.30 may be statistically significative.

2.4.3. Discriminant canonical analysis Efficiency

Wilks' Lambda test was used to evaluate variables that significantly contribute to the discriminant function. When Wilks' Lambda approximates to 0, the contribution of the variable to a discriminant function increases. If significance is below 0.05, the function can be concluded to explain the group adscription well (Anuthama et al., 2011).

2.4.4. Discriminant canonical analysis Model Reliability

Pillai's trace criterion was used in the discriminant function analysis to test the assumption of equal covariance matrices. This is the only acceptable test that must be used in cases of unequal sample sizes (Zhang et al., 2020). Pillai's Trace Criterion was calculated using the discriminant analysis routine of the analyzing data package of XLSTAT 2014 (Pearson Edition). A significance below 0.05 indicates significant statistical differences in the dependent variables across the levels of independent, hence application of DCA is feasible.

2.4.5. Discriminant canonical analysis Model Reliability

A preliminary principal component analysis was computed to minimize overall variables into few meaningful variables that contributed to morphological characterization of males and females in different genotypes.

2.4.6. Canonical coefficients and loadings interpretation and spatial representation

The percentage of allocation of an individual within its group (defined by it genotype) was calculated using a discriminant function analysis. Values of ≥|0.40| in the discriminant loading of a variable were considered to be significantly discriminant. So, non-significant variables were excluded from the function using stepwise procedures. Higher values for absolute coefficients for each particular variable determine better discriminating power. Afterward, data were standardized following the premises reported by Manly and Alberto (2016) and Mahalanobis distances were calculated using the following formula:

$$D_{ij}^2 = (\bar{\Upsilon}_i - \bar{\Upsilon}_j)\, COV^{-1} (\bar{\Upsilon}_i - \bar{\Upsilon}_j)\,,$$

where $D_{ij}^2$ is the distance between population i and j; $\Upsilon_i$ and $\Upsilon_j$ are the means of variable x in the ith and jth populations, respectively; and $COV^{-1}$ is the inverse of the covariance matrix of measured variable x. Squared Mahalanobis distances matrix was converted into a Euclidean distances matrix.

Afterwards, dendrograms were built using the underweighted pair-group method arithmetic averages (UPGMA) from the Rovira i Virgili University, Tarragona, Spain, and the Phylogeny procedure of MEGA X 10.0.5 from the Institute of Molecular Evolutionary Genetics, The Pennsylvania State University, State College, PA, USA.

2.4.7. Discriminant Function Cross-Validation

Percentage of correctly classified cases can be defined as the hit radio. The leave-one-out cross-validation procedure was used to consider if the discriminant functions can be validated. Classification accuracy is achieved when the classification rate is at least 25% higher than obtained by chance.

Press' Q statistic can support these results, since it can be used to compare the discriminating power of the cross-validated function, as follows:

$$Press\ Q' = \frac{[n - (n'K)]^2}{n(K-1)},$$

where n is the number of observations in the sample; n' is the number of observations correctly classified; and K is the number of groups.

The value of Press' Q statistic must be compared with the critical value of 6.63 for $\chi^2$ with a degree of freedom in a significance of 0.01. When Press' Q exceeds the critical value of $\chi^2 = 6.63$, the cross-validated classification can be regarded as significantly better than chance.

*2.5. Data Mining CHAID Decision Tree*

Chi-Squared Automatic Interaction Detection (CHAID) decision tree (DT) data mining method was used for classification, prediction, interpretation, and discrete categorized data manipulation. Each internal node build in the tree around a zoometrics or phaneroptics trait (input variables), a Chi-square test significance split criterion ($P<0.05$ at least) is fulfilled in what is called a prepruning process.

Breiman et al. (1984) suggested pre or post pruning methods implementation prevents overdimension of trees to prevent the failure to pursue the addition of traits (branches) which add significantly to the overall fit. As a result, a tree which exhaustively depicts the significant relationships across independent variables, from which those nodes which do not contribute to the overall prediction have been discarded. Furthermore, CHAID additionally penalizes model complexity. In these regards, Bonferroni inequality significant adjustment for significance levels was used.

Breiman's method uses Chi squared tests to determine to configure the tree building process. Each branch represents an outcome of the test (in a number of two or more), and each leaf node (or terminal node) represents a category level of the target variable (breed/variety). The root node in the tree is the one that is located at the top. The decisions are made at each node and each records of data continues through the tree along a path until the record reaches a leaf or terminal node of the tree (Ceylan et al., 2018).

Afterwards, cross validation was performed to validate the set of predictors considered measuring the differences between the prediction error for a tree applied to a new sample and a training sample. Cross validation of decision tree was

performed using the 'Complexity Parameter' and cross validated error to estimate how accurately the model performs data prediction. Ten-fold cross validation (Baykara, 2015) was performed keeping every sample record in either training sample and study data. Resubstitution error rate measures the proportion of original observations that were misclassified by various subsets of the original tree.

Ten-fold cross-validation was used to obtain a cross-validated error rate, from which the optimal tree is selected to prevent bias and outlier overfitting. The Ten-fold cross-validation involves creating Ten-random subsets of the original data, setting one portion aside as a test set, constructing a tree for the remaining 10-1 portions, and evaluating the tree using the test portion. This is repeated for all portions, and an estimate of the error is evaluated. Adding up the error across the ten portions represents the cross-validated error rate. Afterwards, the tree yielding the lowest cross-validated error rate is selected as the tree that best fits the data.

## 3. Results

### *3.1. Discriminant canonical analysis reliability*

Values of $\rho < 0.05$ obtained for Pillai's trace criterion suggest idoneity of data to perform the DCA (Table 3). Contribution of canonical functions to the meaning of each discriminating function was assessed by Wilks' Lambda statistic (Table 4).

**Table 3.** Summary of the results of Pillai's Trace of Equality of Covariance Matrices of Canonical Discriminant Functions.

| | | |
|---|---|---|
| Females | Pillai's Trace Criterion | 2.8664 |
| | F (Observed value) | 7.1227 |
| | F (Critical value) | 1.1540 |
| | df1 | 261 |
| | df2 | 3978 |
| | *p*-value | < 0.0001 |
| | alpha | 0.05 |
| Males | Pillai's Trace Criterion | 3.8256 |
| | F (Observed value) | 2.7989 |
| | F (Critical value) | 1.1740 |
| | df1 | 252 |
| | df2 | 954 |
| | *p*-value | < 0.0001 |
| | alpha | 0.05 |

**Table 4.** Canonical discriminant analysis efficiency parameters to determine the significance of each canonical discriminant function.

| | Test of Function(s) | Wilks' Lambda | Chi-square | df | Sig. |
|---|---|---|---|---|---|
| **Females** | 1 through 7 | 0.045 | 1436.63 | 63 | 0 |
| | 2 through 7 | 0.411 | 410.85 | 48 | 0 |
| | 3 through 7 | 0.814 | 95.218 | 35 | 0 |
| **Males** | 1 through 4 | 0.017 | 515.527 | 36 | 0 |
| | 2 through 4 | 0.242 | 180.18 | 24 | 0 |
| | 3 through 4 | 0.813 | 26.252 | 14 | 0.024 |

Supplementary Tables S1 and S2 show a summary of the values of tolerance and VIF for each variable across sexes. VIF values over 5 were discarded from further analyses: skull width, anteroposterior tarsus diameter, eyes color, beak index, tarsus color, tarsus length, skull length, lateromedial tarsus diameter, and wingspan were the variables discarded for females, while lateromedial tarsus diameter, ocular width, skull width, beak index, nails color, tail length, eyes color, tarsus color, wattles width, tarsus length, and skull length were the traits discarded prior to DCA in male individuals.

*3.2. Canonical coefficients, loading interpretation and spatial representation*

DCA determined three discriminating canonical functions for both sexes (Tables 4 and 5). Lower Wilk lambda values and respective higher eigenvalues were indicative of higher discriminating power. In females, 90.71% of the total variance was explained by functions F1 and F2 (eigenvalue of 9.66 and 5.17 for F1 and F2, respectively). In males, functions F1 and F2 (eigenvalue of 26.91 and 7.34 for F1 and F2, respectively) explained 88.49% of the total variance.

**Table 5.** Canonical variable functions and percentage of self-explained and cumulative variance.

| Sex | Function | Eigenvalue | Discrimination (%) | Cumulative % |
|---|---|---|---|---|
| **Females** | F1 | 9.6611 | 58.8681 | 58.8681 |
| | F2 | 5.1701 | 31.5034 | 90.3716 |
| | F3 | 0.7705 | 4.6950 | 95.0665 |
| **Males** | F1 | 26.9110 | 69.5353 | 69.5353 |
| | F2 | 7.3362 | 18.9561 | 88.4914 |
| | F3 | 2.7997 | 7.2342 | 95.7256 |

After discarding redundant variables, variables were ranked by the test of equality of group means across groups depending on their discriminating properties (Tables 6 and 7). Lower values of Wilks' Lambda and greater values of F indicate a better discriminating power, which translates into a better position in the rank.

Figure 3 presents a graph of standardized discriminant coefficients across discriminant functions. These analyzes not only allow us to easily identify those variables accounting for higher repercussions on the discriminant power of functions overall, but also the possibility of a reduction in the discriminant power of individual variables as a result of multicollinearity between pairs.

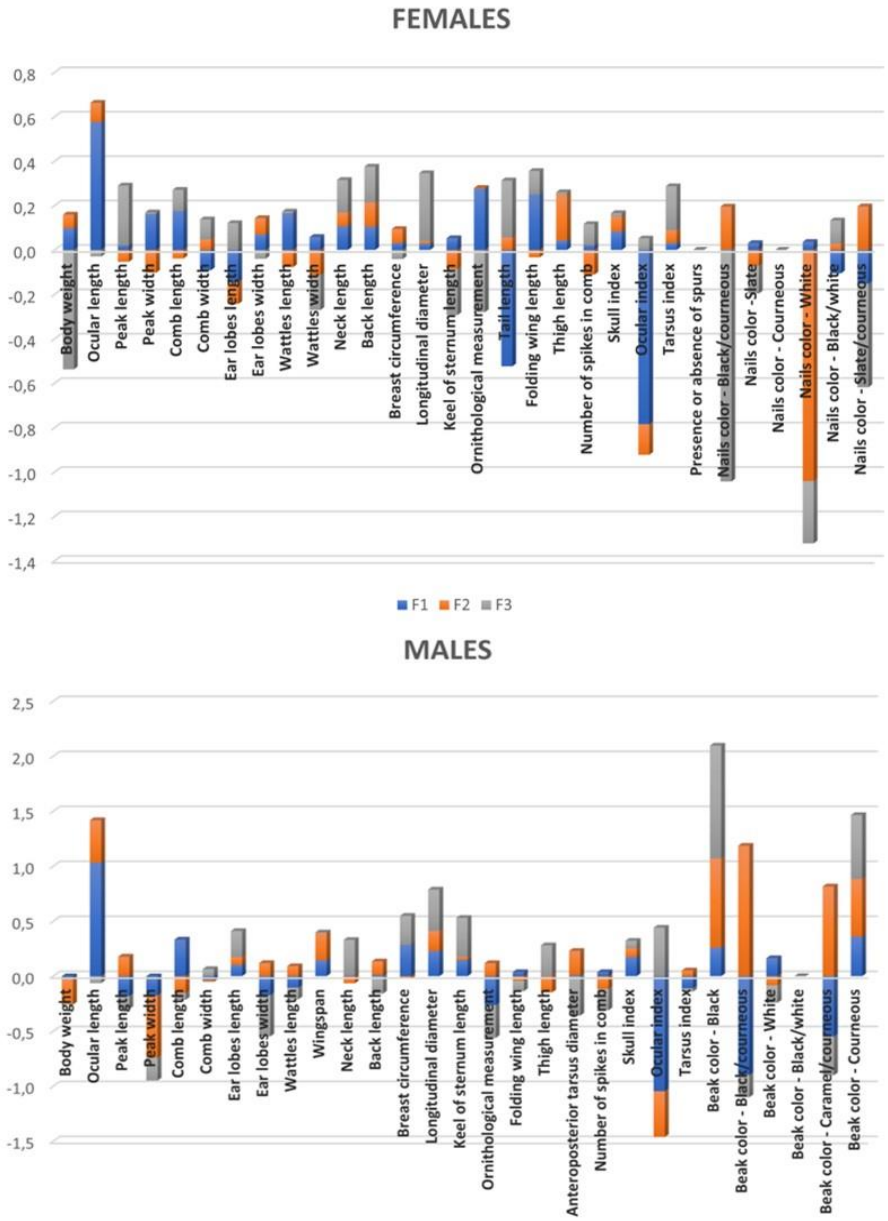


**Figure 3.** Discriminant loadings for biometric quality-related traits determining the relative weight of each trait on each canonical discriminant function.

**Table 6.** Results for the tests of equality of females group means to test for difference in the means across groups once redundant variables have been removed.

| Variables | Lambda | F | DF1 | DF2 | p-value | Rank |
|---|---|---|---|---|---|---|
| Nails color - White | 0.1911 | 217.2864 | 9 | 462 | < 0.0001 | 1 |
| Ocular index | 0.3571 | 92.3999 | 9 | 462 | < 0.0001 | 2 |
| Back length | 0.4291 | 68.3067 | 9 | 462 | < 0.0001 | 3 |
| Body weight | 0.4318 | 67.5522 | 9 | 462 | < 0.0001 | 4 |
| Ocular length | 0.4982 | 51.6983 | 9 | 462 | < 0.0001 | 5 |
| Longitudinal diameter | 0.5184 | 47.6874 | 9 | 462 | < 0.0001 | 6 |
| Keel of esternum length | 0.5262 | 46.2222 | 9 | 462 | < 0.0001 | 7 |
| Wattles length | 0.5381 | 44.0615 | 9 | 462 | < 0.0001 | 8 |
| Folding wing length | 0.5691 | 38.8630 | 9 | 462 | < 0.0001 | 9 |
| Comb length | 0.5828 | 36.7513 | 9 | 462 | < 0.0001 | 10 |
| Wattles width | 0.5986 | 34.4272 | 9 | 462 | < 0.0001 | 11 |
| Breast circumference | 0.6052 | 33.4926 | 9 | 462 | < 0.0001 | 12 |
| Thigh length | 0.6358 | 29.4067 | 9 | 462 | < 0.0001 | 13 |
| Nails color - Black/courneous | 0.6736 | 24.8741 | 9 | 462 | < 0.0001 | 14 |
| Ornithological measurement | 0.6831 | 23.8125 | 9 | 462 | < 0.0001 | 15 |
| Comb width | 0.6868 | 23.4102 | 9 | 462 | < 0.0001 | 16 |
| Peak width | 0.6935 | 22.6921 | 9 | 462 | < 0.0001 | 17 |
| Ear lobes width | 0.7001 | 21.9939 | 9 | 462 | < 0.0001 | 18 |
| Tail length | 0.7660 | 15.6822 | 9 | 462 | < 0.0001 | 19 |
| Peak length | 0.7855 | 14.0167 | 9 | 462 | < 0.0001 | 20 |
| Ear lobes length | 0.8005 | 12.7947 | 9 | 462 | < 0.0001 | 21 |
| Nails color - Slate/courneous | 0.8156 | 11.6036 | 9 | 462 | < 0.0001 | 22 |
| Nails color - Slate | 0.8426 | 9.5928 | 9 | 462 | < 0.0001 | 23 |
| Skull length | 0.8629 | 8.1568 | 9 | 462 | < 0.0001 | 24 |
| Number of speaks in comb | 0.9095 | 5.1094 | 9 | 462 | < 0.0001 | 25 |
| Tarsus index | 0.9416 | 3.1857 | 9 | 462 | 0.0009 | 26 |
| Skull index | 0.9703 | 1.5692 | 9 | 462 | 0.1217 | 27 |
| Nails color - Black/white | 0.9869 | 0.6793 | 9 | 462 | 0.7279 | 28 |
| Presence or absence of spurs | 0.9903 | 0.5005 | 9 | 462 | 0.8743 | 29 |

F, Snedecor's F; df1, numerator degrees of freedom for the F-approximation (groups minus 1); df2, denominator degrees of freedom for the F-approximation (observations minus 1).

**Table 7.** Results for the tests of equality of males group means to test for difference in the means across groups once redundant variables have been removed.

| Variables | Lambda | F | DF1 | DF2 | p-value | Rank |
|---|---|---|---|---|---|---|
| Ocular index | 0.1797 | 63.4040 | 9 | 125 | < 0.0001 | 1 |
| Peak color - Black/courneous | 0.2102 | 52.1922 | 9 | 125 | < 0.0001 | 2 |
| Peal color - White | 0.3489 | 25.9192 | 9 | 125 | < 0.0001 | 3 |
| Wingspan | 0.3765 | 22.9996 | 9 | 125 | < 0.0001 | 4 |
| Peak color - Black | 0.4526 | 16.7993 | 9 | 125 | < 0.0001 | 5 |
| Back length | 0.4547 | 16.6534 | 9 | 125 | < 0.0001 | 6 |
| Ocular length | 0.5279 | 12.4222 | 9 | 125 | < 0.0001 | 7 |
| Longitudinal diameter | 0.5536 | 11.1984 | 9 | 125 | < 0.0001 | 8 |
| Anteroposterior tarsus diameter | 0.5576 | 11.0173 | 9 | 125 | < 0.0001 | 9 |
| Body weight | 0.6399 | 7.8142 | 9 | 125 | < 0.0001 | 10 |
| Breast circumference | 0.6511 | 7.4427 | 9 | 125 | < 0.0001 | 11 |
| Folding wing length | 0.6653 | 6.9859 | 9 | 125 | < 0.0001 | 12 |
| Ear lobes width | 0.7245 | 5.2821 | 9 | 125 | < 0.0001 | 13 |
| Peak color - Courneous | 0.7272 | 5.2092 | 9 | 125 | < 0.0001 | 14 |
| Keel of sternum length | 0.7424 | 4.8184 | 9 | 125 | < 0.0001 | 15 |
| Wattles length | 0.7819 | 3.8731 | 9 | 125 | 0.0002 | 16 |
| Comb length | 0.7899 | 3.6936 | 9 | 125 | 0.0004 | 17 |
| Peak width | 0.7903 | 3.6848 | 9 | 125 | 0.0004 | 18 |
| Peak length | 0.8000 | 3.4712 | 9 | 125 | 0.0007 | 19 |
| Ear lobes length | 0.8194 | 3.0609 | 9 | 125 | 0.0024 | 20 |
| Number of speaks in comb | 0.8225 | 2.9981 | 9 | 125 | 0.0029 | 21 |
| Thigh length | 0.8296 | 2.8519 | 9 | 125 | 0.0043 | 22 |
| Neck length | 0.8707 | 2.0623 | 9 | 125 | 0.0378 | 23 |
| Ornithological measurement | 0.8798 | 1.8980 | 9 | 125 | 0.0580 | 24 |
| Comb width | 0.9029 | 1.4932 | 9 | 125 | 0.1574 | 25 |
| Tarsus index | 0.9072 | 1.4215 | 9 | 125 | 0.1858 | 26 |
| Skull index | 0.9254 | 1.1189 | 9 | 125 | 0.3544 | 27 |
| Peak color - Caramel/courneous | 0.9300 | 1.0460 | 9 | 125 | 0.4077 | 28 |

F, Snedecor's F; df1, numerator degrees of freedom for the F-approximation (groups minus 1); df2, denominator degrees of freedom for the F-approximation (observations minus 1).

The substitution of the values for biometric-related traits in the first three discriminating functions was performed to obtain x, y, and z-axis coordinates, for the first, second, and third dimensions, respectively. In these coordinates, each observation was sorted and classified across the different groups. A territorial map was depicted for each sex (Figure 4).

Mahalanobis distance represents the probability that an observation presenting an unknown background belongs to a particular group (breed/variety). It can be computed through the relative distance of the problem observation to the centroid of its closest group. Then, the hit radio was calculated. The hit ratio is the rate of

successfully classified cases across breed/varieties (which was perform across sexes) (Supplementary Tables S3 and S4). Mahalanobis distances obtained after the evaluation of the discriminant analysis matrix were transformed in squared Euclidean distance and represented in Figures 5 and 6, following the premises in Hair et al. (2010).

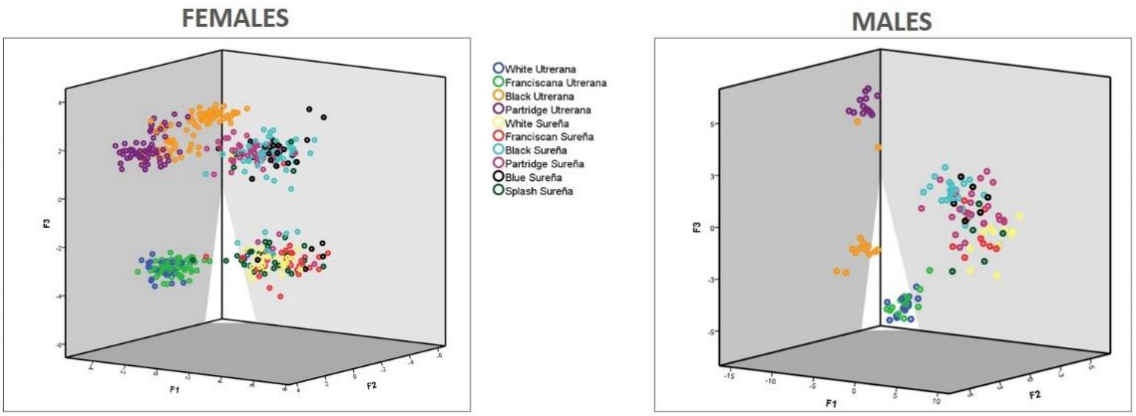


**Figure 4.** Territorial map depicting the observations considered in the canonical discriminant analysis sorted across genotypes.

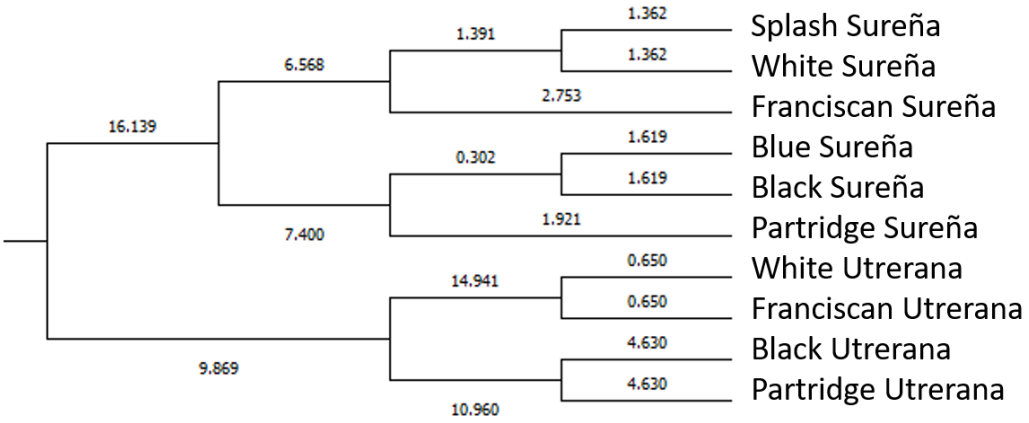


**Figure 5.** Cladogram constructed from Mahalanobis distances between different genotypes (breed/varieties) in female's population.

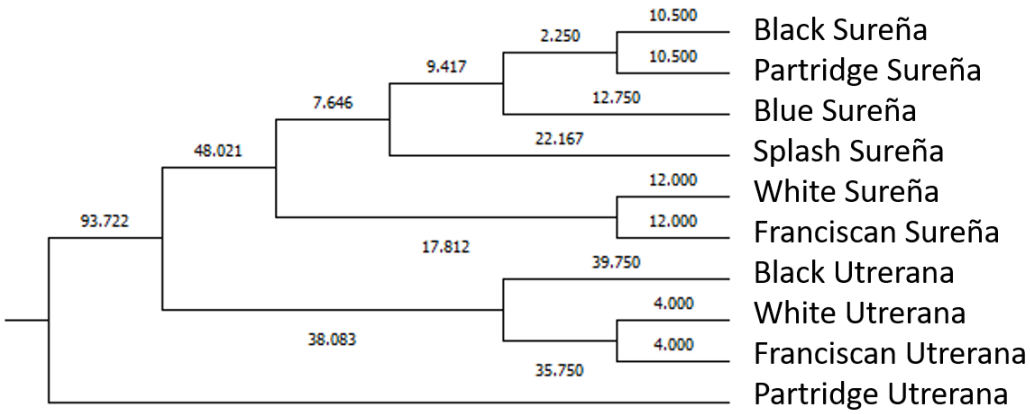


**Figure 6.** Cladogram constructed from Mahalanobis distances between different genotypes (breed/varieties) in male's population.

Supplementary Tables S3, S4, S5, and S6 report the results obtained in the classification and leave-one-out cross-validation for the observations in the present study. 71.82 and 81.48% of original grouped cases were correctly classified for females and males, respectively. From these results, 59.96 and 49.63% of clustered observations were cross-validated. Press' Q values of 2004.41 and 1060.27 were obtained from females and males, respectively; hence, it can be considered that predictions were significantly better than chance at 95% (Chan, 2005).

*3.3. Data Mining CHAID Decision Tree*

The underlying basis for this classification patterns was found after the evaluation of the Data Mining CHAID Decision Tree obtained of chi-square dissimilarity matrix. Classification trees of groups by genotypes produced simple trees with terminal nodes (Supplementary Figures S3 and S4). Chi squared based branch and node distribution suggested females significantly ($P<0.001$) differed depending on their values of nail colour, thus, were classified in four subgroups (black-corneous/slate-corneous, slate, corneous and white). Nail colour was the best discriminant phaneroptics trait and helped to distinguish between black Utrerana, black Sureña, Partridge Utrerana and Franciscan Utrerana). Afterwards, ocular index helped to discriminate across the varieties of Utrerana and Sureña hens ($P<0.001$) with the Utrerana animals presenting ocular indices over 0.986, while Sureña ocular indices were equal to or below 0.986.

By contrast, Chi squared based branch and node distribution suggested males only significantly ($P<0.001$) differed depending on their values of ocular index. Ocular index helped to discriminate between varieties of Utrerana and Sureña roosters ($P<0.001$) with the Utrerana animals presenting ocular indices over 1.015, while Sureña ocular indices were equal to or below 1.015.

Female data mining decision tree ten-fold cross validation reported closely similar resubstitution (probability of misclassifying an unseen instance) and cross-validation error rate estimates of 0.484 and 0.510, for which standard error was 0.023, respectively. For male tree, 0.726 and 0.867 values of resubstitution and cross-validation error rate estimates with respective standard errors of 0.038 and 0.029, respectively. Although data resubstitution can underestimate the classifier error, it has less variability than other methods, such as cross-validation, especially

for small sample sizes. As cross-validation error rate estimates are close to resubstitution ones, albeit lower, trees are not overfitted and the robustness of the results obtained and the validity of the conclusions drawn.

## 4. Discussion

Differential sex-linked hormonal and genetic regulation patterns of the expression of growth has been reported to occur in local poultry breeds (Coyne et al., 2008; Yakubu and Salako, 2009). Dimorphism and dichromatism could be consequence of sexual selection and might provide an adaptative advantage of one population over others. For instance, in the context of the conditions found in rustic backyard environments, even if a lower selective pressure focused towards production, male-to-male competition have induced roosters to increase the size, giving an advantage against the opponent (Desta, 2019).

In the context of multizoometric and phaneroptics analyses, it has been suggested that it is necessary to check for the different relationships across explanatory variables and select independent variables that do not overlap when deciding on the factors which determine the efficiency of predictive models (Marín Navas, et al., 2021). High correlations between skull length and skull width with skull index were revealed by the multicollinearity analysis since the formula for skull index calculation comprises the aforementioned measurements. The same happens with anteroposterior (in both sexes) and lateromedial (only in hens) tarsus diameters as the elements which determine the tarsus index. The calculation formula of beak index, which includes the rest of beak measurements, was eliminated from further analysis due to multicollinearity problems (VIF>5).

Finally, ocular width variable was discarded from the analysis of male individuals since this variable is contained within the formula of ocular index (VIF>5). These results are supported by those by Ning et al. (2019), who found multicollinearity problems when formulae are developed after the inclusion of explanatory variables which have already been included.

Phaneroptics have been reported to be highly significantly interrelated (Assefa and Melesse, 2018). Even if most qualitative variables were discarded after the multicollinearity analysis, nails colour in hen and beak colour in roosters were the only qualitative variables that remained in the DCA. Thus, results suggest that

multicollinearity problems between different qualitative measurements in birds may have occurred.

White colour in hen nails reported to be the best discriminating feature in hens (Table 6). Only seven individuals of White, Splash and Franciscan Sureña showed dark color in nails, while no hen of White and Franciscan varieties showed a different color than white on nails. In roosters, the black/corneous and white colors in beak reported to have high discriminant power too.

Previous studies have reported that phaneroptical features are somehow correlated in native chicken breed provided they may derive from the expression of a same gene background across the body parts (Yaemkong and Ngoc, 2019). Additionally, it has been suggested that these qualitative traits have significant effects on other quantitative traits such as body weight and daily gain in chicken (Patbandha et al., 2018; Yaemkong and Ngoc, 2019).

Our results are indicative of the fact that qualitative variables, with high discriminant ability to discern among local hen genotypes, must be considered as efficient selection criteria in breeding programs, as an effective method to identify the individuals presenting the most desirable production related characteristics at the most convenient earlier age.

Furthermore, certain phaneroptics may be associated with consumers trends and their cultural preference. For instance, while North American consumers have strong preferences for white skinned meat (Wideman et al., 2016), meat form dark poultry are preferred by producers and consumers in South America (Toalombo, et al., 2019). Hence, multivariety breeds accounting for a wide variety of feather and skin colour patterns such as Utrerana and Sureña could satisfy the needs of a wider scope of targets in different market niches.

Ocular index was ranked second and first regarding its discriminant ability in hens and roosters, respectively. The relevance of ocular index may be ascribed to a higher adaptability to the environment and improve capacity to seek for food as a result of improved vision skills. Indeed, except for certain occasions, birds have highly developed vision.

The avian eye, in relation to the size of the skull, is very large. While human have an eye relative size of 5% respect the skull, in hens 50% of cranial volume is occupied by orbit (Wisely et al., 2017). High visual acuity is advantageous for hens relying heavily on their ability to navigate surroundings to find and acquire food, to identify potential mates, and to quickly escape from predators (Brooke et al., 1999; Jones et al., 2007). Hall and Ross (2007) reported that the light level, which is highly correlated with bird activity pattern, has a higher significant influence in eye shape and body size than other factors, such as phylogeny.

Birds with a higher adaptation to darkness habits, like brooder and nesting abilities, exhibit larger axial lengths and corneal, and therefore, a higher eye size diameter than the rest of birds (Hall and Ross, 2007; Podkowa and Surmacki, 2017). On the other hand, larger individuals with larger eyes have the potential for more sensitive and acute vision than smaller individuals with smaller eyes. This could suggest that Sureña breed, with a significantly larger eye size, has a sharper vision. However, each breed has developed an ideal eye design for conditions in which in which it is produced. Larger eyes need more brain space for information processing. Therefore, evaluation of ocular size in each breed must be performed taking into account body size (Møller and Erritzøe, 2010). Thus, the higher size in Sureña's eye should be mainly ascribed to a proportionally larger body shape.

It has also been suggested that lower values for ocular index may act as an adaptation to optimal anti-predator behavior since larger ocular width could suppose an advantage in the lateral visual field (Fernández-Juricic et al., 2008; Banks et al., 2015). Thus, results obtained in the present study may suggest that Utrerana eyes make it more adapted to survival in free-range systems. Furthermore, smaller birds have developed rather improved adaptative qualities like hardiness, agility, scavenging ability and need less time to flight (Tätte et al., 2018). Utrerana breed, with lower body weight and ocular index may be better adapted to free-range systems through their enhanced rusticity, even if literature indicates both breeds are able to easily thrive and are well-adapted in the environmental conditions present in these alternative production systems (De la Cruz Blanco et al., 2011; González Ariza, et al., 2019b).

Back length was the third best discriminant variable in hens. These results agree those reported by previous research (Ukwu et al., 2014; Tyasi et al., 2020), since back length has been reported to be highly correlated to other important traits, thus play an important role as a linear body measurement when intending to predict for body weight, develop and implement productive selection strategies during breeding in laying hens.

Size-related parameters like body weight (in hens) and wingspan (in roosters) played a pivotal role in the classification of individuals (Tables 6 and 7). These traits allow us to delimitate and differ those animals belonging to the Sureña breed. Sureña individuals typically account for a larger body size than that of Utrerana breed individuals.

Lighter hens have been reported to present higher egg productions and lower feed conversion rates, therefore, a better laying ability (Lacin et al., 2008). On the other hand, breeds characterized by larger individuals may be prone to become dual-purpose genotypes in alternative production systems, in which both sexes are reared together, to later, at an advanced age, separate males for final fattening and slaughtering, while females are kept during several laying cycles (Jasouri et al., 2017; Lambertz et al., 2018). Bearing this in mind, focusing efforts on the selection of Utrerana breed towards an egg production aptitude and Sureña as a dual-purpose breed may be the most effective and profitable productive alternative.

Although Sureña and Utrerana breeds were presumably selected from a common origin (Ocaña, et al., 2008), the graphic representation of the observations assessed in the present study (Figure 4) reports a clear differentiation of morphological characteristics between the two breeds. While three clear clusters are shown in Utrerana breed (Partridge, Black and Franciscan/White varieties), the closeness between the six varieties of Sureña avian breed suggest a likely lack of reproductive management and a crossbreeding among the different varieties of this breed.

This proves that, once official breed recognition occurs, an incorrect application of a breeding program in local breeds can lead to a deterioration of the phenotypic and genotypic identity of their individuals, which directly repercuss on the partial or total loss of the genetic pool of these local resources (Wang et al., 2017; Delgado Bermejo et al., 2019).

Contextually, Partridge Utrerana was reported to be the most differentiated variety from all studied varieties. These results are supported by those in Macrì et al. (2019), who reported Partridge Utrerana individuals to be placed the farthest away from the rest of Utrerana varieties.

More than 75% of hens in each Utrerana variety were correctly classified (Supplementary Table S3), except for the individuals of the White variety, given 50% of hens were notably classified as Franciscan Utrerana hens. This Utrerana White/Franciscan misclassification is supported by the results in Figures 5 and 6. Franciscan and White Utrerana varieties were closely clustered (Figures 5 and 6). This finding may indirectly indicate reminiscences of hybridization between White and Franciscan Utrerana varieties, with both presenting white legs and beak, which may be the result of the attempts of breeders to decrease the consanguinity within the White Utrerana variety, given this variety has historically been the subpopulation accounting with the smallest census and the one that faces the highest endargement risk (González Ariza, et al., 2021a).

Blue Sureña variety females were those for which a rather frequent misclassification rate occurred (Supplementary Table S3). This finding may derive from the fact that breeding practices performed in the area may seek the obtention of individuals presenting blue plumage patterns through the cross between other varieties, such as Black or Splash (Campo, 2007).

Biometric studies have been performed worldwide to make breed characterization feasible and for this to be considered during the implementation of conservation strategies and policies (Brito, et al., 2021). This suggests that the preservation for the breed diversity may be one of the motor elements which may ensure the future survival of a breed. This future survival may rely on the enhancement of breeds ability to cover a wider scope of market demands, hence to reach a broader audience (González Ariza et al., 2021b). The present methodological proposal is framed into the context of opportunity and resurgence of a potential production industry which intends to lay the base for a sustainable selective breeding programs in avian breeds. Certain easily measurable traits, like phaneroptics and ocular index efficiently play a pivotal role in the classification of birds. In this context, the discriminant tool designed in the present research allows to efficiently classify individuals

considering biometric and phaneroptics related traits. This is supported on the 71.82 and 81.48% of individuals correctly ascribed to their prior hen breed/variety cluster.

## 5. Conclusions

Sexual selection of larger males in backyard production systems may evidence clear sexual dimorphism in Utrerana and Sureña breeds. The use of these multivariety breeds is productively advantageous since a broader scope of market demands could be satisfied in terms of carcass organoleptic characteristics. This research confirms that native breeds in south of Spain may be well adapted to extensive and backyard systems, but also that their differential zoometric adaptation may make them more suited for the aptitude that they were selected to perform. Nevertheless, Utrerana breed showed a better morphological adaptation to optimal anti-predator behavior and rusticity. In any case, both breeds should follow different breeding programs considering to alternative routes: Sureña has greater potential as a dual-purpose breed while morphometric traits of Utrerana breed may be indicative of a higher profitability in egg-producing farms. The present research validates the efficiency of the discriminant tool designed while performing individual selection and breed ascription considering easily measurables traits such as ocular index and phaneroptics, which at the same time, may ensure the survival of these local genetic resources.

**Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Figure S1: Illustration of classification trees in females populations, Figure S2: Illustration of classification trees in males populations, Table S1: Multicollinearity analysis of biometric-related traits in females, Table S2: Multicollinearity analysis of biometric-related traits in males, Table S3: Appropriately classified females into their groups, Table S4: Appropriately classified males into their groups, Table S5: Leave-one-out cross-validation of females into their genotypes, Table S6: Leave-one-out cross-validation of males into their genotypes.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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**Supplementary Table S1.** Multicollinearity analysis of biometric-related traits in females.

| Statistics/Parameters | Tolerance (1 - $R^2$) | VIF |
|---|---|---|
| Comb length | 0.2048 | 4.8821 |
| Wattles length | 0.2085 | 4.7959 |
| Nails color - White | 0.2160 | 4.6306 |
| Nails color - Courneous | 0.2297 | 4.3534 |
| Body weight | 0.2376 | 4.2082 |
| Wattles width | 0.2464 | 4.0593 |
| Comb width | 0.2815 | 3.5524 |
| Ornithological measurement | 0.2916 | 3.4294 |
| Back length | 0.3067 | 3.2600 |
| Longitudinal diameter | 0.3359 | 2.9766 |
| Nails color - Slate | 0.3454 | 2.8950 |
| Ocular length | 0.3483 | 2.8708 |
| Breast circumference | 0.3715 | 2.6916 |
| Ocular index | 0.3798 | 2.6330 |
| Keel of esternum length | 0.4141 | 2.4149 |
| Thigh length | 0.4378 | 2.2841 |
| Folding wing length | 0.4627 | 2.1610 |
| Nails color - Black/Courneous | 0.4899 | 2.0413 |
| Peak width | 0.4973 | 2.0108 |
| Ear lobes length | 0.5090 | 1.9647 |
| Tail length | 0.5441 | 1.8381 |
| Ear lobes width | 0.5490 | 1.8215 |
| Peak length | 0.6767 | 1.4777 |
| Neck length | 0.7105 | 1.4075 |
| Skull index | 0.7746 | 1.2910 |
| Presence/absence of spurs | 0.7913 | 1.2637 |
| Number of spikes in comb | 0.8174 | 1.2234 |
| Tarsus index | 0.8748 | 1.1431 |
| Nails color - Black/white | 0.9089 | 1.1002 |

Interpretation thumb rule: VIF = 1 (Not correlated); 1 < VIF < 5 (Moderately correlated); VIF ≥ 5 (Highly correlated).

**Supplementary Table S2.** Multicollinearity analysis of biometric-related traits in males.

| Statistics/Parameters | Tolerance (1 - $R^2$) | VIF |
|---|---|---|
| Comb length | 0.2237 | 4.4707 |
| Wingspan | 0.2270 | 4.4059 |
| Anteroposterior tarsus diameter | 0.2598 | 3.8492 |
| Body weight | 0.2866 | 3.4894 |
| Peak color - White | 0.2955 | 3.3841 |
| Back length | 0.3065 | 3.2631 |
| Wattles length | 0.3197 | 3.1276 |
| Comb width | 0.3267 | 3.0613 |
| Ear lobes length | 0.3403 | 2.9384 |
| Ocular index | 0.3406 | 2.9361 |
| Peak color - Black | 0.3634 | 2.7518 |
| Longitudinal diameter | 0.3842 | 2.6027 |
| Peak width | 0.3982 | 2.5115 |
| Folding wing length | 0.4102 | 2.4377 |
| Thigh length | 0.4105 | 2.4359 |
| Peak color - Black/corneous | 0.4111 | 2.4328 |
| Ocular length | 0.4118 | 2.4286 |
| Breast circumference | 0.4764 | 2.0990 |
| Keel of esternum length | 0.4896 | 2.0423 |
| Ear lobes width | 0.4981 | 2.0078 |
| Ornitological measurements | 0.5341 | 1.8722 |
| Tarsus index | 0.5843 | 1.7113 |
| Peak color - Caramel/courneous | 0.6274 | 1.5939 |
| Skull index | 0.6297 | 1.5882 |
| Peak length | 0.6874 | 1.4547 |
| Neck length | 0.6937 | 1.4415 |
| Peak color - Black/white | 0.6967 | 1.4353 |
| Number of spikes in comb | 0.7008 | 1.4269 |

Interpretation thumb rule: $VIF = 1$ (Not correlated); $1 < VIF < 5$ (Moderately correlated); $VIF \geq 5$ (Highly correlated).

**Supplementary Table S3.** Appropriately classified females into their groups.

| from \ to | White Sureña | Splash Sureña | Blue Sureña | Franciscan Sureña | Black Sureña | Partridge Sureña | White Utrerana | Franciscan Utrerana | Black Utrerana | Partrige Utrerana | Total | % correct |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **White Sureña** | 17 | 6 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 27 | 62.96% |
| **Splash Sureña** | 7 | 15 | 2 | 3 | 3 | 0 | 0 | 2 | 0 | 0 | 32 | 46.88% |
| **Blue Sureña** | 1 | 0 | 14 | 4 | 6 | 6 | 0 | 0 | 1 | 0 | 32 | 43.75% |
| **Franciscan Sureña** | 3 | 3 | 1 | 27 | 0 | 0 | 1 | 0 | 0 | 0 | 35 | 77.14% |
| **Black Sureña** | 1 | 5 | 5 | 1 | 49 | 9 | 0 | 0 | 0 | 0 | 70 | 70.00% |
| **Partridge Sureña** | 3 | 1 | 4 | 1 | 4 | 32 | 0 | 0 | 0 | 0 | 45 | 71.11% |
| **White Utrerana** | 1 | 0 | 0 | 0 | 0 | 0 | 14 | 15 | 0 | 0 | 30 | 46.67% |
| **Franciscan Utrerana** | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 56 | 0 | 0 | 61 | 91.80% |
| **Black Utrerana** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 56 | 18 | 74 | 75.68% |
| **Partrige Utrerana** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 59 | 66 | 89.39% |
| **Total** | 33 | 30 | 26 | 39 | 63 | 47 | 20 | 73 | 64 | 77 | 472 | 71.82% |

**Supplementary Table S4.** Appropriately classified males into their groups.

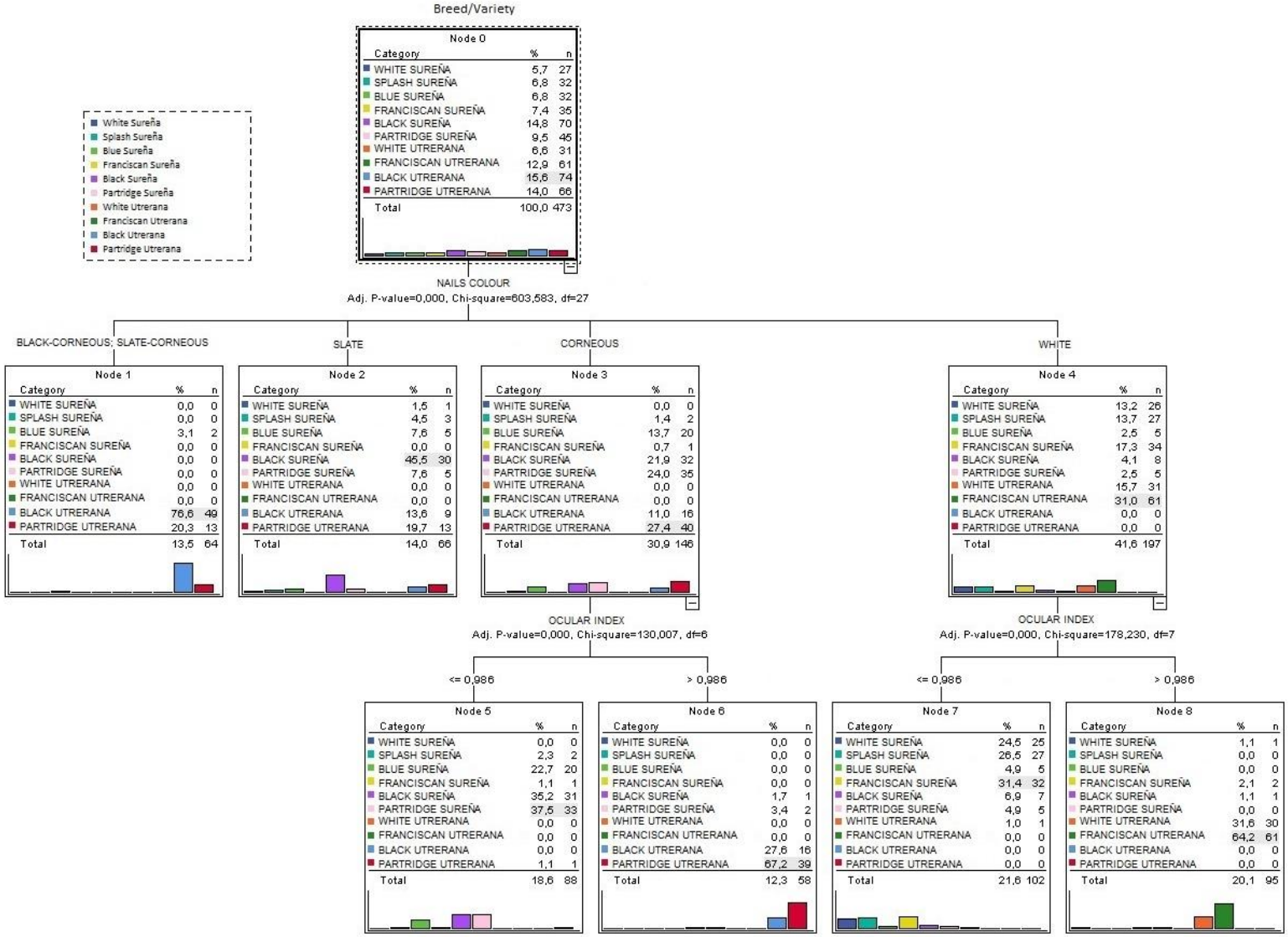
| from \ to | White Sureña | Splash Sureña | Blue Sureña | Franciscan Sureña | Black Sureña | Partridge Sureña | White Utrerana | Franciscan Utrerana | Black Utrerana | Partrige Utrerana | Total | % correct |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| White Sureña | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 100.00% |
| Splash Sureña | 0 | 4 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 6 | 66.67% |
| Blue Sureña | 0 | 0 | 4 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 6 | 66.67% |
| Franciscan Sureña | 1 | 0 | 0 | 7 | 1 | 2 | 0 | 0 | 0 | 0 | 11 | 63.64% |
| Black Sureña | 0 | 0 | 0 | 1 | 19 | 3 | 0 | 0 | 0 | 0 | 23 | 82.61% |
| Partridge Sureña | 1 | 0 | 2 | 1 | 5 | 14 | 0 | 0 | 0 | 0 | 23 | 60.87% |
| White Utrerana | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 1 | 0 | 0 | 14 | 92.86% |
| Franciscan Utrerana | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 12 | 0 | 0 | 14 | 85.71% |
| Black Utrerana | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 1 | 14 | 92.86% |
| Partrige Utrerana | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 13 | 100.00% |
| Total | 13 | 4 | 6 | 9 | 27 | 21 | 15 | 13 | 13 | 14 | 135 | 81.48% |

**Supplementary Table S5.** Leave-one-out cross-validation of females into their genotypes.

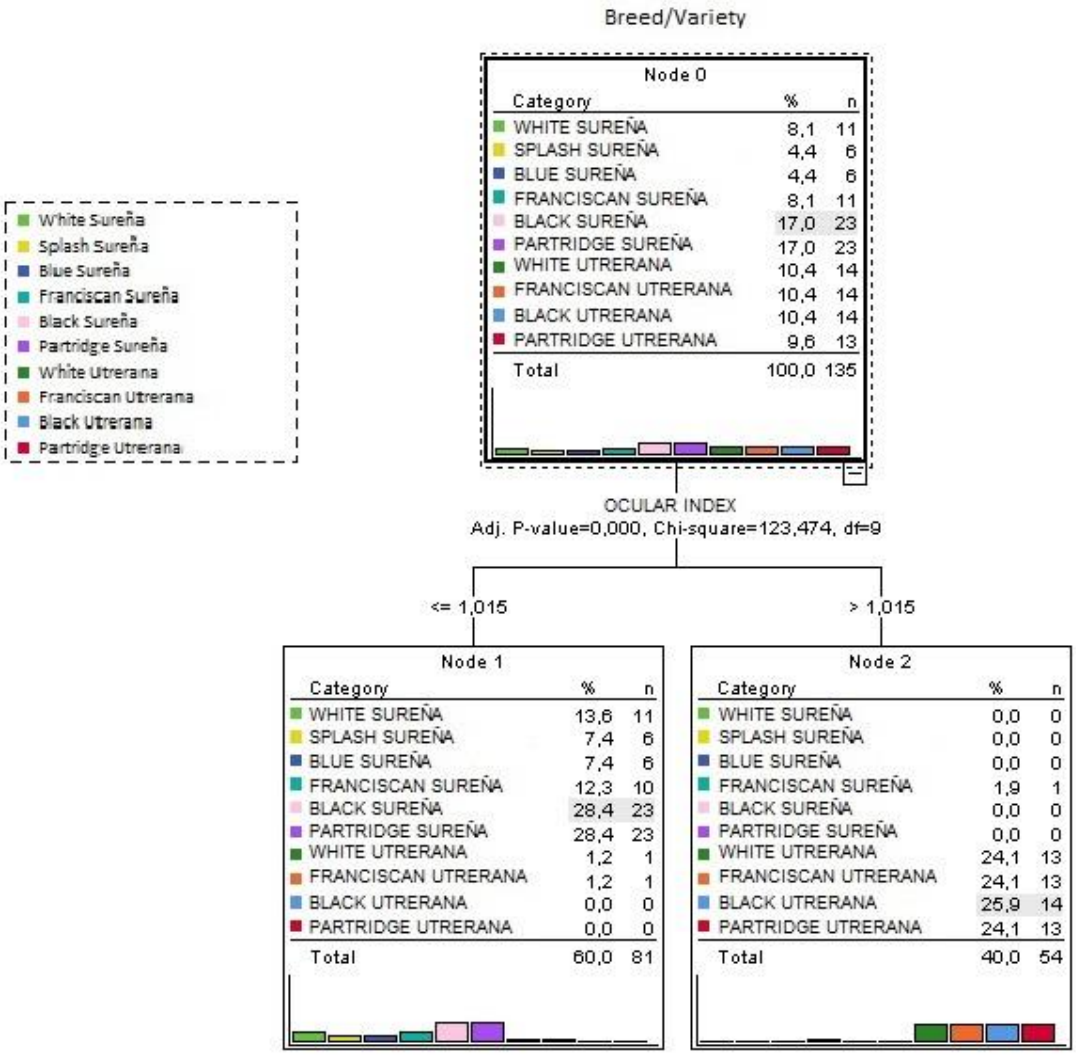
| from \ to | White Sureña | Splash Sureña | Blue Sureña | Franciscan Sureña | Black Sureña | Partridge Sureña | White Utrerana | Franciscan Utrerana | Black Utrerana | Partrige Utrerana | Total | % correct |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| White Sureña | 12 | 10 | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 27 | 44.44% |
| Splash Sureña | 10 | 11 | 2 | 4 | 3 | 0 | 0 | 2 | 0 | 0 | 32 | 34.38% |
| Blue Sureña | 1 | 0 | 10 | 4 | 8 | 8 | 0 | 0 | 1 | 0 | 32 | 31.25% |
| Franciscan Sureña | 6 | 6 | 1 | 21 | 0 | 0 | 1 | 0 | 0 | 0 | 35 | 60.00% |
| Black Sureña | 1 | 5 | 9 | 3 | 40 | 12 | 0 | 0 | 0 | 0 | 70 | 57.14% |
| Partridge Sureña | 3 | 1 | 6 | 1 | 8 | 25 | 0 | 0 | 0 | 1 | 45 | 55.56% |
| White Utrerana | 1 | 0 | 0 | 0 | 0 | 0 | 7 | 22 | 0 | 0 | 30 | 23.33% |
| Franciscan Utrerana | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 50 | 0 | 0 | 61 | 81.97% |
| Black Utrerana | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 51 | 23 | 74 | 68.92% |
| Partrige Utrerana | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 56 | 66 | 84.85% |
| Total | 35 | 33 | 28 | 37 | 60 | 45 | 19 | 74 | 61 | 80 | 472 | 59.96% |

**Supplementary Table S6.** Leave-one-out cross-validation of males into their genotypes.

| from \ to | White Sureña | Splash Sureña | Blue Sureña | Franciscan Sureña | Black Sureña | Partridge Sureña | White Utrerana | Franciscan Utrerana | Black Utrerana | Partrige Utrerana | Total | % correct |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| White Sureña | 5 | 2 | 0 | 2 | 0 | 1 | 0 | 1 | 0 | 0 | 11 | 45.45% |
| Splash Sureña | 1 | 0 | 0 | 3 | 0 | 2 | 0 | 0 | 0 | 0 | 6 | 0.00% |
| Blue Sureña | 0 | 0 | 0 | 0 | 2 | 4 | 0 | 0 | 0 | 0 | 6 | 0.00% |
| Franciscan Sureña | 3 | 1 | 0 | 4 | 1 | 2 | 0 | 0 | 0 | 0 | 11 | 36.36% |
| Black Sureña | 0 | 0 | 2 | 2 | 13 | 6 | 0 | 0 | 0 | 0 | 23 | 56.52% |
| Partridge Sureña | 2 | 3 | 5 | 3 | 7 | 3 | 0 | 0 | 0 | 0 | 23 | 13.04% |
| White Utrerana | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 3 | 0 | 0 | 14 | 78.57% |
| Franciscan Utrerana | 0 | 1 | 0 | 0 | 0 | 0 | 7 | 6 | 0 | 0 | 14 | 42.86% |
| Black Utrerana | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 2 | 14 | 85.71% |
| Partrige Utrerana | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 13 | 100.00% |
| Total | 11 | 7 | 7 | 14 | 23 | 18 | 18 | 10 | 12 | 15 | 135 | 49.63% |




**Supplementary Figure S1:** Illustration of classification trees in females populations.




**Supplementary Figure S2:** Illustration of classification trees in males populations.

# Chapter 2.

## Characterization of the productive capacity of Utrerana Avian Breed.

- *González Ariza, A., S. Nogales Baena, T. M. Lupi, A. Arando Arbulu, F. J. Navas González, J. M. León Jurado, J. V. Delgado Bermejo, and M. E. Camacho Vallejo.* ***Characterization of biological growth curves of different varieties of an endangered native hen breed kept under free range conditions.*** *Italian Journal of Animal Science 1:806-813.*

- *González Ariza A., A. Arando Arbulu, J. M. León Jurado, F. J. Navas González, S. Nogales Baena, and M. E. Camacho Vallejo.* ***Mathematical modeling of egg production curve in a multivariety endangered hen breed.*** *Submitted to Research in Veterinary Science.*

# Characterization of biological growth curves of different varieties of an endangered native hen breed kept under free range conditions

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**Abstract:** The aim of this study is to model the growth samples of four varieties (White, Black, Partridge, Franciscan) of Spanish Utrerana hen breed, which is endangered, by using Brody, Von Bertalanffy, Verhulst, Logistic and Gompertz models. For this purpose, a total of 16235 weight data observations from 2004 animals reared in free range system were collected. Logistic was the best suited model for predicting the biological growth curve of White variety in both sexes, while Von Bertalanffy was the best fitting model for the rest of individuals of the breed, based on the 5 goodness-of-fit and flexibility criteria: Pseudo-$R^2$, mean squared error, Akaike information criterion, Bayesian information criterion and the biological coherence of the estimated parameters. Black variety was the heaviest, with values of 2605.96 and 2032.61 g (for males and females, respectively) for *a* parameter, while White variety presented the lowest maturity weight (*a* = 2442.99 and 1874.24 g, for males and females, respectively). Conclusively, this growth characterization is essential for the conservation of the Utrerana hen, to search for new market niches and a greater profitability to this differentiated product.

**Keywords:** Growth curves; non-linear models; growth parameters; local breeds; variety

**Highlights**

- Non-linear models can explain the Utrerana hen growth.
- Females reach maturity earlier than males.
- Utrerana hen shows a strong sexual dimorphism.

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## 1. Introduction

Utrerana hen is an endangered Mediterranean light breed created during the first half of the 20th century in Andalusia (southern Spain) (Orozco 1989). It has four different varieties: White, Black, Partridge and Franciscan (Figure 1). Its initial productive orientation was as a laying hen, raised on family farms, but the birth of approximately 50% of males on each incubation batch has promoted the traditional use of the meat carcass of this breed for self-consumption (Campo 2007). Utrerana poultry breed has shown moderate genetic diversity among the individuals that compose the breed (Macrì et al., 2019). Moreover, Utrerana hen and its varieties can be considered as differentiated populations from other Spanish poultry breeds (Vega-Plá et al., 2019).

There is a growing interest among consumers in animal products obtained through sustainable production systems, the purpose of which is obtain quality food, with less impact on the environment and human health, considering animal welfare (Barba et al., 2016). Alternative forms of farming, involving local breeds, are necessary to avoid the loss of biodiversity, the disappearance of animal genetic resources, the search of economic sustainability and the linkage of population to rural areas (Alderson, 2018; Toalombo et al., 2019). Utrerana breed is adapted to these systems due to its high rusticity and low disease prevalence (Del Castillo, 1951). Native poultry breeds have a genome that makes them more resistant than commercial hybrid lines to conditions caused by climate change in a specific geographical area (Mpenda et al., 2019).


**Figure 1.** Hen and rooster of each Utrerana variety. A: White; B: Black; C: Partridge; D: Franciscan.

Within the cycle life of animals, the total growth duration can be divided in three phases: an acceleration phase, a deceleration phase, and a stabilization phase for ripening (Nogales et al., 2017). So, growth pattern usually is typically fitted by models with sigmoidal structure. The study of the growth of a breed and how certain factors such as variety or sex influence it, is necessary to establish the potential for meat productivity (Sariyel et al., 2017). Growth can be explained through mathematical functions, which could also predict for the age of sexual maturity or the suitable age for commercial slaughter, while helping monitoring general health conditions and nutritional requirements (Kaplan and Gürcan 2018).

There are previous bibliographic references on the productive performance of the growth of some local Spanish breeds (Francesch, 1998; Cubiló et al., 1999; Sánchez et al., 2000; Muriel, 2003; Miguel et al., 2009; Franco et al. 2012; Cajal and Francesch, 2014). Non-linear growth models (Brody, Von Bertalanffy, Verhulst, Logistic, Gompertz, and others) have been studied in other native breeds, geographically and genetically separated (Yang et al., 2006; Kennedy, 2007; Magothe et al., 2010; Rizzi et al., 2013; Osei-Amponsah et al., 2014; Zhao et al., 2015; Mata-Estrada et al., 2020). In the Utrerana poultry breed, research with the aim to describe the quality-related

characteristics and the sensory preference of eggs has been performed (González Ariza et al., 2019a; González Ariza et al., 2019b; González Ariza et al., 2021).

Consequently, the present study aimed to determine the best fitting non-linear growth curve models for growth performance of the Utrerana poultry breed, and the characterization of their biological curve while evaluating the relationship between body weight and age.

## 2. Materials and methods

### *2.1.Chicken flock and Environmental Conditions*

The weight-age data for this study were obtained from 2004 individuals reared under free-range conditions during the years 2018 and 2019 in a public hatchery located at the Agropecuary Provincial Centre of Diputación of Córdoba, Spain.

The chickens were hatched during the first half in both years, since this breed describes seasonal laying patterns regarding the period of eggs (González Ariza Antonio et al., 2019b). The chickens were sorted into incubation batches according to age in 20 batches. Feed and water were available ad libitum in all rearing phases (Table 1). At hatching, the chicks were placed, sorted per incubation batch, in rearing rooms (5 birds/$m^2$ with electric heaters (Copele LGA, Copele, Murcia, Spain) in each room). Animals had access to the outside (1 bird/$m^2$) from 2 month of age on.

**Table 1.** Chemical composition of the compound feed used for feeding the chicken batches in the study.

| Chemical composition (%) | 0-4 weeks | 4-32 weeks | >32 weeks |
|---|---|---|---|
| Crude protein | 20.50 | 18.00 | 15.70 |
| Crude fat and oils | 2.10 | 3.00 | 4.60 |
| Crude fiber | 2.80 | 3.00 | 3.20 |
| Crude ashes | 6.30 | 5.70 | 14.00 |
| Calcium | 0.90 | 1.00 | 4.10 |
| Phosphorus | 0.69 | 0.50 | 0.46 |
| Sodium | 0.15 | 0.18 | 0.19 |
| Methionine | 0.48 | 0.32 | 0.31 |
| Lysine | 1.13 | 0.90 | 0.72 |

*2.2. Recording for the biological growth curve*

The weights were individually measured: on hatching day, weekly during the first month of life, every two weeks from 1 to 3 months and every 28 days from the age of 3 months on. An electronic scale (measurement precision = 0,01 g; CSB-600C, Cobos, Barcelona, Spain) was used to measure weights below 600 g, while a suspended electronic scale (measurement precision = 5 g; Kern CH50K100, Kern & Sohn, Balingen, Germany) was used for animals which exceeded 600 g.

*2.3.Curve fitting*

The data file was purged as described by Lupi et al. (2015). Finally, data of 98.5% of the total of animals were retained for this study. A total of 16235 weights were kept. A slightly higher number of observations were sampled in females (56.04%) compared to males (44.96%), due to the fact that 749 males were used versus 1255 females in the present study (Table 2).

**Table 2.** Number of animals (n) and weight observations (N) used for each variety in both sexes in the study.

| Variety | Females | | Males | | Total | |
|---|---|---|---|---|---|---|
| | n | N | n | N | n | N |
| White | 108 | 967 | 76 | 757 | 184 | 1724 |
| Black | 421 | 3456 | 277 | 2874 | 698 | 6330 |
| Partridge | 373 | 2123 | 175 | 1442 | 548 | 3565 |
| Franciscan | 353 | 2552 | 221 | 2064 | 574 | 4616 |
| Total | 1255 | 9098 | 749 | 7137 | 2004 | 16235 |

Five non-linear functions were evaluated in the present study: Brody, Von Bertalanffy, Verhulst (a variation of Logistic model; frequently called as Logistic model in the literature), Logistic and Gompertz (Table 3). Data were processed with the non-linear regression procedure from the SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016). The results for best-fitted model in each variety were compared with real weight data and animal ages.

The parameter *a* is defined as the asymptotic or maximum growth response of the adult bird. The parameter *b* is related to initial weight (hatch weight). The parameter *k* represents the relative growth rate (exponential growth rate) and indicate the maturity of individuals. Finally, the parameter *m* shapes the growth

**Table 3.** Mathematical description of growth models, biological parameters and growth evaluators.

| | Mathematical expression | Inflection weight | Inflection age | Growth rate | Age to maturity (y≈a) | Maturity degree |
|---|---|---|---|---|---|---|
| Brody | y=*a**(1-*b**exp(-*k**t)) | - | - | $v_c = ka\left(1-\frac{y}{a}\right)$ | $-\frac{ln\left(\frac{a-y}{ba}\right)}{k}$ | |
| Von Bertalanffy | y=*a**(1-*b**exp(-*k**t))**3 | $y_i = \frac{8a}{27}$ | $t_i = \frac{\ln(3b)}{k}$ | $v_c = 3ky\left[\left(\frac{a}{y}\right)^{1/3}-1\right]$ | $-\frac{ln\left(\frac{1-\sqrt[3]{\frac{y}{a}}}{b}\right)}{k}$ | |
| Verhulst | y=*a*/(1+*b**exp(-*k**t)) | $y_i = \frac{a}{2}$ | $t_i = \frac{\ln(b)}{k}$ | $v_c = ky\left(1-\frac{y}{a}\right)$ | $-\frac{ln\left(\frac{a-y}{y*b}\right)}{k}$ | $u = \frac{y}{a}$ |
| Logistic | y=*a**(1+exp(-*k**t))**(-*m*) | $y_i = \frac{a}{2}$ | $t_i = \frac{-\ln(2^{1/m}-1)}{k}$ | $v_c = mk\frac{y_c}{2}\left(\frac{e^{-kt}}{1+e^{-kt}}\right)$ | $-\frac{ln\left[\left(\frac{a}{y}\right)^{1/m}-1\right]}{k}$ | |
| Gompertz | y=*a**exp(-*b**exp(-*k**t)) | $y_i = \frac{a}{e}$ | $t_i = \frac{\ln(b)}{k}$ | $v_c = kyln\left(\frac{a}{y}\right)$ | $-\frac{ln\left(\frac{\ln\left(\frac{y}{a}\right)}{-b}\right)}{k}$ | |

y = weight, in kg, at age t; t = age in days; *a*, *b*, *k* and *m* = parameters

curve, thus determining its inflection point (Loaiza-Echeverri et al., 2013; Tariq et al., 2013; Lupi et al., 2015). For the choice of the best fit models for each variety and sex, the following criteria were used (Lupi et al., 2015; Pizarro Inostroza et al., 2020):

1. The use of the coefficient of determination ($R^2$) in linear regression models determine the quality of the fit of the used model, but in non-linear regression models could overestimate higher values. So, the mathematical approach is Pseudo- $R^2$ and is determined by:

$$Pseudo - R^2 = 1 - \frac{SSResidual}{SSTotal_{corrected}}$$

   where SS = sum of squares.
2. The lowest mean square of the error (MSE) of the studied equation, as a measure that includes the variability of factors not considered by research.
3. The lowest value of the Akaike information criterion (AIC). This tool can consider changes in the fitness quality and the number of parameters between models:

$$AIC = N\ ln\left(\frac{SSResidual}{N}\right) + 2K$$

   where N = numbers of observations; SSResidual = sum of squares of the residuals and K = number of parameters.

4. The lowest value of Bayesian information criterion (BIC), that is a model-order selection criterion:

$$BIC = N \ln\left(\frac{SSResidual}{N}\right) + K * \ln(N)$$

   where N = numbers of observations; SSResidual = sum of squares of the residuals and K = number of parameters.
5. Biological coherence of the estimated parameters.

The five fitting models used in the present study were ranked considering the goodness-of-fit and flexibility criteria individually. The highest score in the rank was given to the model obtaining the most desirable value for each particular criterion.

Afterwards, as goodness-of-fit and flexibility criteria may differ in terms of which their most desirable values are and what their magnitude is, a combined selection index (ICO) was developed following the premises in Van Vleck (1993) to summarize the position in the rank for each of the goodness-of-fit and flexibility criteria determined for each model. The combined index used (ICO) was as follows:

$$ICO = \frac{Pseudo{-}R^2\ Rank\ Position{*}\mathrm{W1}+\ MSE\ Rank\ Position{*}\mathrm{W2}+\ AIC\ Rank\ Position{*}\mathrm{W3}+\ BIC\ Rank\ Position{*}\mathrm{W4}}{4},$$

All criteria were given the same relevance in the ICO, hence, no coefficient was used, that is the proportion of 1:1:1:1 was followed. As a result, the models presenting greater ICO values were those presenting the best-fitting, explanatory and predictive properties for each variety and sex (Supplementary Tables S1, S2, S3, S4 and S5).

## 3. Results

A summary of biological curve shape parameters, fitness and accuracy statistics for the different models that were tested across the Utrerana breed and its varieties are shown in Supplementary Tables S1, S2, S3, S4 and S5. The White genotype, reported the best fitting values as verified with all models, excluding Brody model, which presents, in a generalized way, lower values for Pseudo-R2 across all varieties. Brody model overestimates asymptotic weight, in both sexes, however the rest of models showed convergence and very similar adjustment values. Except for White genotype, Von Bertalanffy model fitted growth data better than the rest of studied models. In the White variety, the Logistic model was the most suitable model. Supplementary Tables S6, S7, S8, S9 and S10 show the observed and predicted weights for the best fitting model for both sexes of Utrerana poultry breed and its varieties.

Table 4 shows the estimated parameters for each variety and sex using the best fitting model in the study of the biological growth curve in Utrerana breed. Males showed a higher body weight in all growth stages, however these differences become clearly noticeable from 45 days of life, as can be seen in Figures 2 and 3, where growth of Utrerana and its varieties are graphically represented.

**Table 4.** Estimated parameters for the best-fitting model for both sexes of Utrerana poultry breed.

| Breed/ Variety | Sex | Model | *a* (s.e.) | *k* (s.e.) | *b/m* (s.e.) | Pseudo-$R^2$ | MSE | AIC | BIC |
|---|---|---|---|---|---|---|---|---|---|
| Utrerana | F | Von Bertalanffy | 1979.00 (4.87) | 0.014 (0.000) | 0.786 (0.004) | 0.971 | 26591.00 | 92696.41 | 92717.76 |
| | M | Von Bertalanffy | 2560.30 (10.00) | 0.014 (0.000) | 0.854 (0.004) | 0.976 | 19613.76 | 70535.13 | 70555.75 |
| White | F | Logistic | 1874.24 (11.12) | 0.022 (0.000) | 5.525 (0.138) | 0.953 | 25213.26 | 9803.69 | 9818.28 |
| | M | Logistic | 2442.99 (25.92) | 0.021 (0.000) | 5.864 (0.110) | 0.963 | 18097.62 | 7424.27 | 7438.16 |
| Black | F | Von Bertalanffy | 2032.61 (8.74) | 0.013 (0.000) | 0.788 (0.006) | 0.943 | 22839.18 | 34688.22 | 34706.66 |
| | M | Von Bertalanffy | 2605.98 (18.01) | 0.014 (0.000) | 0.862 (0.006) | 0.958 | 16114.04 | 27844.72 | 27862.61 |
| Partridge | F | Von Bertalanffy | 1937.83 (9.84) | 0.014 (0.000) | 0.781 (0.011) | 0.933 | 32957.96 | 22055.54 | 22105.52 |
| | M | Von Bertalanffy | 2484.10 17.46) | 0.015 (0.000) | 0.845 (0.009) | 0.958 | 19922.59 | 14277.23 | 14293.05 |
| Franciscan | F | Von Bertalanffy | 2024.45 (9.25) | 0.012 (0.000) | 0.752 (0.007) | 0.945 | 24677.40 | 25813.02 | 25830.55 |
| | M | Von Bertalanffy | 2595.94 (19.50) | 0.013 (0.000) | 0.822 (0.007) | 0.947 | 22827.04 | 20740.70 | 20733.58 |

F: female; M: male; s.e.: standard error; Pseudo-R2: non-linear determinative coefficient; MSE: mean square error; AIC: Akaike information criteria; BIC: Bayesian information criteria.

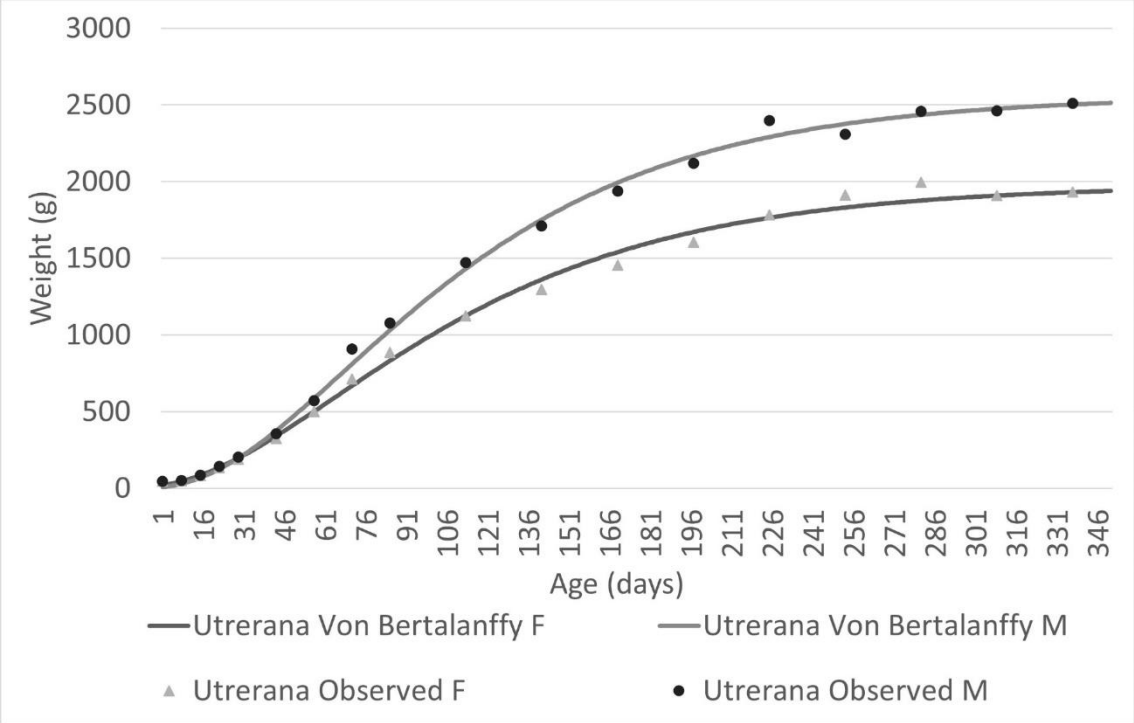


**Figure 2.** Growth curves for both sexes of Utrerana poultry breed predicted with the best fitting model in comparison with the observed data.

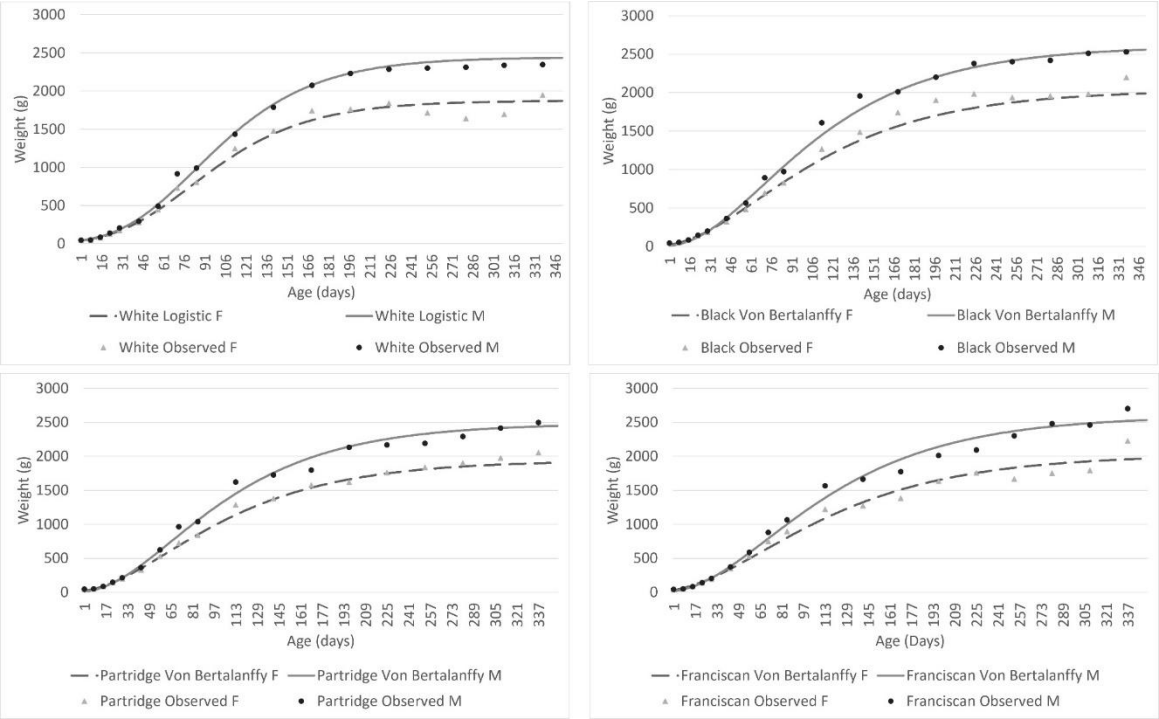


**Figure 3.** Growth curves for both sexes of Utrerana poultry breed predicted with the best fitting model in each variety in comparison with the observed data.

## 4. Discussion

Hatching weight predicted by the Logistic model in the White genotype (41.95 and 40.71 g for males and females, respectively) have higher accuracy respect to the measured hatching weight that the hatching weight predicted by Von Bertalanffy model in the rest of varieties (Table 5). Predictions of hatching weight were between 6.90 and 41.95 g, so slightly some authors supported constraining hatching weight in order to improve fitting ($R^2$) of the data. Barbato (1990) reported that hatching weight should be measured, but not estimated, while others authors suggested to make a data correction: Mignon-Grasteau et al. (1999) suggested to constrain hatching weight within two standard deviations of the mean and Pasternak and Shalev (1994) suggested weighting hatching weight by the inverse of the variance.

Utrerana is a slow-growing breed characterized by clear sexual dimorphism which became evident on the base of the body weight from 45 days onwards. Utrerana can be defined as a light breed because the early age of inflection in the growth curve, the high precocity and the low maturity weight of the individuals, compared with other chicken breeds. Males and females of the White genotype reported lower maturity weights, while for the rest of the varieties (Partridge, Franciscan and Black), both sexes reported more similar values (Table 4). Maturity was reached

later in the Franciscan genotype ($k$ = 0.013 in males and $k$ = 0.012 in females), while an earliest growth was reported for the White variety ($k$ = 0.021 in males and $k$ = 0.022 in females). A negative correlation between parameters a and k can be observed across the different varieties of the Utrerana breed. Some authors have suggested that there is a high probability that larger and heavier animals are less precocious than smaller and lighter ones (Bathaei and Leroy, 1998).

Regarding other Spanish breeds, Francesch (1998) collected weights of chickens of Empordanesa Roja, Penedesenca Negra and Prat Leonada breeds, from hatching to 20 weeks of age, obtaining values of 2840, 2660 and 2675 g, respectively. In addition, Cubiló et al. (1999) reported values of 2482 g in Penedesenca Negra breed at 16 weeks. These weights are above those estimated for Utrerana breed for the same age. Although Utrerana poultry breed has been classified as a dual-purpose hen, a lower potential for meat-production than these other breeds has also been reported (Fernández et al., 2009). Sánchez et al. (2000) using the Mos hen, a native breed with a clear orientation towards meat production reported higher values for chicken growth (4434 and 3641 g in males and females at 300 days of life).

Contrastingly, it has been reported weights of 1752.60 g (Black variety) and 1740.90 g (brunette variety) of weight in females at adulthood in Sobrarbe hen breed, weights of 2491.96 g in cocks at 32 weeks in Extremeña Azul breed, and values of k of 0.153 and a of 2660.91 g using the Gompertz model for cocks of Castellana Negra breed (Francesch, 1998; Muriel, 2003; Miguel et al., 2009). These results agree with the findings of the present study. Sobrarbe, Extremeña Azul, Castellana Negra and Utrerana hen breeds have been genetically selected towards the egg production in extensive systems, great rusticity and resistance to extreme weather situations. Besides, Castellana Negra and Utrerana poultry breeds have a great geographical proximity and literature indicates that have a common genetic origin (Orozco, 1989).

Predicted $k$ parameters in the present study agree with those by other authors who used Von Bertalanffy models in slow-growing broilers and native creole chickens (Narinç et al., 2010; Mata-Estrada et al., 2020). Still these are slightly lower when compared with local breeds or slow-growing genotypes using Logistic and Gompertz models, respectively (Rizzi et al., 2013; Aksoy et al., 2021). However,

Topal and Bolukbasi (2008) obtained much higher values of *k* in fast-growing broilers using Von Bertalanffy model. The relative growth could be slower in native breeds than in fast-growing lines due to the lower productive selection and the environmental conditions of these breeds.

Inflection age estimated by Logistic model in local chicken of Ghana (Osei-Amponsah et al., 2014) are close to the values reported in the present study. Both sexes of Utrerana hen breed present similar results (<4 days between males and females) at the estimated inflection age, in contrast with the results obtained by Mata-Estrada et al. (2020), who reported a difference between males and females of approximately 10 and 8 days for Von Bertalanffy and Logistic models. Therefore, the slaughter age for Utrerana breed must be similar in both sexes. In any case, in native breeds with low inflection weight, the slaughter age must be delayed until the birds reach a weight close to the weight at maturity, seeking in the chicken carcass a differentiated product for the market (Franco et al., 2012).

Regarding to the best models for describing growth on poultry, some authors suggested that Von Bertalanffy growth model was the best model to fit growth in local breeds (Yang et al., 2006; Mata-Estrada et al., 2020). Nevertheless, Atil et al. (2007) suggested that logistic growth model reports the best fit in broilers when compared to Von Bertalanffy and Gompertz models. In any case, the results obtained in the present research are also agree with those reported by Narinç et al. (2017), since they reported that Gompertz was a suitable model to fit the growth curve in slow-growing chickens.

## 5. Conclusions

The non-linear growth models used in this study are suitable to describe the biological growth of the Utrerana breed, with Logistic and Von Bertalanffy models standing out as the best fitting models in different Utrerana varieties, in accordance to the goodness-of-fit and flexibility criteria. Utrerana growth curves are very similar to the rest of light breeds, with a clear sexual dimorphism, hence males of this breed could be profitable from a meat production point of view. The obtained results can be useful for making zootechnical decisions like determine a slaughter age, the nutritional requirements and control the health status of the batch and may support the breeding program for these endangered breeds. Finally, further studies

are needed to estimate genetic parameters of the growth curve of this breed and make a genetic selection of individuals based on growth characteristics.

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**Ethical approval:** The study follows the national guidelines and premises described in the Declaration of Helsinki. Protocols applicated were permitted by the regulations of the European Union (2010/63/EU) in their transposition to the Royal Decree-Law 53/2013 and its credited entity the Ethics Committee of Animal Experimentation from the University of Córdoba.

**Declaration of interest statement:** The authors declare no conflict of interest.

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**Supplementary Table S1.** Estimated parameters for each model in the study of the biological growth curve for both sexes of Utrerana poultry breed (not classified by varieties).

| Model | Sex | *a* (s.e.) | *k* (s.e.) | *b*/*m* (s.e.) | Pseudo-$R^2$ | MSE | AIC | BIC | ICO Rank |
|---|---|---|---|---|---|---|---|---|---|
| Brody | F | 2104.43 (6.81) | 0.007 (0.000) | 1.055 (0.002) | 0.963 | 32685.285 | 94573,80 | 94595,15 | 1 |
| | M | 3045.65 (21.48) | 0.006 (0.000) | 1.056 (0.002) | 0.955 | 35253.235 | 74719,15 | 74739,77 | 1 |
| Von Bertalanffy | F | 1979.00 (4.87) | 0.014 (0.000) | 0.786 (0.004) | 0.971 | 26590.998 | 92696,41 | 92717,76 | 5 |
| | M | 2560.30 (10.00) | 0.014 (0.000) | 0.854 (0.004) | 0.976 | 19613.755 | 70535,13 | 70555,75 | 5 |
| Verhulst | F | 1912.50 (4.87) | 0.028 (0.000) | 15.373 (0.241) | 0.964 | 31833.691 | 94333,62 | 94354,96 | 2 |
| | M | 2259.43 (8.10) | 0.033 (0.000) | 21.040 (0.311) | 0.969 | 24803.793 | 72210,41 | 72231,03 | 2 |
| Logistic | F | 1943.19 (4.73) | 0.019 (0.000) | 4.817 (0.034) | 0.969 | 28088.389 | 93194,83 | 93216,18 | 3 |
| | M | 2415.64 (8.77) | 0.021 (0.000) | 5.424 (0.032) | 0.974 | 20532.430 | 70861,77 | 70882,39 | 3 |
| Gompertz | F | 1955.57 (4.75) | 0.017 (0.000) | 3.608 (0.027) | 0.970 | 27303.796 | 92937,08 | 92958,42 | 4 |
| | M | 2465.30 (9.14) | 0.018 (0.000) | 4.086 (0.026) | 0.975 | 19869.004 | 70627,40 | 70648,01 | 4 |

F: female; M: male; s.e,: standard error; Pseudo-$R^2$: non-linear determinative coefficient; MSE: mean square error; AIC: Akaike information criterion; BIC: Bayesian information criterion; ICO: combined selection index.

**Supplementary Table S2.** Estimated parameters for each model in the study of the biological growth curve for both sexes of white variety of Utrerana poultry breed.

| Model | Sex | *a* (s.e.) | *k* (s.e.) | *b*/*m* (s.e.) | Pseudo-$R^2$ | MSE | AIC | BIC | ICO Rank |
|---|---|---|---|---|---|---|---|---|---|
| Brody | F | 1980.85 (17.30) | 0.008 (0.000) | 1.080 (0.007) | 0.926 | 39687.45 | 10242.36 | 10256.98 | 1.00 |
| | M | 3093.69 (80.77) | 0.006 (0.000) | 1.064 (0.006) | 0.905 | 46143.16 | 8132.80 | 8146.69 | 1.00 |
| Von Bertalanffy | F | 1894.83 (11.83) | 0.016 (0.000) | 0.904 (0.020) | 0.951 | 26189.26 | 9840.39 | 9855.01 | 3.00 |
| | M | 2572.00 (32.35) | 0.015 (0.000) | 0.927 (0.016) | 0.959 | 19911.16 | 7496.56 | 7510.45 | 2.75 |
| Verhulst | F | 1851.15 (11.52) | 0.032 (0.001) | 20.277 (1.090) | 0.950 | 26621.83 | 9856.23 | 9870.85 | 2.00 |
| | M | 2293.84 (22.09) | 0.034 (0.001) | 24.315 (1.054) | 0.959 | 20062.97 | 7502.31 | 7516.20 | 2.00 |
| Logistic | F | 1874.24 (11.12) | 0.022 (0.000) | 5.525 (0.138) | 0.953 | 25213.26 | 9803.69 | 9818.28 | 4.75 |
| | M | 2442.99 (25.92) | 0.021 (0.000) | 5.864 (0.110) | 0.963 | 18097.62 | 7424.27 | 7438.16 | 4.75 |
| Gompertz | F | 1880.95 (11.30) | 0.019 (0.000) | 4.250 (0.118) | 0.953 | 25291.17 | 9806.65 | 9821.27 | 4.00 |
| | M | 2484.54 (27.81) | 0.019 (0.000) | 4.498 (0.095) | 0.962 | 18275.05 | 7431.66 | 7445.54 | 3.75 |

F: female; M: male; s.e,: standard error; Pseudo-$R^2$: non-linear determinative coefficient; MSE: mean square error; AIC: Akaike information criterion; BIC: Bayesian information criterion; ICO: combined selection index.

**Supplementary Table S3.** Estimated parameters for each model in the study of the biological growth curve for both sexes of black variety of Utrerana poultry breed.

| Model | Sex | *a* (s.e.) | *k* (s.e.) | *b*/*m* (s.e.) | Pseudo-$R^2$ | MSE | AIC | BIC | ICO Rank |
|---|---|---|---|---|---|---|---|---|---|
| Brody | F | 2196.90 (12.67) | 0.006 (0.000) | 1.052 (0.003) | 0.928 | 32320.94 | 35492.29 | 35510.74 | 1.00 |
| | M | 3297.10 (46.30) | 0.005 (0.000) | 1.052 (0.002) | 0.917 | 28821.98 | 29845.09 | 29862.98 | 1.00 |
| Von Bertalanffy | F | 2032.61 (8.74) | 0.013 (0.000) | 0.788 (0.006) | 0.943 | 22839.18 | 34688.22 | 34706.66 | 5.00 |
| | M | 2605.98 (18.01) | 0.014 (0.000) | 0.862 (0.006) | 0.958 | 16114.04 | 27844.72 | 27862.61 | 4.75 |
| Verhulst | F | 1941.01 (8.77) | 0.028 (0.000) | 15.805 (0.371) | 0.929 | 28528.08 | 35456.87 | 35475.32 | 2.00 |
| | M | 2204.93 (12.37) | 0.035 (0.000) | 22.828 (0.510) | 0.949 | 19953.74 | 28458.97 | 28476.86 | 2.00 |
| Logistic | F | 1984.12 (8.48) | 0.019 (0.000) | 4.858 (0.050) | 0.939 | 24524.62 | 34934.29 | 34952.73 | 3.00 |
| | M | 2403.53 (14.62) | 0.021 (0.000) | 5.558 (0.049) | 0.957 | 16627.01 | 27934.78 | 27952.67 | 3.00 |
| Gompertz | F | 2000.93 (8.51) | 0.017 (0.000) | 3.633 (0.040) | 0.940 | 23672.57 | 34812.08 | 34830.52 | 4.00 |
| | M | 2470.34 (15.68) | 0.019 (0.000) | 4.182 (0.040) | 0.958 | 16148.01 | 27850.77 | 27868.66 | 4.00 |

F: female; M: male; s.e,: standard error; Pseudo-$R^2$: non-linear determinative coefficient; MSE: mean square error; AIC: Akaike information criterion; BIC: Bayesian information criterion; ICO: combined selection index.

**Supplementary Table S4.** Estimated parameters for each model in the study of the biological growth curve for both sexes of partridge variety of Utrerana poultry breed.

| Model | Sex | A (s.e.) | k (s.e.) | b/m (s.e.) | Pseudo-$R^2$ | MSE | AIC | BIC | ICO |
|---|---|---|---|---|---|---|---|---|---|
| Brody | F | 2045,31 (13,10) | 0,007 (0,000) | 1,054 (0,005) | 0,922 | 38122,984 | 22397,63 | 22414,60 | 1 |
| | M | 2819,66 (33,88) | 0,006 (0,000) | 1,060 (0,004) | 0,927 | 34483,718 | 15068,36 | 15084,18 | 1 |
| Von Bertalanffy | F | 1937,83 (9,84) | 0,014 (0,000) | 0,781 (0,011) | 0,933 | 32957,961 | 22055,54 | 22105,52 | 4,75 |
| | M | 2484,10 17,46) | 0,015 (0,000) | 0,845 (0,009) | 0,958 | 19922,587 | 14277,23 | 14293,05 | 5 |
| Verhulst | F | 1883,42 (9,76) | 0,029 (0,000) | 15,042 (0,552) | 0,922 | 38105,155 | 22396,62 | 22413,61 | 1,75 |
| | M | 2295,48 (16,22) | 0,032 (0,000) | 19,107 (0,608) | 0,944 | 26062,851 | 14664,63 | 14680,46 | 2 |
| Logistic | F | 1908,15 (9,56) | 0,020 (0,000) | 4,774 (0,080) | 0,930 | 34479,239 | 22184,34 | 22201,32 | 2,75 |
| | M | 2392,70 (16,14) | 0,021 (0,000) | 5,278 (0,070) | 0,955 | 21122,808 | 14361,59 | 14377,41 | 3 |
| Gompertz | F | 1918,37 (9,61) | 0,017 (0,000) | 3,571 (0,065) | 0,931 | 33699,521 | 22135,78 | 22152,76 | 3,75 |
| | M | 2424,32 (16,45) | 0,019 (0,000) | 3,984 (0,057) | 0,957 | 20331,421 | 14306,52 | 14322,35 | 4 |

F: female; M: male; s.e,: standard error; Pseudo-$R^2$: non-linear determinative coefficient; MSE: mean square error; AIC: Akaike information criterion; BIC: Bayesian information criterion; ICO: combined selection index.

**Supplementary Table S5.** Estimated parameters for each model in the study of the biological growth curve for both sexes of franciscan variety of Utrerana poultry breed.

| Model | Sex | *a* (s.e.) | *k* (s.e.) | *b*/*m* (s.e.) | Pseudo-$R^2$ | MSE | AIC | BIC | ICO Rank |
|---|---|---|---|---|---|---|---|---|---|
| Brody | F | 2162.63 (13.11) | 0.006 (0.000) | 1.048 (0.003) | 0.936 | 28629.47 | 26192.11 | 26209.65 | 2.25 |
| | M | 3075.00 (37.92) | 0.005 (0.000) | 1.051 (0.003) | 0.922 | 34003.94 | 21539.26 | 21556.15 | 1 |
| Von Bertalanffy | F | 2024.45 (9.25) | 0.012 (0.000) | 0.752 (0.007) | 0.945 | 24677.40 | 25813.02 | 25830.55 | 5 |
| | M | 2595.94 (19.50) | 0.013 (0.000) | 0.822 (0.007) | 0.947 | 22827.04 | 20740.70 | 20733.58 | 4.75 |
| Verhulst | F | 1957.95 (9.45) | 0.025 (0.000) | 13.727 (0.373) | 0.929 | 31560.07 | 26440.82 | 26458.35 | 1.25 |
| | M | 2268.61 (16.82) | 0.032 (0.000) | 19.557 (0.577) | 0.928 | 31120.09 | 21356.33 | 21373.23 | 2 |
| Logistic | F | 1985.99 (9.058) | 0.018 (0.000) | 4.570 (0.055) | 0.939 | 27088.96 | 26050.96 | 26068.49 | 3.25 |
| | M | 2438.69 (17.83) | 0.020 (0.000) | 5.217 (0.061) | 0.941 | 25378.60 | 20935.39 | 20952.28 | 3 |
| Gompertz | F | 1999.34 (9.06) | 0.015 (0.000) | 3.401 (0.044) | 0.944 | 33699.52 | 25941.34 | 25958.88 | 3.25 |
| | M | 2495.05 (18.36) | 0.017 (0.000) | 3.897 (0.048) | 0.942 | 20331.42 | 20933.66 | 20850.56 | 4.25 |

F: female; M: male; s.e,: standard error; Pseudo-$R^2$: non-linear determinative coefficient; MSE: mean square error; AIC: Akaike information criterion; BIC: Bayesian information criterion; ICO: combined selection index.

**Supplementary Table S6.** Comparison between estimated weights for the best fitting model and observed weights for both sexes of Utrerana poultry breed.

| Age (d) | Females | | Males | |
|---|---|---|---|---|
| | Von Bertalanffy (g) | Observed (g) | Von Bertalanffy (g) | Observed (g) |
| 0 | 19.32 | 43.66 ± 4.35 | 8.00 | 44.12 ± 4.52 |
| 7 | 45.85 | 49.07 ± 8.45 | 29.69 | 49.88 ± 9.71 |
| 14 | 84.82 | 83.62 ± 21.11 | 68.42 | 84.31 ± 21.68 |
| 21 | 135.34 | 137.90 ± 33.78 | 124.27 | 142.55 ± 37.36 |
| 28 | 195.88 | 188.88 ± 51.98 | 195.72 | 202.90 ± 59.17 |
| 42 | 339.57 | 324.81 ± 69.15 | 375.58 | 355.11 ± 80.54 |
| 56 | 501.14 | 500.90 ± 110.68 | 586.75 | 572.71 ± 116.28 |
| 70 | 668.10 | 713.36 ± 119.78 | 809.98 | 909.12 ± 125.23 |
| 84 | 831.28 | 884.42 ± 132.00 | 1030.76 | 1078.99 ± 180.63 |
| 112 | 1124.96 | 1124.70 ± 151.70 | 1430.53 | 1471.44 ± 218.10 |
| 140 | 1361.06 | 1296.50 ± 174.79 | 1751.34 | 1710.00 ± 303.73 |
| 168 | 1540.12 | 1453.72 ± 219.80 | 1992.66 | 1938.45 ± 269.08 |
| 196 | 1371.13 | 1605.05 ± 207.91 | 2167.26 | 2120.00 ± 285.51 |
| 224 | 1764.83 | 1782.50 ± 265.82 | 2290.55 | 2399.17 ± 374.99 |
| 252 | 1830.85 | 1911.25 ± 169.53 | 2376.25 | 2310.10 ± 313.35 |
| 280 | 1876.90 | 1994.17 ± 393.90 | 2435.21 | 2457.25 ± 315.09 |
| 308 | 1908.83 | 1996.17 ± 285.45 | 2475.51 | 2460.00 ± 301.95 |
| 336 | 1930.85 | 1937.83 ± 292.93 | 2502.93 | 2510.84 ± 301.35 |

**Supplementary Table S7.** Comparison between estimated weights for the best fitting model and observed weights for both sexes of white variety of Utrerana poultry breed.

| Age (d) | Females | | Males | |
|---|---|---|---|---|
| | Logistic (g) | Observed (g) | Logistic (g) | Observed (g) |
| 0 | 40.71 | 45.34 ± 4.34 | 41.95 | 45.51 ± 4.15 |
| 7 | 60.73 | 47.69 ± 7.35 | 63.86 | 50.15 ± 7.34 |
| 14 | 87.85 | 78.95 ± 22.06 | 94.11 | 84.45 ± 20.19 |
| 21 | 123.23 | 125.87 ± 32.96 | 134.37 | 139.61 ± 32.79 |
| 28 | 167.81 | 174.19 ± 55.49 | 186.02 | 204.89 ± 55.65 |
| 42 | 285.99 | 277.16 ± 50.34 | 326.54 | 293.93 ± 56.23 |
| 56 | 439.89 | 445.88 ± 95.37 | 515.10 | 493.58 ± 102.03 |
| 70 | 619.11 | 730.00 ± 59.26 | 740.50 | 915.00 ± 89.30 |
| 84 | 808.79 | 806.67 ± 76.81 | 984.53 | 992.25 ± 154.68 |
| 112 | 1164.35 | 1245.00 ± 108.28 | 1454.70 | 1433.34 ± 200.02 |
| 140 | 1436.79 | 1478.75 ± 143.90 | 1825.25 | 1786.67 ± 240.07 |
| 168 | 1617.77 | 1742.50 ± 223.29 | 2076.53 | 2072.50 ± 213.44 |
| 196 | 1728.27 | 1760.00 ± 213.44 | 2232.34 | 2230.00 ± 346.48 |
| 224 | 1792.56 | 1840.00 ± 326.00 | 2324.09 | 2285.50 ± 357.50 |
| 252 | 1828.97 | 1716.67 ± 279.30 | 2376.57 | 2300.00 ± 360.08 |
| 280 | 1849.28 | 1642.50 ± 284.25 | 2406.10 | 2313.34 ± 371.15 |
| 308 | 1860.52 | 1695.00 ± 300.52 | 2422.57 | 2337.50 ± 384.55 |
| 336 | 1866.71 | 1945.00 ± 301.30 | 2431.70 | 2347.50 ± 404.26 |

**Supplementary Table S8.** Comparison between estimated weights for the best fitting model and observed weights for both sexes of black variety of Utrerana poultry breed.

| Age (d) | Females | | Males | |
|---|---|---|---|---|
| | Von Bertalanffy (g) | Observed (g) | Von Bertalanffy (g) | Observed (g) |
| 0 | 19.48 | 43.13 ± 4.24 | 6.90 | 43.89 ± 4.85 |
| 7 | 45.88 | 49.41 ± 8.40 | 27.44 | 50.20 ± 9.90 |
| 14 | 84.60 | 81.91 ± 17.43 | 65.17 | 83.01 ± 22.41 |
| 21 | 134.82 | 136.61 ± 33.92 | 120.37 | 141.86 ± 40.25 |
| 28 | 195.08 | 184.90 ± 43.79 | 191.62 | 196.63 ± 60.65 |
| 42 | 338.51 | 318.96 ± 62.37 | 372.47 | 361.35 ± 77.54 |
| 56 | 500.52 | 480.92 ± 80.87 | 586.19 | 561.97 ± 105.47 |
| 70 | 668.76 | 692.88 ± 109.91 | 813.03 | 892.25 ± 156.51 |
| 84 | 834.02 | 826.66 ± 104.13 | 1037.99 | 971.75 ± 151.12 |
| 112 | 1133.67 | 1266.66 ± 75.21 | 1446.41 | 1607.50 ± 120.94 |
| 140 | 1376.96 | 1485.00 ± 104.4 | 1774.93 | 1955.00 ± 134.35 |
| 168 | 1563.21 | 1738.75 ± 89.76 | 2022.44 | 2010.00 ± 216.79 |
| 196 | 1700.73 | 1900.00 ± 155.46 | 2201.70 | 2200.00 ± 224.52 |
| 224 | 1799.94 | 1982.50 ± 213.76 | 2328.38 | 2377.50 ± 286.38 |
| 252 | 1870.42 | 1935.00 ± 292.42 | 2416.50 | 2400.00 ± 306.28 |
| 280 | 1920.00 | 1955.00 ± 290.15 | 2477.16 | 2417.50 ± 305.02 |
| 308 | 1954.63 | 1982.50 ± 285.45 | 2518.63 | 2510.00 ± 362.99 |
| 336 | 1978.71 | 2197.50 ± 301.27 | 2546.85 | 2526.66 ± 384.45 |

**Supplementary Table S9.** Comparison between estimated weights for the best fitting model and observed weights for both sexes of partridge variety of Utrerana poultry breed.

| Age (d) | Females | | Males | |
|---|---|---|---|---|
| | Von Bertalanffy (g) | Observed (g) | Von Bertalanffy (g) | Observed (g) |
| 0 | 20.49 | 46.25 ± 3.94 | 9.29 | 46.10 ± 4.13 |
| 7 | 48.45 | 49.57 ± 9.14 | 33.25 | 50.99 ± 13.09 |
| 14 | 89.28 | 86.09 ± 21.74 | 75.18 | 86.93 ± 25.81 |
| 21 | 141.95 | 145.45 ± 35.17 | 134.82 | 146.18 ± 43.06 |
| 28 | 204.73 | 199.83 ± 57.82 | 210.27 | 212.01 ± 70.65 |
| 42 | 352.56 | 328.33 ± 81.64 | 397.53 | 362.05 ± 78.92 |
| 56 | 517.13 | 528.16 ± 142.15 | 613.89 | 623.33 ± 127.26 |
| 70 | 685.54 | 722.25 ± 147.39 | 839.30 | 963.33 ± 150.32 |
| 84 | 848.60 | 840.50 ± 177.74 | 1059.26 | 1038.33 ± 165.10 |
| 112 | 1138.15 | 1285.50 ± 195.18 | 1450.25 | 1622.14 ± 172.98 |
| 140 | 1367.01 | 1376.00 ± 178.49 | 1756.86 | 1723.75 ± 134.44 |
| 168 | 1537.80 | 1575.00 ± 178.03 | 1982.63 | 1797.50 ± 162.86 |
| 196 | 1660.87 | 1618.33 ± 285.30 | 2142.75 | 2132.50 ± 204.76 |
| 224 | 1747.63 | 1760.33 ± 297.50 | 2253.70 | 2167.50 ± 247.42 |
| 252 | 1807.91 | 1835.00 ± 283.77 | 2329.45 | 2190.00 ± 311.75 |
| 280 | 1849.41 | 1900.00 ± 300.71 | 2380.67 | 2290.00 ± 311.75 |
| 308 | 1877.80 | 1975.00 ± 301.47 | 2415.10 | 2415.00 ± 378.28 |
| 336 | 1897.14 | 2052.50 ± 301.78 | 2438.14 | 2496.00 ± 378.59 |

**Supplementary Table S10.** Comparison between estimated weights for the best fitting model and observed weights for both sexes of franciscan variety of Utrerana poultry breed.

| Age (d) | Females | | Males | |
|---|---|---|---|---|
| | Von Bertalanffy (g) | Observed (g) | Von Bertalanffy (g) | Observed (g) |
| 0 | 30.90 | 41.73 ± 3.58 | 14.74 | 42.50 ± 3.70 |
| 7 | 60.69 | 49.00 ± 8.53 | 40.88 | 48.66 ± 7.56 |
| 14 | 100.92 | 87.27 ± 24.40 | 82.36 | 83.96 ± 18.90 |
| 21 | 150.67 | 141.21 ± 32.97 | 138.76 | 142.61 ± 31.62 |
| 28 | 208.67 | 194.61 ± 51.09 | 208.60 | 204.52 ± 50.68 |
| 42 | 343.50 | 348.30 ± 61.23 | 380.26 | 372.32 ± 84.45 |
| 56 | 493.86 | 534.60 ± 105.21 | 579.66 | 585.67 ± 109.28 |
| 70 | 649.83 | 747.50 ± 143.44 | 790.71 | 877.50 ± 125.28 |
| 84 | 803.86 | 895.00 ± 148.33 | 1001.02 | 1066.33 ± 165.99 |
| 112 | 1087.16 | 1221.50 ± 172.30 | 1388.35 | 1566.25 ± 181.64 |
| 140 | 1322.97 | 1272.50 ± 173.58 | 1707.81 | 1664.17 ± 180.44 |
| 168 | 1508.60 | 1385.00 ± 186.38 | 1955.34 | 1772.50 ± 197.94 |
| 196 | 1649.68 | 1633.33 ± 245.27 | 2139.91 | 2010.00 ± 234.75 |
| 224 | 1754.46 | 1755.00 ± 262.34 | 2274.18 | 2094.17 ± 260.79 |
| 252 | 1831.08 | 1667.00 ± 270.48 | 2370.30 | 2300.00 ± 291.02 |
| 280 | 1886.53 | 1750.00 ± 287.97 | 2438.37 | 2480.00 ± 368.75 |
| 308 | 1926.36 | 1793.33 ± 316.31 | 2486.22 | 2460.00 ± 370.89 |
| 336 | 1954.83 | 2225.00 ± 330.25 | 2519.69 | 2700.00 ± 427.50 |

# Mathematical modeling of egg production curve in a multivariety endangered hen breed

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**Abstract:** This study aimed to compare the egg laying performance of the four varieties (white, franciscan, black and partridge) of a Spanish endangered avian breed: Utrerana hen. A flock of 60 Utrerana hens (15 per variety) were housed individually allowing the daily egg trazability. For the study of the biological laying curves, seven nonlinear regression models were used: compartmental, Gamma, linear hyperbolic, logistic curvilinear, McNally, Narushin-Takma and quadratic logarithmic. In order to select the best model to fit the different laying curves, five goodness-of-fit and flexibility criteria were calculated: mean square of the error, Akaike information criteria, corrected Akaike information criteria, Bayesian information criteria and the coefficient of determination ($R^2$). Best fit values were reached by the six-parameter model of Narushin-Takma to fit the laying curve of white ($R^2$=0.828) franciscan ($R^2$=0.888) and black ($R^2$=0.899) varieties, while quadratic logarithmic was the best-fitted model in the laying performance of partridge Utrerana hen ($R^2$=0.917). Conclusively, the productive characterization and the generation of knowledge allow to establish livestock models that adapt to the breed and thus, improve the economic profitability that ensures the conservation of local genetic resources.

**Keywords:** Laying curves; nonlinear models; local breeds; variety; zootechny

**Highlights:**

- Some nonlinear models can explain the Utrerana egg performance.
- The egg production of Utrerana hen is strongly affected by its genotype.
- Utrerana hen shows a productive seasonality, despite the heat stress resistance.

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## 1. Introduction

Eggs contribute a large percentage of proteins of animal origin to the human diet worldwide (Dhama et al., 2014). This is due to the nutritional benefits, low cost and culinary versality of this product (Conrad et al., 2017). In the last years, it has been observed a growing interest in hen welfare, product closeness and the consumption of eggs obtained in alternative production systems. These attitudes of costumers towards eggs produce greater acceptance of products obtained from local breeds than those obtained through the use of commercial hybrids (González Ariza et al., 2019a). In addition, local breeds are perfectly suited to free-range farms, since the great rusticity, ability to search food in the wild and resistance to extreme weather conditions that present these genetic groupings (Lordelo et al., 2020).

Utrerana avian breed was created in the middle of the 20th century, from a selection of a heterogeneous population of local hens from different family farms located through the Andalusian countryside, that has four feather varieties: partridge, franciscan, black and white (Orozco, 1989). In the beginning, it was selected for its aptitude as a laying hen and its rusticity (Campo, 2007), but the emergence of genetically selected breeds of laying hens in Europe caused the displacement, the hybridization, and a reduction in the census of Utrerana breed individuals (Garcia and Cordero, 2006; Lyimo et al., 2014). All this relegated the Utrerana hen to an ornamental and morphological selection that caused a drastic decrease in its productive indices (Fernández et al., 2009).

This breed is classified as an endangered breed, according to the Royal Decree Law 45/8 February 2019, and presented a census of 1548 individuals on 31st December, 2019 (MAPA, 2021). Therefore, the characterization of the breed becomes necessary

to implement programs for the recovery, conservation and productivity improvement.

The modelling for knowledge, analysis and interpretation of the egg production curves over time allows to make behavioral predictions, to know the productive performance with respect to the expected at a given moment, making herd balances and analysis of laying curve shape, peak and persistence (Savegnago et al., 2012).

Nonlinear regression models are widely used to fit egg production data. Mathematically, the egg production curves can be divided in three phases: the first phase is the increase in laying from the lay of the first egg, at the age of sexual maturity, until the hen reaches the maximum point, following by the second phase, in which a linear trend is reached after a while, an then, in the third phase, the laying curve decreases to zero (Narinç et al., 2014). The laying curve in hen closely resembles the milk production curve of dairy cows and small ruminants, so multiple functions can be used to model both production systems (Pizarro Inostroza et al., 2020).

The number of eggs and the time of the laying period are related by the egg production curves (Yang et al., 1989). It has been shown that mathematical models are suitable to characterize the laying performance of hen flocks. Through the use of nonlinear regression functions, an alternative method has been developed in order to carry out genetic studies of poultry farms, as well as to be able to evaluate and improve the zootechnical management, like the flocks performance and the forecast income (Miyoshi et al., 1996).

Differences between the internal and external physical characteristics and the chemical composition of the egg have already been observed between the different varieties of the Utrerana avian breed (González Ariza et al., 2019c; González Ariza et al., 2020). In addition, it is possible to compare the egg production between the four varieties of Utrerana hen by using nonlinear models to fit the laying curve. Consequently, the present study objective was to fit the weekly egg production rate of the four varieties of Utrerana hen, using nonlinear regression models (compartmental, Gamma, linear hyperbolic, logistic curvilinear, McNally, Narushin-Takma and quadratic logarithmic functions).

## 2. Material and methods

### *2.1. Institutional animal care and use committee statement*

Codes of good practice were followed by the center in which experiments were carried out. This research follows the national guidelines for care and use of laboratory and farm animals and conducted in accordance with the Declaration of Helsinki. Protocols applicated were permitted by the regulations of the European Union (2010/63/EU) in their transposition to the Royal Decree-Law 53/2013, the Ethics Committee of Animal Experimentation from the University of Córdoba.

### *2.2 Flock, management and egg production*

**Table 1.** Flock management information. All cages were chosen according to Council Directive 1999/74/EC of 19 July, 1999, laying down minimum standards for the protection of laying hens.

| Flock Management | Utrerana varieties | | | |
|---|---|---|---|---|
| Parameter | White | Franciscan | Black | Partridge |
| Laying hens | 15 | 15 | 15 | 15 |
| Hens (70 weeks old) | 8 | 8 | 8 | 8 |
| Pullets (28 weeks old) | 7 | 7 | 7 | 7 |
| Stocking density | 4 animals per each $m^2$ | | | |
| Nest box density | 29 animals per each $m^2$ | | | |
| Waterer allotment/space | Circle waterers of 5 cm of diameter per animal | | | |
| Feeder allotment/space | 41 cm per animal | | | |
| Floor substrate | Wood shavings covering the floor at a depth of approximately 1 cm | | | |
| Nest box substrate | Plastic sturf mats covering the floor at a depth of approximately 1 cm | | | |

The present study was carried out in the public hatchery located at the Agropecuary Provincial Centre of Diputación of Córdoba (Andalusian; south Spain; 37°54′50.9″ N 4°42′40.4″ W). The data were collected on 60 hens, distributed depending on their age and variety as shown in Table 1. The information about the origin of each hen (livestock and parentage) was provided by the National Association of Utrerana Hen Breeders (ANCGU). Animals were fed with the same commercial mixture (15.20% crude protein, 4.60% crude fat and oils, 3.20% crude fiber, 14.00% crude ashes, 4.1% calcium, 0.66% phosphorus, 0.19% sodium, 0.31% methionine, 0.72% lysine) during all the experimental period (391 days, from February 4$^{th}$, 2019 to March 1$^{st}$, 2020). Feed and water were available ad libitum. Hens characteristics of laying pattern can be found in a previous experiment

(González Ariza et al., 2019c). Oviposition for each hen was collected daily and the cumulative were managed in individual cages (50 x 62 x 41 cm) following Council Directive 1999/74/EC of 19 July 1999, setting the minimum standards for the protection of laying hens in the experimental center. Additional information regarding the weekly egg rate was calculated for each hen separately. During the experimental period 5 hens were removed because of mortality.

*2.3. Curve fitting*

The weekly egg production rate for each hen was used to fit the mean population curve for the different Utrerana breed varieties. Convergence criterion was defined as the sum of squares between successive iterations. The iterative Gauss-Newton least-squares method was used (Hartley, 1961) with a nonlinear regression procedure (NLIN) within the SAS 9.4 software (SAS, 2014). A maximum of 2000 iteration rounds were used for each analysis. Many studies have been carried out to find the best nonlinear function for the laying characterization of hen breeds. Among the most used models used for this purpose (Gómez-Cuello et al., 2017; Narinç et al., 2014; Otwinowska-Mindur et al., 2016; Savegnago et al., 2012), seven nonlinear regression models were evaluated in the present experiment: compartmental, Gamma, linear hyperbolic, logistic curvilinear, McNally, Narushin-Takma and quadratic logarithmic:

- Compartmental (McMillan, 1981):

$$y_t = a(e^{-bt} - e^{-ct}),$$

  where $y_t$ represents the egg production rate at $t$ weeks of laying; $e$ is the random error; a is the maximum potential of egg production; $b$ is the rate of increase to the peak and $c$ is the rate of decrease after the peak. In this function, it is assumed that production of hens begins at maximum laying rate.

- Gamma (Wood, 1967):

$$y_t = at^b e^{(-ct)},$$

  where $y_t$ represents the egg production rate at $t$ weeks of laying; $e$ is the random error; $a$ is the initial production; $b$ is the rate of increase to the peak and $c$ is the rate of decrease after the peak.

- Linear hyperbolic (Gómez-Cuello et al., 2017):

$$y_t = a - b\frac{c}{t},$$

where $y_t$ represents the egg production rate at $t$ weeks of laying; $e$ is the random error; $a$ is the initial production; $b$ is the rate of increase to the peak and $c$ is the rate of decrease after the peak.

- Logistic Curvilinear (Cason et al., 1988):

$$y_t = a(e^{-bt})\left[\frac{1}{1+e^{c+dt}}\right],$$

where $y_t$ represents the egg production rate at $t$ weeks of laying; $e$ is the random error and $a$, $b$, $c$ and $d$ represent constants to be determined by a least square error nonlinear curvilinear program.

- McNally (McNally, 1971):

$$y_t = at^b e^{(-ct+dt^{0.5})},$$

where $y_t$ represents the egg production rate at $t$ weeks of laying; $e$ is the random error; $a$ is the initial production; $b$ is the rate of increase to the peak, $c$ is the rate of decrease after the peak and $d$, that is proportional to the square root of time.

- Narushin-Takma (Narushin and Takma, 2003):

$$y_t = \frac{at^3 + bt^2 + ct + d}{t^3 + et^2 + ft + g},$$

where $y_t$ represents the egg production rate at $t$ weeks of laying; $e$ is the random error and $a$, $b$, $c$, $d$, $f$ and $g$ are parameters no biologically interpretable.

- Quadratic logarithmic (Malhotra et al., 1980):

  - $y_t = a + b + ct^2 + d\ lnt,$

where $y_t$ represents the egg production rate at $t$ weeks of laying; $e$ is the random error and $a$, $b$, $c$ and $d$ are parameters no biologically interpretable.

Graphical residual analysis was performed to test for the heteroscedastidity assumption. Heteroscedastidity assumption in residuals was detected, hence the nonlinear regression approach was justified. From the various models used and

fitted for each Utrerana hen variety, the following goodness-of-fit-criteria were used:

- The lowest mean square of the error (MSE) of the analyzed model, as a measure which includes the variability of factors not considered by the investigator:

$$MSE = \frac{SSResidual}{N - K},$$

  where N represents the numbers of observations; SSResidual the sum of squares of the residuals and K is the number of parameters.
- The lowest value of the Akaike information criteria (AIC): this criterion considers the changes in the fitness quality between models:

$$AIC = N\ ln\left(\frac{SSResidual}{N}\right) + 2K,$$

  where N represents the numbers of observations; SSResidual the sum of squares of the residuals and K is the number of parameters.
- The lowest value of Corrected Akaike information criteria (AICc), as a more precise tool when a large N is not present or when models with a large number of parameters are used:

$$AICc = AIC + 2K\frac{(K+1)}{N(N+1)},$$

  where N represents the numbers of observations and K is the number of parameters.
- The lowest value of Bayesian information criterion (BIC), which is a model-order selection criterion and penalizes models with a large number of parameters:

$$BIC = N * N\ln\left(\frac{SSResidual}{N}\right) + K * \ln(N),$$

  where N represents the numbers of observations; SSResidual the sum of squares of the residuals and K is the number of parameters.
- The highest level of the coefficient of determination ($R^2$) that is a measure to determine the proportion of the variance explained by the model:

$$R^2 = 1 - \frac{SSResidual}{SSTotal}$$

### *2.4. Comparison of fitting models*

The nonlinear fitting models used in the present research were ranked considering the goodness-of-fit and flexibility criteria of MSE, AIC, AICc, BIC and $R^2$ individually. The first position in the rank was awarded to the model with the most desirable values for each particular criterion. So, the rest of position in the rank were determined in ascending order from the most desirable values to the lowest desirable ones.

For this, a combined selection index (ICO) was developed following the premises in Van Vleck (1993) in order to rank the models. It allowed summarize the position in the rank for each of the goodness-of-it criteria estimated for each model. The formula used to calculate the ICO was as follow:

$$ICO = \frac{MSE\ (RP) * \mathrm{W1} + AIC\ (RP) * \mathrm{W2} + \mathrm{AICc}\ (RP) * W3 + BIC\ (RP) * \mathrm{W4} + R^2\ (RP) * W5}{5},$$

where RP is the rank position within the ICO, W1 is the weight for MSE rank position, W2 for AIC rank position, W3 for AICc rank position, W4 for BIC rank position and W5 for $R^2$ rank position.

All criteria were given the same relevance in the ICO, so the proportion 1 : 1 : 1 : 1 : 1 was followed and no coefficient was used. Therefore, the models with greater ICO values showed the best-fitting and predictive properties for each variety of Utrerana avian breed (Supplementary Tables S1, S2, S3 and S4).

## 3. Results

Supplementary Tables S1, S2, S3 and S4 show the fitness and accuracy statistics for the different models tested in the present study across the four Utrerana varieties. A summary of goodness-of-fit and flexibility criteria of the best model for each variety are shown in Table 2. The partridge variety reported the highest fit values for most models, like Gama, McNally, Narushin-Takma and Quadratic Logarithmic. However, the white variety was the variety that presented worst fitted criteria (Narushin-Takma model: MSE=2.202; AIC=1154.69; AICc=1238.75; BIC=1186.39 and $R^2$=0.828). Except for the partridge variety, Narushin-Takma model fitted the egg laying curve better than the rest of studied models, based on the 5 goodness-of-fit and flexibility criteria (MSE, AIC, AICc, BIC and $R^2$). In the

partridge variety, Quadratic Logarithmic model was the most suitable to describe the annual egg production (MSE=2.041; AIC=964.28; AICc=1004.31; BIC=985.10 and $R^2$=0.917).

**Table 2.** Estimated goodness-of-fit and flexibility criteria for the best-fitting model in each Utrerana variety.

| Variety | Model | MSE | AIC | AICc | BIC | $R^2$ | Iterations |
|---|---|---|---|---|---|---|---|
| White | N-T | 2.202 | 1154.69 | 1238.75 | 1186.39 | 0.828 | 1 |
| Franciscan | N-T | 1.904 | 1083.66 | 1167.72 | 1116.20 | 0.888 | 1 |
| Black | N-T | 2.011 | 1109.36 | 1193.41 | 1141.55 | 0.899 | 1 |
| Partridge | Q-L | 2.041 | 964.28 | 1004.31 | 985.10 | 0.917 | 1 |

MSE: Median Square Error; AIC: Akaike Information criterion; AICc: Corrected Akaike Information criterion; BIC: Bayesian Information criterion; $R^2$: coefficient of determination; N-T: Narushin-Takma model; Q-L: Quadratic logarithmic model.

The estimated parameters (*a*, *b*, *c*, *d*, *f* and *g*) for each variety and model are showed in Supplementary Tables S5, S6, S7 and S8. Table 3 reports a summary of the estimated parameters for each Utrerana variety using the best fitting model of the laying curve.

**Table 3.** Estimated parameters criteria for the best-fitting model in each Utrerana variety.

| Variety | Model | a (s.e.) | b (s.e.) | c (s.e.) | d (s.e.) | f (s.e.) | g (s.e.) |
|---|---|---|---|---|---|---|---|
| White | N-T | 0.0033± 0.0003 | 107.100± 0.031 | 1.909± 0.788 | -2.597± 9.571 | 3.470± 0.001 | 18.902± 5.517 |
| Franciscan | N-T | 0.0032± 0.0003 | 107.100± 0.031 | 2.630± 0.786 | -17.210± 9.547 | 3.470± 0.001 | 21.325± 5.503 |
| Black | N-T | 0.0033± 0.0003 | 107.100± 0.031 | 0.503± 0.789 | -25.092± 9.530 | 3.470± 0.000 | 42.909± 5.493 |
| Partridge | Q-L | 9.820± 5.260 | -10.319± 0.640 | 0.105± 0.008 | 63.439± 4.688 | - | - |

a, b, c, d, f, g: parameters; N-T: Narushin-Takma model; Q-L: Quadratic logarithmic model.

Table 4 shows the values of the total number of eggs produced by each variety (N=15) during the entire research period (n), the peak yield (production and week) and the persistency of the laying curve for the best fitting model in each of the four varieties. The Quadratic Logarithmic model predicted a higher and earlier peak of production and a lower persistency that Narushin-Takma model in the rest of Utrerana varieties, as can be seen in Figure 1, where a comparison of laying performance of the different varieties is graphically represented. The partridge variety (94.09 eggs/hen/year) proved to be the least productive variety in relation

to the rest of the varieties (white: 109.20 eggs/hen/year; franciscan: 121.36 eggs/hen/year; black: 106.84 eggs/hen/year).

**Table 4.** Number of observations, egg production at peak yield, week of peak yield and persistence estimates for each variety curve laying.

| Variety | Model | n | Peak yield (eggs) | Peak yield (week) | Persistency (days) |
|---|---|---|---|---|---|
| White | N-T | 1455 | 49.52 | 13 | 69.43 |
| Franciscan | N-T | 1674 | 59.00 | 14 | 104.52 |
| Black | N-T | 1579 | 60.24 | 9 | 74.25 |
| Partridge | Q-L | 1346 | 66.19 | 7 | - |

n: number of observations on the study period; N-T: Narushin-Takma model; Q-L: Quadratic logarithmic model.

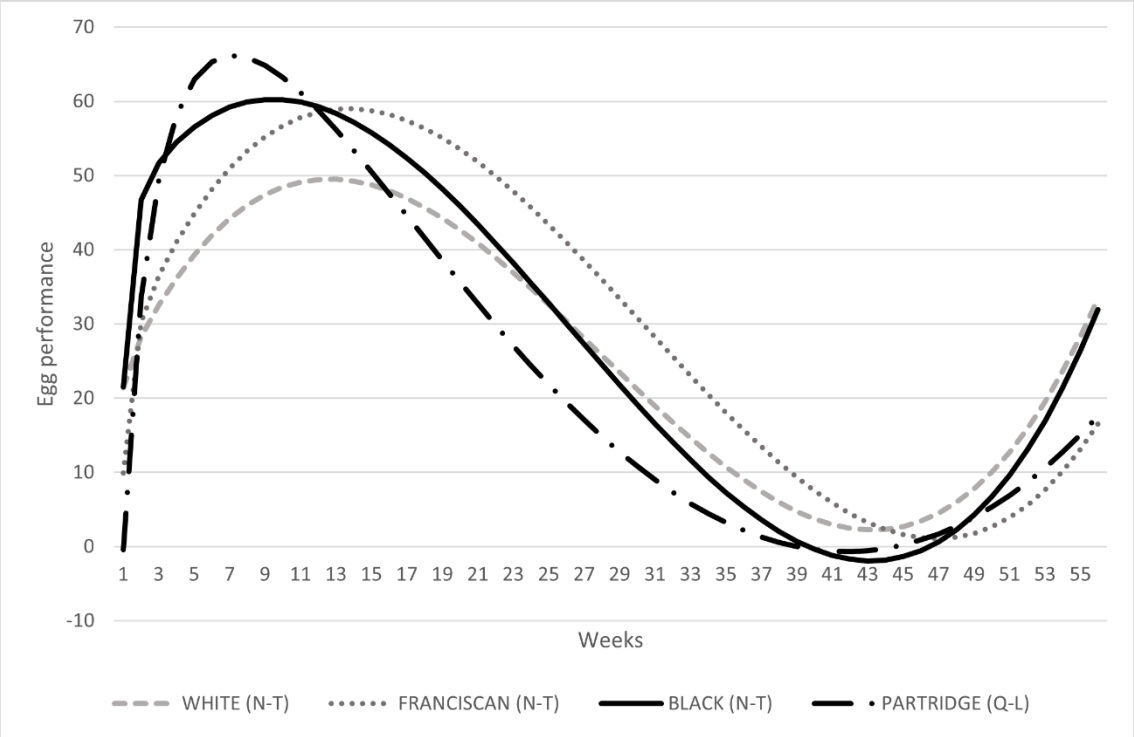


**Figure 1.** Comparison of biological laying curves for different varieties of the Utrerana avian breed, with egg performance predicted by the best-fitted model in each variety (N-T: Narushin-Takma, Q-L: quadratic logarithmic.

## 4. Discussion

Laying parameters allows to predict the annual egg production and economic projections for large flocks (Yang et al., 1989). However, in endangered breeds, with a lower number of available individuals and observations, the studied models show differences in the goodness-of-fit and flexibility criteria values among them (Miyoshi et al., 1996). Some mathematical models normally used in laying hens (compartmental, linear hyperbolic, logistic curvilinear or McNally models) (Cason, 1990; Gavora et al., 1971; McNally, 1971) were not suitable for Utrerana hen, due to

the differences between the model parameters and the real Utrerana varieties egg laying curve, the lack of predictive capacity and the variations in the maximum egg production rate between high productive hybrid strains and a autochthonous hen breed. It is assumed that a flock of commercial hybrid strains of laying hens present a maximum laying rate of 80% (Yang et al., 1989) or up to 100% (Adams and Bell, 1980; McMillan, 1981), according to the genetic and productive improvements used in each investigation. In the present research, the peak yield was around 60-70%, depending on the variety. Utrerana avian breed suffered a decrease in production rates due to the fact that in recent years its conservation has been linked to ornamental poultry farming, based on the morphological selection of breeding animals (Campo, 2007; González Ariza et al., 2019b). Genetic improvement in egg performance is necessary to guide Utrerana breed towards productive systems, since it has been shown that the productive indices are lower than those of other Spanish native breeds, whether they are Atlantic type (Sobrarbe hen and Galiña de Mos breeds) or Mediterranean type (Castellana Negra hen and Menorca hen breeds). Annual egg production of Utrerana avian breed can only be compared with that of the Menorca breed (120 eggs/hen/year) (Cajal and Francesch, 2014; Miguel et al., 2007; Rivera et al., 2009; Villalba et al., 2007). Nevertheless, Utrerana egg presents different characteristics, both in their internal and external physical quality, as well as in their chemical and sensory characteristics (González Ariza et al., 2019a; González Ariza et al., 2019c; González Ariza et al., 2020), which can give the breed an advantage over other breeds or strains and may cover the currently increasing demand from markets for non-conventional quality products linked to specific breeds adapted to more sustainable farming practices (Lordelo et al., 2016).

In previous research, it has been indicated that poultry egg performance is affected by a series of intrinsic factors, like genotype and age of the hens (Tůmová et al., 2017). However, several extrinsic factors also influence the productive parameters in poultry farms, including: environmental factors, like temperature (Dorn et al., 2014) and photoperiod (Guo et al., 2019), and nutrition intake (Ding et al., 2016). In the present study, all extrinsic conditions and the age of the different flocks (according to variety) were the same, so the only factor that could affect the laying rate of hens was the genotype.

Narushin-Takma model gave the most accurate results in prediction of the most of varieties (white, franciscan and black) laying curve, based on MSE, AIC, AICc, BIC and $R^2$. These findings are in accordance with those of other authors (Faridi et al., 2011; Rahimzadeh et al., 2017), who found the Narushin-Takma model to be the best model to fit egg performance in broiler breeding and in Japanese quail flocks, both with lower annual productive yields than the commercial laying hen strains. Narushin-Takma model has demonstrated to be a flexible nonlinear equation to predict the poultry production in any type of production system (Faridi et al., 2011). Increasing the coefficients does not greatly complicate the calculations, since the function can be simplified by implementing iteration procedures with specialized statistical programs (Narushin and Takma, 2003).

On the other hand, quadratic logarithmic model was the best-fitting model for describing the egg performance in partridge variety. The majority of the works utilizing the quadratic logarithmic model are in estimation of lactation curve in ruminants (Biswal et al., 2017; Gupta et al., 2016; Malhotra et al., 1980; Pizarro Inostroza et al., 2020), however, few references in bibliography can be found on the application of this model in poultry research. In recent studies (Gómez-Cuello et al., 2017), this model has been used in the estimation of the laying curve in a commercial strain of laying hen, with acceptable values of $R^2$ (0.953). In contrast, these authors obtained less flexibility of the model when compared it with McNally and linear hyperbolic models.

Although Gamma model was not the best model to fit the biological curve of egg production of any of the for varieties, according to the goodness of fit criteria, it obtained acceptable fitness values (values of $R^2$ of 0.668, 0.870, 0.811 and 0.891 for white, franciscan, black and partridge varieties, respectively; Tables S1, S2, S3 and S4). These results agree with those obtained in previous research (Atta et al., 2010; Miyoshi et al., 1996; Narinç et al., 2014), since the laying curve was successfully fitted by using Gamma model.

Biological coherence of the estimated parameters and graphical evaluation of curve fitting are indicators of the flexibility of the biological curves fitting (Lupi et al., 2015; Nogales et al., 2017; Savegnago et al., 2012). In Figure 2, it can be observed the fitted curves for weekly egg production rate in the four varieties. The graphical analysis

showed that the best-fitting model in each Utrerana variety present enough flexibility to property fit the egg performance during all the yearly laying cycle.

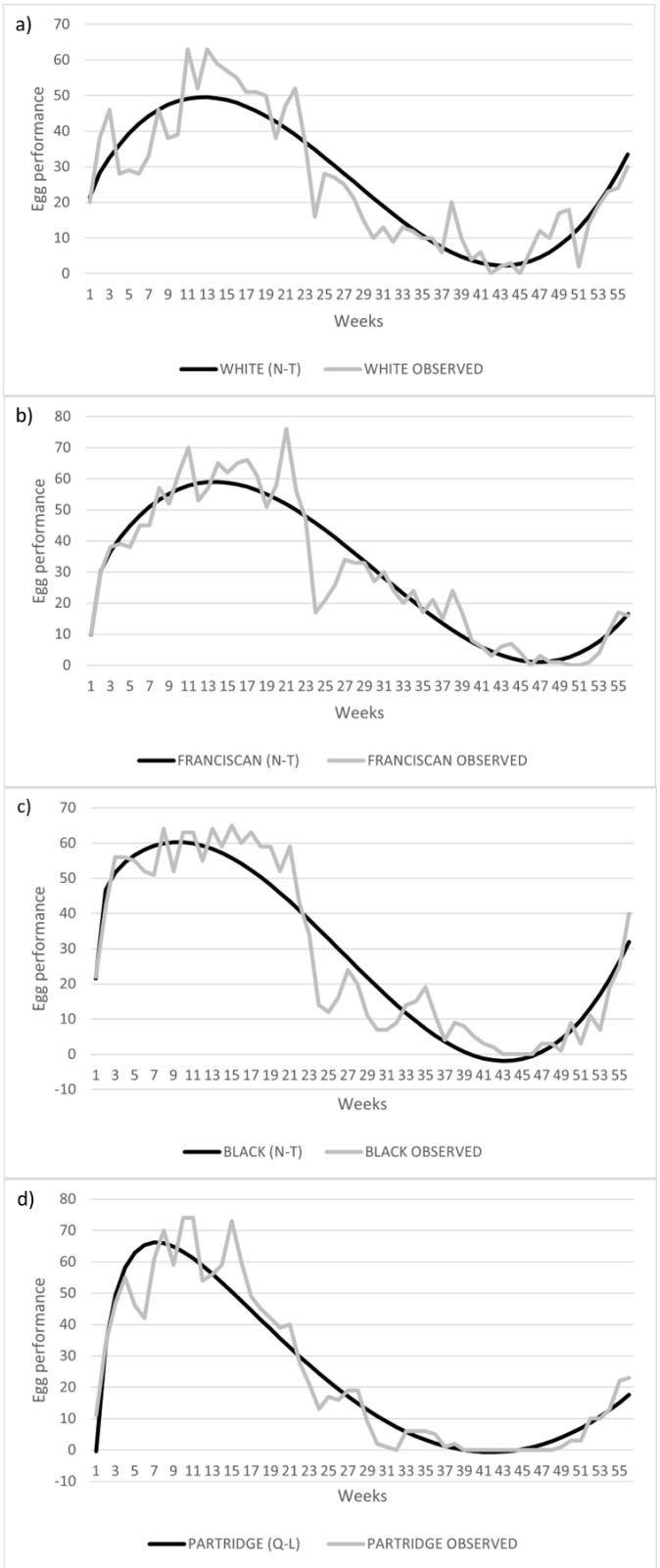


**Figure 2.** Fitted curves for weekly egg performance rate for white (a), franciscan (b), black (c) and partridge (d) varieties using the best-fitted model in each variety (N-T: Narushin-Takma, Q-L: quadratic logarithmic).

When the predicted laying curve are represented in Figure 1, it can be observed that the peak yield is higher in partridge variety than in the rest of varieties, while the laying decrease after the peak is faster in partridge variety too, causing a sharp decrease in production rates compared to the rest of the varieties (Table 4). Thus, these results suggest that the partridge variety present greater seasonality, influenced by its genotype-season interaction, in which temperature and photoperiod greatly influence the egg production (Jesuyon and Oseni, 2015; Xu et al., 2011). It has been shown that the partridge variety involves a genetic subdivision, far from the rest of varieties, within the breed (Macrì et al., 2019). Although heat stress reduces significantly the egg production of laying hens (Kilic and Simsek, 2013), the Utrerana egg performance was acceptable until the end of the summer (corresponding to week 33 of study). Córdoba, the place where the research was developed, is influenced by Mediterranean weather: maximum temperatures above 40 °C were reached during summer 2019, as reported by the State Meteorological Agency (AEMET) from Spain. The laying results obtained in the present research suggest that the Utrerana breed tolerates high-temperatures induced stress, so the use of this breed could be an interesting alternative to production systems in which animals adapted to the environment are necessary, such as extensive or free-range systems. These results agree with previous studies on the quality of Utrerana egg, since it was observed that heat stress did not significantly affect the external and internal qualities of the egg (González Ariza et al., 2019c).

The models used in the present research presented parameter estimates with biological interpretations of importance for research and its transfer to the livestock sector, like peak egg yield, persistence of egg laying after the peak and the week in which egg production reached the peak (Table 4). From these parameters and the knowledge of the laying curve of each bird genotype, the selection of hens for egg production can be efficient in the first month of laying and allows to improve the peak egg production and persistence of egg curve in the flock (Savegnago et al., 2012).

## 5. Conclusions

Some nonlinear functions are suitable to describe the biological laying curve of the Utrerana avian breed, an endangered breed with a low number of individuals and observations, with Narushin-Takma and quadratic logarithmic models standing out as the best fitting models in the different Utrerana varieties, in accordance to the goodness-of-fit and flexibility criteria used in the present research (MSE, AIC, AICc, BIC and $R^2$). Utrerana egg production rates are low, however, the flocks showed acceptable rates during the hottest months of the year. An effort to improve the productive indices of the flocks, which together with the differentiated characteristics of organoleptic, physical and chemical quality that Utrerana egg has shown, allows obtaining greater profitability of the laying hen farms. The characterization of laying performance of this breed can be useful for making zootechnical decisions like the nutritional requirements and control of the health status of the batch and can be an important tool to support the breeding program.

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**Ethical approval:** The study follows the national guidelines and premises described in the Declaration of Helsinki. Protocols applicated were permitted by the regulations of the European Union (2010/63/EU) in their transposition to the Royal Decree-Law 53/2013 and its credited entity the Ethics Committee of Animal Experimentation from the University of Córdoba.

**Declaration of interest statement:** The authors declare no conflict of interest.

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**Supplementary Table S1.** Estimated goodness-of-fit, flexibility criteria, and rank for each model in the study of the laying curve of white variety of Utrerana avian breed.

| Model | MSE | AIC | AICc | BIC | $R^2$ | ICO Rank | Iterations |
|---|---|---|---|---|---|---|---|
| Compartmental | 7.072 | 2849.15 | 2873.17 | 2865.00 | 0.446 | 3.00 | 36 |
| Gamma | 4.237 | 2103.90 | 2127.92 | 2119.75 | 0.668 | 5.00 | 17 |
| Linear Hyperbolic | 12.411 | 3667.48 | 3691.50 | 3683.33 | 0.028 | 1.00 | 1 |
| Logistic Curvilinear | 7.203 | 2876.98 | 2917.01 | 2898.16 | 0.436 | 2.00 | 7 |
| McNally | 6.199 | 2658.48 | 2698.51 | 2679.62 | 0.515 | 4.00 | 2 |
| Narushin-Takma | 2.202 | 1154.69 | 1238.75 | 1186.39 | 0.828 | 7.00 | 1 |
| Quadratic Logarithmic | 3.972 | 2010.77 | 2050.80 | 2031.91 | 0.689 | 6.00 | 1 |

MSE: median square error; AIC: Akaike information criterion; AICc: corrected Akaike information criterion; BIC: Bayesian information criterion; $R^2$: coefficient of determination; ICO: combined selection index.

**Supplementary Table S2.** Estimated goodness-of-fit, flexibility criteria, and rank for each model in the study of the laying curve of franciscan variety of Utrerana avian breed.

| Model | MSE | AIC | AICc | BIC | $R^2$ | ICO Rank | Iterations |
|---|---|---|---|---|---|---|---|
| Compartmental | 7.245 | 3318.10 | 3342.11 | 3334.37 | 0.572 | 3.00 | 19 |
| Gamma | 2.207 | 1327.86 | 1351.87 | 1344.12 | 0.870 | 6.00 | 17 |
| Linear Hyperbolic | 16.832 | 4729.14 | 4753.15 | 4745.41 | 0.005 | 1.00 | 1 |
| Logistic Curvilinear | 8.922 | 3667.59 | 3707.61 | 3689.28 | 0.473 | 2.00 | 7 |
| McNally | 3.907 | 2285.33 | 2325.35 | 2307.02 | 0.769 | 4.00 | 2 |
| Narushin Takma | 1.904 | 1083.66 | 1167.72 | 1116.20 | 0.888 | 7.00 | 1 |
| Quadratic Logarithmic | 2.781 | 1716.12 | 1756.14 | 1737.81 | 0.836 | 5.00 | 1 |

MSE: median square error; AIC: Akaike information criterion; AICc: corrected Akaike information criterion; BIC: Bayesian information criterion; $R^2$: coefficient of determination; ICO: combined selection index.

**Supplementary Table S3.** Estimated goodness-of-fit, flexibility criteria and rank for each model in the study of the laying curve of black variety of Utrerana avian breed.

| Model | MSE | AIC | AICc | BIC | $R^2$ | ICO Rank | Iterations |
|---|---|---|---|---|---|---|---|
| Compartmental | 7.948 | 3276.16 | 3300.17 | 3292.25 | 0.602 | 3.00 | 122 |
| Gamma | 3.768 | 2097.45 | 2121.46 | 2113.54 | 0.811 | 5.00 | 11 |
| Linear Hyperbolic | 18.771 | 4633.13 | 4657.14 | 4649.22 | 0.059 | 1.00 | 1 |
| Logistic Curvilinear | 8.210 | 3328.41 | 3368.44 | 3349.87 | 0.589 | 2.00 | 9 |
| McNally | 6.539 | 2969.03 | 3009.06 | 2990.49 | 0.673 | 4.00 | 2 |
| Narushin Takma | 2.011 | 1109.36 | 1193.41 | 1141.55 | 0.899 | 7.00 | 1 |
| Quadratic Logarithmic | 2.845 | 1654.99 | 1695.01 | 1676.45 | 0.858 | 6.00 | 1 |

MSE: median square error; AIC: Akaike information criterion; AICc: corrected Akaike information criterion; BIC: Bayesian information criterion; $R^2$: coefficient of determination; ICO: combined selection index.

**Supplementary Table S4.** Estimated goodness-of-fit, flexibility criteria and rank for each model in the study of the laying curve of partridge variety of Utrerana avian breed.

| Model | MSE | AIC | AICc | BIC | $R^2$ | ICO Rank | Iterations |
|---|---|---|---|---|---|---|---|
| Compartmental | 10.188 | 3127.33 | 3151.34 | 3142.94 | 0.587 | 3.00 | 76 |
| Gamma | 2.680 | 1330.07 | 1354.09 | 1345.69 | 0.891 | 5.00 | 13 |
| Linear Hyperbolic | 23.625 | 4259.45 | 4283.47 | 4275.06 | 0.043 | 1.00 | 1 |
| Logistic Curvilinear | 10.641 | 3186.87 | 3226.90 | 3207.69 | 0.569 | 2.00 | 13 |
| McNally | 7.757 | 2761.36 | 2801.39 | 2782.18 | 0.686 | 4.00 | 2 |
| Narushin Takma | 2.454 | 1214.19 | 1298.25 | 1245.41 | 0.901 | 6.00 | 1 |
| Quadratic Logarithmic | 2.041 | 964.28 | 1004.31 | 985.10 | 0.917 | 7.00 | 1 |

MSE: median square error; AIC: Akaike information criterion; AICc: corrected Akaike information criterion; BIC: Bayesian information criterion; $R^2$: coefficient of determination; ICO: combined selection index.

**Supplementary Table S5.** Estimated parameters for each model in the study of the laying curve of white variety of Utrerana avian breed.

| Model | a | b | c | d | f | g |
|---|---|---|---|---|---|---|
| Compartmental | 51.3880±5.9970 | 0.0221±0.0083 | -0.0339±0.0270 | - | - | - |
| Gamma | 11.7140± 4.0300 | 1.0790±0.2090 | -0.1007±0.0140 | - | - | - |
| Linear Hyperbolic | 24.2963±2.7880 | 3.0781±2.4591 | -6.6510±0.000 | - | - | - |
| Logistic Curvilinear | 51.7530±5.3110 | 0.0270±0.0049 | 1.3271E14±0.0000 | -3.0800E13±0.0000 | - | - |
| McNally | 66.1920±30.9010 | -0.0001±0.0001 | 1.3328E9±1.4892E9 | -25949.4000±72496.4000 | - | - |
| Narushin-Takma | 0.0033±0.0003 | 107.1000±0.0308 | 1.9090±0.7882 | -2.597±9.5710 | 3.4700±0.0009 | 18.9021±5.5167 |
| Quadratic Logarithmic | 11.9364±7.6305 | -6.3283±0.9284 | 0.0610±0.0109 | 41.8725±6.8001 | - | - |

**Supplementary Table S6.** Estimated parameters for each model in the study of the laying curve of franciscan variety of Utrerana avian breed.

| Model | a | b | c | d | f | g |
|---|---|---|---|---|---|---|
| Compartmental | 56.7315±5.8008 | 0.0139±0.0058 | -0.0617±0.0066 | - | - | - |
| Gamma | 8.4076 ±2.2397 | 1.3806 ±0.1582 | -0.1170±0.0106 | - | - | - |
| Linear Hyperbolic | 28.9909±3.4831 | 1.6469±3.0722 | -6.6510±0.000 | - | - | - |
| Logistic Curvilinear | 61.4653±6.3862 | 0.0277±0.0050 | 3.1830E14±0.000 | -1.3600E14±0.0000 | - | - |
| McNally | 66.1917±30.9012 | -0.0001±0.0001 | 1.3328E9±1.4892E9 | -25949.4000±72496.4000 | - | - |
| Narushin Takma | 0.0032±0.0003 | 107.1000±0.0308 | 2.6300±0.7863 | -17.2100±9.5472 | 3.4700±0.0009 | 21.3249±5.5030 |
| Quadratic Logarithmic | 3.3715±6.8497 | -6.1639±0.8334 | 0.0462±0.0098 | 49.5931±6.1043 | - | - |

**Supplementary Table S7.** Estimated parameters for each model in the study of the laying curve of black variety of Utrerana avian breed.

| Model | a | b | c | d | f | g |
|---|---|---|---|---|---|---|
| Compartmental | 69.3963±7.0317 | 0.0317±0.0076 | -0.0371±0.0203 | - | - | - |
| Gamma | 21.4524±5.0735 | 0.9773±0.1612 | -0.1138±0.0136 | - | - | - |
| Linear Hyperbolic | 25.0085±3.5722 | 5.8206±3.1508 | -6.651±0.000 | - | - | - |
| Logistic Curvilinear | 70.3801±6.5902 | 0.0373±0.0054 | 1.6940E13±0.0000 | -1.4100E13±0.0000 | - | - |
| McNally | 25.8948±3.0658 | -0.00037±0.0001 | 4.2228E8±2.9864E8 | 26160.4000±5636.8000 | - | - |
| Narushin-Takma | 0.0033±0.0003 | 107.1000±0.0307 | 0.5031±0.7894 | -25.0915±9.5299 | 3.4700±0.0000 | 42.9094±5.4931 |
| Quadratic Logarithmic | 19.7281±6.7284 | -8.9957±0.8187 | 0.0895±0.0096 | 54.4411±5.9961 | - | - |

**Supplementary Table S8.** Estimated parameters for each model in the study of the laying curve of partridge variety of Utrerana avian breed.

| Model | a | b | c | d | f | g |
|---|---|---|---|---|---|---|
| Compartmental | 66.7773±7.6043 | 0.0359±0.0087 | -0.0392±0.0175 | - | - | - |
| Gamma | 11.2570±2.8774 | 1.5201±0.1820 | -0.1704±0.0164 | - | - | - |
| Linear Hyperbolic | 21.2537±3.6995 | 5.0794±3.2630 | -6.651±0.000 | - | - | - |
| Logistic Curvilinear | 67.8758±7.3154 | 0.0427±0.0068 | 2.2250E14±0.000 | -3.8600E13±0.000 | - | - |
| McNally | 6.5644±33.2428 | -0.0005±0.0001 | 2.7991E8±2.0175E8 | 29122.0000±11975.6000 | - | - |
| Narushin Takma | 0.0030±0.0003 | 107.2000±0.0313 | -0.3145±0.8001 | -39.6877±9.7153 | 3.4700±0.000 | 45.9589±5.5999 |
| Quadratic Logarithmic | 9.8198±5.2604 | -10.3193±0.6401 | 0.1053±0.0075 | 63.4394±4.6879 | - | - |

# Chapter 3.

## Analysis of the Utrerana egg quality-related characteristics of Utrerana Avian Breed.

- *González Ariza A., F. J. Navas González, A. Arando Arbulu, J. M. León Jurado, C. J. Barba Capote, and M. E. Camacho Vallejo.* ***Non-Parametrical Canonical Analysis of Quality-Related Characteristics of Eggs of Different Varieties of Native Hens Compared to Laying Lineage.*** *Animals 2019, 9, 153.*
- *González Ariza A., A. Arando, F. J. Navas González, J. M. León, J. V. Delgado, and M. E. Camacho.* ***Egg Quality-related Data Mining based Discriminant Analysis Tool for Native Hen Breed Productive Characterization.*** *Submitted to Poultry Science.*
- *González Ariza A., F. J. Navas González, A. Arando Arbulu, J. V. Delgado Bermejo, and M. E. Camacho Vallejo.* ***Hen breed and variety factors as a source of variability for the chemical composition of eggs.*** *Journal of Food Composition and Analysis 2021, 95, 103673.*

# Non-Parametrical Canonical Analysis of Quality-Related Characteristics of Eggs of Different Varieties of Native Hens Compared to Laying Lineage

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**Simple Summary:** The development of new more productive lines of laying hens has displaced native breeds to second place; therefore, new lines of research that ensure the conservation of local breeds and biodiversity are increasingly necessary. The aim of the present study is to characterize the productive capability of Utrerana and to compare the relationships among parameters determining the internal and external quality of the egg, through canonical correlation analysis. We used a flock of 68 Utrerana hens with animals of each of its four varieties (white, black, Franciscan and partridge), and a group of 17 Leghorn hens as a control group. The breed and variety significantly affected egg characteristics. The external and internal quality parameters of the egg were evaluated and reported results providing consistent data for the characterization of the products from this breed. This productive characterization could benefit the conservation of the Utrerana breed, the establishment of livestock models that adapt to it and the search for a market in which this product could be used.

**Abstract:** The aim of the present study is to characterize the productive capability of Utrerana and to compare the relationships among parameters determining the internal and external quality of the egg, through canonical correlation analysis. A

flock of 68 Utrerana hens and a control group of Leghorn hens (n = 17) were housed individually to allow individual identification of eggs and for the assessment of egg quality characteristics. Almost all variables showed differences when both breeds were compared, except for white height, yolk diameter, yolk$^{L*}$ and yolk pH ($p > 0.05$). Only minor diameter, white height, yolk$^{L*}$, yolk$^{a*}$, and shell weight reported significant differences between laying age groups. White height, yolk color, and almost all yolk color coordinates were significantly different ($p < 0.05$) for period and month. Egg and white weight reached highest significantly different levels for the fourth and fifth time that the hens laid an egg. External quality-related traits are better predictors of internal quality-related traits than vice versa, enabling the implementation of an effective noninvasive method for internal quality determination and egg classification aimed at suiting the needs of consumers.

**Keywords**: Egg quality; color coordinate decomposition; internal quality traits; external quality traits; DSM color fan

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## 1. Introduction

Along with other food such as milk, eggs represent a great contribution of proteins of animal origin to the human diet (Dhama et al., 2014). In 2017, the world production of eggs exceeded 1416 trillion tons of eggs, equivalent to 80 million metric tons, 30% higher than production in 2000 (FAOSTAT, 2019). In the European Union, the production of food in alternative production systems is on the rise; in 2017, free range and organic egg production accounted for 20% of the total production of eggs (Agri, 2019).

Currently, almost all of the consumed eggs are produced by commercial hybrid lines, which are characterized by high productive performance and a good feed conversion index (Mottet and Tempio, 2017; Tallentire et al., 2018). However, the exploitation of these highly productive lines causes a decrease in the genetic variability of the species and has negative effects on the development of sustainable practices based on local breeds (Hoffmann, 2011).

The emergence of new commercial lines of laying hens with a much greater productive capacity throughout the twentieth century caused the displacement of

the autochthonous breed. In many cases, their hybridization with more productive lines relegated autochthonous breeds, including the Utrerana avian breed, to a form of ornamental poultry farming, based on the morphological selection of breeding animals. As a result, a reduction in the census of animals of this breed occurred in addition to a decrease in the productive indices (Garcia and Cordero, 2006).

The Utrerana hen breed was created in the first half of the 20th century, starting with the selection of a heterogeneous population of chickens from the Andalusian countryside (Orozco, 1989). Its initial productive orientation was to be a laying hen, with an annual output of 120-180 eggs, white in color and with an average weight of 62-64 g. It has four different varieties, characterized by the color of the plumage and the legs: white, Franciscan, black, and partridge (Campo, 2007).

The need for the characterization of the products of the Utrerana hen breed is largely due to the situation that it faces. This breed is classified as an endangered breed, according to the Royal Decree Law 2129/26 December, 2008, which establishes the national program of conservation, breeding, and promotion of livestock breeds, and presented a census of 1309 animals on 31 December, 2018 (MAPA, 2019). Therefore, facing this alarming situation, the implementation of programs for the recovery, conservation, and productivity improvement of the breed, trying to provide it with an identity and again, a productive role able to satisfy the demands of the market is required. Thus, the assessment of local products may be a strategy for the conservation of local breeds, for instance, avoiding the loss of linkage between local products and their area of production, as is the case of industrial products (Vaarst et al., 2015).

Biodiversity must not only be considered as the genetic conservation of animal resources but also the search economic sustainability and the maintenance of the hen population in rural areas (Alderson, 2018). The increasing concern of the society about animal welfare has allowed the development of alternative forms of livestock, including extensive local farms (Verbeke, 2009). The Utrerana hen breed, as a local breed, is perfectly suited to this operating system, since it presents great rusticity and resistance to extreme weather situations, with great ability to search for food in the wild (Lordelo et al., 2016).

In general, the quality of the egg is related to characteristics that affect the acceptability of eggs by the consumer (Stadelman, 1977 ). Among the considerable number of characteristics of egg quality that can be measured, external factors such as egg weight are the most important (Song et al., 2000; Adeogun and Amole, 2004; Dudusola, 2010; Hanusova et al., 2015). The internal egg quality is also an important aspect to consider, especially when approaching the marketing opportunities of the product. A dense albumen height is among the most important determinants of the internal quality (Scott and G Silversides, 2001; Monira et al., 2003). In addition to these factors, other parameters such as the major and minor diameters of the egg, eggshell, yolk color and the weight and pH of the white and yolk allows a more complete characterization of the quality of the egg (Bain, 2007; Islam et al., 2017; Sirri et al., 2018b). It has been shown that breed genotype can significantly affect most of these features: egg shape, yolk and albumen quality, shell and egg weight and amount of yolk (Zita et al., 2009).

The first objective of this study is to characterize the productive capacity of the four varieties of Utrerana hens compared to a globally distributed laying lineage, as a means of demonstrating the benefits of greater genetic diversity on the quality of products derived from sustainable native breeds. In addition, we quantified the explanatory power of the variance by factors such as the laying month, laying order, period, laying age, variety, and breed found in two sets of parameters of external and internal egg quality. Secondly, we compared the relationships among determining parameters of the internal and external quality of the egg of endangered native hens through a canonical correlation analysis to develop a predictive tool that may enable indirect scoring of the internal quality of the egg from the set of external quality variables.

## 2. Materials and Methods

### *2.1. Animal Sample and Diets*

A total of 85 hens were used in the present study, distributed depending on their age and variety as shown in Table 1. The birds were housed in individual cages (50 × 62 × 41 cm) following Council Directive 1999/74/EC of 19 July, 1999, laying down minimum standards for the protection of laying hens at the Centro Agropecuario Provincial de Cordoba (Spain), for 6 months (January to June 2018). All the animals

were fed on the same commercial feed (15.2% crude protein, 4.1% calcium, 0.66% available phosphorus) for the whole experimental period. Feed and water were available ad libitum. All the birds were reared according to the regulations of the European Union (2010/63/EU) in their transposition to the Spanish law (RD 53/2013).

**Table 1.** Flock management information. All cages were chosen according to Council Directive 1999/74/EC of 19 July, 1999, laying down minimum standards for the protection of laying hens.

| Flock Management Parameter | Utrerana varieties | | | | Leghorn (Control) |
|---|---|---|---|---|---|
| | White | Franciscan | Black | Partridge | |
| Breeding hens | 17 | 17 | 17 | 17 | 17 |
| Hens (70 weeks old) | 12 | 12 | 12 | 12 | 12 |
| Pullets (28 weeks old) | 5 | 5 | 5 | 5 | 5 |
| Stocking density | 4 animals per each $m^2$ | | | | |
| Nest box density | 29 animals per each $m^2$ | | | | |
| Waterer allotment/space | Circle waterers of 5 cm of diameter per animal | | | | |
| Feeder allotment/space | 41 cm per animal | | | | |
| Floor substrate | Wood shavings covering the floor at a depth of approximately 1 cm | | | | |
| Nest box substrate | Plastic sturf mats covering the floor at a depth of approximately 1 cm | | | | |

*2.2. Work Sample*

All statistical tests were carried out using an egg sample comprising 194 eggs laid from March to June 2018 by the animal sample described above. A total of 147 eggs had been laid by Utrerana hens, while 47 belonged to Leghorn laying hens. The same information registration protocol was followed for all the eggs comprising the sample except for yolk and white pH determination. Due to economic reasons, 97 eggs were chosen at random to perform yolk and white pH analysis.

*2.3. External and Internal Quality-Related Traits Set Description*

Two sets of variables were measured. The first set of variables comprised external quality-related traits, those characteristics that can be measured externally without the need to break the eggs. This first set comprised the variables of egg weight, length and breadth, shell color lightness and shell color coordinates (Shell L*, Shell a*, Shell b*, lightness, red/green and yellow/blue coordinates, respectively). In contrast, the second set of traits, considered internal quality-related variables, required the egg to be broken so as to be scored. This second set

comprises albumen height, yolk color, yolk lightness and color coordinate decomposition (YolkL*, Yolka*, Yolkb*), yolk diameter, shell weight, yolk weight, albumen weight, yolk pH and white pH.

*2.4. Information Registration*

Laying lasted for 120 days. All eggs were divided into three periods of 40 days with a mean number of 64.67 eggs per period. Periods ran from second half March to first half April, second half April to first half May, and second half May to first half June. Egg temperature at the time of egg quality assessment was 22 °C ± 1 °C. Individual collection of the eggs of each hen was carried out and all the required variables for the external characterization of the egg were studied daily during the 24 hours following oviposition. Every egg was weighed with a weighing scale (Cobos, CSB-600C, Barcelona, Spain). Major and minor diameters of the egg were measured following a Vernier scale (Electro DH M 60.205, Barcelona, Spain). The color of the shell was determined using a portable spectrophotometer (CM 700d, Konica Minolta Holdings Inc., Tokyo, Japan), and the results were expressed using the International Commission on Illumination (CIE) L*a*b* system color profile (CIE, 1976).

The traits measured to describe the internal quality of the eggs were as follows: weight of the egg, shell, egg yolk and egg white, white height, the diameter of the yolk, pH of the white and yolk and the color of the yolk. These measurements were taken every fifteen days in all the eggs that the flock of hens laid on the day of collection, evaluating a total of 194 eggs. Then, a sample of 97 eggs was tested at random for yolk and white pH.

To determine internal quality-related traits, the eggshell was broken and the egg contents were deposited on a glass surface. The diameter of the yolk was measured with a Vernier scale. The intensity of the yellow color of the yolk of the egg was measured with the portable spectrophotometer and with a DSM® fan (formerly Roche color fan). The pH of the yolk and white was measured using reactive strips. The height of the white was computed as the mean of three measurements obtained with a Haugh digital micrometer (Baxlo, Barcelona, Spain). Finally, eggshell, egg white, and the yolk were weighed separately using a precision balance.

### *2.5. Statistical Analysis*

All variables recorded were separated into two variable sets. The first set included external egg quality-related parameters, such as egg weight, major diameter, minor diameter, shell$^{L*}$, shell$^{a*}$, shell$^{b*}$ and white height, respectively. The second set was internal egg quality-related parameters, such as yolk color, yolk$^{L*}$, yolk$^{a*}$, yolk$^{b*}$, yolk diameter, shell weight, yolk weight, white weight, yolk pH, and white pH.

Levene's test for equality of error variance was run to test for homoskedasticity. Mauchly's W Test was run to test for sphericity. All assumptions except for Shapiro Wilk Francia's normality tests were carried out using SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016). Shapiro Wilk Francia's normality tests were carried out with the sfrancia routine of StataCorp Stata version 14.2. Skewness and Kurtosis statistics were tested to support the reports by Shapiro Wilk Francia's normality tests. As the factors (month of laying, laying order, controlled period, laying age, variety, and breed) and variables in the model had violated most of the common parametric assumptions, the decision to follow a non-parametric approach was made. The Mann-Whitney U test was used to compare differences between the two independent groups of the laying age (laying hens and laying pullets) and breed (Utrerana and Leghorn) variables (Supplementary Tables S1 and S2). Similarly, a Kruskal-Wallis H test was performed to study the potentially existing differences between-levels of the same factor when three or more groups existed within the same independent variable (the rest of independent variables) in Table 2 and Supplementary Table S3.

After conducting the Mann-Whitney U test, we assessed the relationship between the factors of laying age and breed and the internal and external quality-related variables tested. Simultaneously, we used the Kruskal-Wallis H test to assess the relationship with the same variables and those factors with three or more categories or groups (k). Then, we computed the strength of the effects of these factors using r and partial eta squared ($\eta p^2$) as quantification measures depending on whether Mann-Whitney U or Kruskal-Wallis H tests had been carried out beforehand (Table 2 and Supplementary Table S3). According to Fritz et al. (2012), r can be calculated

**Table 2.** Summary of the results for the Kruskal-Wallis H test and the determinative coefficient through r or partial eta squared ($\eta p^2$), for the fixed effects for internal and external egg quality traits from the model, excluding yolk and white pH in Utrerana hens (n = 194).

| Variable | Parameter | Egg weight | Major diameter | Minor diameter | Shell L* | Shell a* | Shell b* | White height | Yolk colour | Yolk L* | Yolk a* | Yolk b* | Yolk diameter | Shell weight | Yolk weight | White weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Month | $\chi^2$ | 5.156 | 6.423 | 1.139 | 15.831 | 1.415 | 10.753 | 8.854 | 52.954 | 67.718 | 82.856 | 89.812 | 1.056 | 7.675 | 3.071 | 4.411 |
| | dfn | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | p-value | 0.161 | 0.093 | 0.768 | 0.001 | 0.702 | 0.013 | 0.031 | 0.000 | 0.000 | 0.000 | 0.000 | 0.788 | 0.053 | 0.381 | 0.220 |
| | dfd | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 |
| | F | 1.719 | 2.141 | 0.380 | 5.277 | 0.472 | 3.584 | 2.951 | 17.651 | 22.573 | 27.619 | 29.937 | 0.352 | 2.558 | 1.024 | 1.470 |
| | $\eta p^2$ | 0.026 | 0.032 | 0.006 | 0.076 | 0.007 | 0.053 | 0.044 | 0.216 | 0.261 | 0.301 | 0.319 | 0.005 | 0.038 | 0.016 | 0.022 |
| Order | $\chi^2$ | 13.672 | 8.945 | 10.682 | 5.844 | 3.418 | 1.777 | 9.268 | 2.178 | 1.245 | 1.702 | 6.035 | 4.371 | 7.241 | 4.262 | 25.574 |
| | dfn | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | p-value | 0.018 | 0.111 | 0.058 | 0.322 | 0.636 | 0.879 | 0.099 | 0.824 | 0.940 | 0.889 | 0.303 | 0.497 | 0.203 | 0.512 | 0.000 |
| | dfd | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 |
| | F | 2.734 | 1.789 | 2.136 | 1.169 | 0.684 | 0.355 | 1.854 | 0.436 | 0.249 | 0.340 | 1.207 | 0.874 | 1.448 | 0.852 | 5.115 |
| | $\eta p^2$ | 0.067 | 0.045 | 0.053 | 0.030 | 0.018 | 0.009 | 0.047 | 0.011 | 0.007 | 0.009 | 0.031 | 0.022 | 0.037 | 0.022 | 0.119 |
| Period | $\chi^2$ | 1.366 | 2.360 | 0.350 | 1.211 | 0.022 | 5.345 | 7.700 | 6.149 | 10.135 | 11.999 | 4.589 | 2.684 | 7.185 | 3.296 | 0.422 |
| | dfn | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | p-value | 0.505 | 0.307 | 0.840 | 0.546 | 0.989 | 0.069 | 0.021 | 0.046 | 0.006 | 0.002 | 0.101 | 0.261 | 0.028 | 0.192 | 0.810 |
| | dfd | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 |
| | F | 0.683 | 1.180 | 0.175 | 0.606 | 0.011 | 2.673 | 3.850 | 3.075 | 5.068 | 6.000 | 2.295 | 1.342 | 3.593 | 1.648 | 0.211 |
| | $\eta p^2$ | 0.007 | 0.012 | 0.002 | 0.006 | 0.000 | 0.027 | 0.038 | 0.031 | 0.050 | 0.059 | 0.023 | 0.014 | 0.036 | 0.017 | 0.002 |
| Essay | $\chi^2$ | 3.666 | 1.657 | 4.491 | 3.065 | 0.15 | 0.291 | 1.078 | 2.879 | 6.163 | 4.382 | 0.831 | 0.379 | 11.707 | 0.323 | 3.754 |
| | dfn | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | p-value | 0.056 | 0.198 | 0.034 | 0.08 | 0.699 | 0.590 | 0.299 | 0.09 | 0.013 | 0.036 | 0.362 | 0.538 | 0.001 | 0.57 | 0.053 |
| | dfd | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 |
| | F | 3.666 | 1.657 | 4.491 | 3.065 | 0.150 | 0.291 | 1.078 | 2.879 | 6.163 | 4.382 | 0.831 | 0.379 | 11.707 | 0.323 | 3.754 |
| | r | 0.019 | 0.008 | 0.023 | 0.016 | 0.001 | 0.001 | 0.006 | 0.015 | 0.031 | 0.022 | 0.004 | 0.002 | 0.057 | 0.002 | 0.019 |

**Table 2.** *Cont.*

| Variable | Parameter | Egg weight | Major diameter | Minor diameter | ShellL* | Shella* | Shellb* | White height | Yolk colour | YolkL* | Yolka* | Yolkb* | Yolk diameter | Shell weight | Yolk weight | White weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variety | $\chi^2$ | 34.300 | 39.881 | 29.270 | 36.567 | 65.585 | 98.046 | 15.421 | 15.238 | 5.696 | 16.295 | 15.891 | 21.296 | 62.090 | 54.267 | 59.063 |
| | dfn | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | p-value | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.004 | 0.004 | 0.223 | 0.003 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 |
| | dfd | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 |
| | F | 8.575 | 9.970 | 7.318 | 9.142 | 16.396 | 24.512 | 3.855 | 3.810 | 1.424 | 4.074 | 3.973 | 5.324 | 15.523 | 13.567 | 14.766 |
| | $\eta p^2$ | 0.152 | 0.173 | 0.133 | 0.161 | 0.256 | 0.339 | 0.075 | 0.074 | 0.029 | 0.079 | 0.077 | 0.100 | 0.245 | 0.221 | 0.236 |
| Breed | $\chi 2$ | 22.546 | 8.765 | 21.607 | 31.945 | 49.3 | 92.019 | 11.455 | 8.597 | 0.873 | 7.845 | 10.748 | 3.502 | 55.027 | 23.651 | 29.992 |
| | dfn | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | p-value | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.350 | 0.005 | 0.001 | 0.061 | 0.000 | 0.000 | 0.000 |
| | dfd | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 |
| | F | 22.546 | 8.765 | 21.607 | 31.945 | 49.300 | 92.019 | 11.455 | 8.597 | 0.873 | 7.845 | 10.748 | 3.502 | 55.027 | 23.651 | 29.992 |
| | r | 0.104 | 0.043 | 0.100 | 0.141 | 0.203 | 0.322 | 0.056 | 0.042 | 0.004 | 0.039 | 0.052 | 0.018 | 0.221 | 0.109 | 0.134 |

$\eta p^2$ can be benchmarked against Cohen (1969) criteria of small (0.01), medium (0.09), and large (0.25) effects as suggested in Richardson (2011). In Cohen's terminology, a small effect size is one in which there is a real effect but which you can only see through careful study. By contrast, a 'large' effect size is an effect which is big enough, and/or consistent enough, that you may be able to see it 'with the naked eye'.

as an effect size for the Mann-Whitney U test using the formula:

$$r = z/\sqrt{N}$$

Cohen's guidelines (Cohen, 1988) for *r* are that a small effect is 0.1, a medium effect is 0.3, and a large effect is 0.5 (Coolican, 2009). Calculation of *r*, $r^2$, or $\eta^2$ from these z values is possible because

$$r = z/\sqrt{N} \text{ and } r^2 \text{ or } \eta\text{^}2 = z^2/N$$

The literature recommends the use of partial eta square instead of classical eta square when using a multifactor design. The reason for this is that, through the use of partial eta square, we report an index of the strength of association between an independent variable and a dependent variable that excludes the variance produced by other variables (Brown, 2008). The Kruskal-Wallis H test produces chi2 values with k − 1 degrees of freedom. We can transform $chi^2$ into an F value with k − 1 numerator degrees of freedom (dfn) and N-k denominator degrees of freedom (dfd) using the expression F(dfn,dfd) = $chi^2$/(k − 1).

In Cohen's terminology, a small effect size (0.01) is one in which there is a real effect but which you can only see through careful study. By contrast, a 'large' effect size (0.25) is an effect which is big enough, and/or consistent enough that one may be able to see it 'with the naked eye'.

As almost all the variables have been previously reported to be non-normally distributed (Table 1) (Shapiro-Wilk Francia's tests ($p < 0.001$), an independent-sample median test was carried out to assess the differences in the median between categories within the same factor (Supplementary Tables S4 and S5). Supplementary Table S6 shows descriptive statistics for external and internal egg quality-related traits in Utrerana hens compared to the laying lineage in two models, including (n = 97) and excluding (n = 194) yolk and white pH.

Afterward, we studied the pairwise comparisons for any dependent variables for which the Kruskal-Wallis test is significant, aiming at assessing whether there were statistically significant differences between groups of the same factor concerning the external and internal quality-related variables using Dunn's test (Supplementary Tables S1 and S2). Then to provide a quantifiable measure of such differences, we provide within-group (level) medians in Supplementary Table S7.

We estimated the Pearson product-moment correlation coefficient among variables from both sets using a bivariate procedure from the Correlate package of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016) to avoid the severe multicollinearity or linear dependency between several variables, aiming at excluding those with multiple correlation coefficients higher than 0.80 according to Montgomery et al. (2012) (Supplementary Table S8). Canonical correlation analysis was performed to analyze the relation between the two sets of traits (internal quality and external quality) (Hotelling, 1935; Hotelling, 1936). Therefore, it is possible to define the linear combination of the two sets of variables as (Johnson and Wichern, 2007):

$$U_1 = a_{11}X_1 + a_{12}X_2 + \cdots + a_{1p}X_p$$

$$V_1 = b_{11}Y_1 + b_{12}Y_2 + \cdots + b_{1p}Y_p$$

Canonical variables $U_1$ and $V_1$ belong to the ith canonical pair associated with the first canonical correlation, expressed for:

$$r_i = \frac{c\hat{o}v(U_i,\ V_i)}{\sqrt{\hat{V}(U_i) \cdot \hat{V}(V_i)}}$$

The percentage of variance explained by the canonical variable $U_x^2$ and its opposite $V_y^2$ is determined by:

$$U_{x_i}^2 = \frac{\sum_{j=1}^{p} a_{ij}^2}{p}$$

$$V_{y_i}^2 = \frac{\sum_{j=1}^{q} b_{ij}^2}{q}$$

p and q are the number of variables from X and Y, respectively. To check for significance of canonical correlation, maximum likelihood ratio test was performed, considering Lambda (Λ) from Wilk's statistics following the equations reported in Khattree and Naik (2000).

All nonparametric tests were carried out using the independent samples package from the non-parametrical task of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016). Canonical correlation analysis was carried out using Canonical correlation procedure from the Correlate package of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016) .

### *2.6. Publication Ethics Statement*

All farms included in the study followed specific codes of good practices and, therefore, the animals received humane care in compliance with the national guidelines for the care and use of laboratory and farm animals in research. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki. The Spanish Ministry of Economy and Competitivity through the Royal Decree-Law 53/2013 and its credited entity the Ethics Committee of Animal Experimentation from the University of Córdoba permitted the application of the protocols present in this study as cited in the fifth section of its second article, as the animals assessed were used for credited zootechnical use. This national Decree follows the European Union Directive 2010/63/UE, from the 22 September 2010.

## 3. Results

### *3.1. Parametric Nature Assumption Testing*

The data was non-normally distributed (Shapiro Wilk's Francia W, $p < 0.001$) in all cases except for egg weight, major diameter, and white weight. Skewness statistics reported values between −1/2 and 1/2, which suggested that almost all variables were approximately symmetric, except for egg weight and white weight which were moderately skewed. All variables presented a distribution with kurtosis <3 (excess kurtosis <0) or platykurtic. Compared to a normal distribution, the central peak of the data distribution is lower and broader, and its tails are shorter and thinner.

Levene's test for equality of error variance reported that the error variance around the predicted scores was not the same for all the predicted values ($p < 0.05$), except for minor diameter, thus there was no homogeneity of variances for each combination of the levels of the independent variables (species, month, year, and pathology diagnosed); hence, the assumption of homoscedasticity was violated. Mauchly's W Test of Sphericity (Mauchly's W = 0.001), $\chi^2(104) = 2985.402$, $p < 0.05$) indicated that the variances of the differences were not equal; hence, the assumption of sphericity was also violated.

### *3.2. Factor Variance Explanatory Power and Whitin Between-Level Differences*

The Mann-Whitney U test was used to compare differences between the two independent groups of the laying age (Laying hens and Laying pullets) and breed variables (Utrerana and Leghorn). Almost all variables showed differences when the two breeds were compared, except for white height, yolk diameter, yolk$^{L*}$ and yolk pH. However, only minor diameter, white height, yolk$^{L*}$, yolk$^{a*}$, and shell weight reported a significant difference between the different laying age groups (Table 2 and Supplementary Tables S1-S3).

The study reports the results from the Kruskal-Wallis H test for all the variables and levels considered in the study and r and partial eta squared ($\eta p^2$) as a measure of the strength of the factors the variables tested (Table 2 and Supplementary Table S3). Supplementary Tables S4 and S5 show the differences between the median of the categories of the factors; month of laying, laying order, controlled period, laying age, variety, and breed reported by the independent-sample median test. Supplementary Tables S1 and S2 show the results for Dunn's tests pairwise comparisons between the different levels of the factors and variables.

Dunn's test pairwise comparisons and the independent-sample median test showed the white variety of the Utrerana hen and Leghorn were not significantly different ($p > 0.05$) for all variables except for egg weight, minor diameter and eggshell, with Leghorns reporting the highest median for all the three variables and varieties. The same tests reported a generalized significant difference between eggs from the first lay and the rest of the lays regarding egg and albumen/white weight. There were significant differences between March, April, May and June for almost all the variables measured except for shell$^{L*}$ (between March-May and April-June), shell$^{b*}$ (among any of the months compared), white height (between March and May themselves and between the months of March and May and April), Yolk color (March-June) and Yolk$^{L*}$ (between June and May themselves and between the months of June and May and April). Minor diameter, yolk$^{L*}$, yolk$^{a*}$, and shell weight were significantly different ($p < 0.05$) when hens and pullets were compared, with hens having a significantly higher median than pullets for all the variables except for yolk$^{L*}$.

### *3.3. External and Internal Quality-Related Variables Canonical Correlation Analysis*

The results of the canonical correlation produced three significant canonical correlations, when yolk and white pH were included and four significant correlations when such factors were not considered as shown in Table 3 and Supplementary Table S9, respectively.

**Table 3.** Standardized canonical coefficients of variables, canonical correlations between two sets of variables (r), squared canonical correlation ($r^2$) and their probabilities (F) for internal and external egg quality-related traits, excluding yolk and white pH, in Utrerana hens compared to laying lineage (n = 194).

| Canonical pairs | 1st | 2nd | 3rd | 4th | 5th | 6th |
|---|---|---|---|---|---|---|
| r ($R_c$) | 0.936 | 0.616 | 0.394 | 0.314 | 0.260 | 0.068 |
| $r^2$ ($R_c^2$) | 0.876 | 0.379 | 0.155 | 0.099 | 0.068 | 0.05 |
| F | 12.999 | 4.071 | 2.348 | 1.856 | 1.382 | 0.214 |
| Degrees of Freedom | 54 | 40 | 28 | 18 | 10 | 4 |
| Sig. | 0.000 | 0.000 | 0.000 | 0.017 | 0.187 | 0.931 |
| Standardized canonical coefficients of External quality related traits | | | | | | |
| Egg weight | **-0.972** | 0.139 | -0.065 | 0.240 | -0.934 | 1.888 |
| Major diameter | 0.025 | -0.345 | -0.337 | **0.575** | 0.606 | -1.464 |
| Minor diameter | -0.066 | 0.091 | **0.520** | **-0.859** | 0.597 | -1.059 |
| Shell$^{L*}$ | -0.021 | 0.134 | -1.327 | **-0.787** | 0.763 | 0.167 |
| Shell$^{a*}$ | -0.076 | 0.251 | -0.322 | -0.104 | -1.092 | -0.443 |
| Shell$^{b*}$ | 0.015 | **-0.967** | **-0.929** | **-0.838** | 0.993 | 0.545 |
| Standardized canonical coefficients of Internal quality related traits | | | | | | |
| White height | -0.037 | 0.229 | -0.219 | -0.311 | -0.194 | 0.462 |
| Yolk colour | -0.030 | -0.189 | 0.139 | **-0.543** | 0.224 | -1.428 |
| Yolk$^{L*}$ | -0.010 | **0.616** | **-0.944** | **0.670** | 0.136 | -0.308 |
| Yolk$^{a*}$ | 0.040 | 0.198 | **-1.370** | **0.449** | -0.206 | 1.537 |
| Yolk$^{b*}$ | 0.093 | -0.031 | **0.543** | **0.479** | -0.630 | -1.367 |
| Yolk diameter | -0.026 | 0.010 | -0.09 | -0.139 | -0.089 | 0.102 |
| Shell weight | -0.305 | **0.551** | 0.188 | **-0.405** | -0.607 | -0.595 |
| Yolk weight | **-0.400** | **-0.470** | **-0.538** | -0.098 | -0.132 | 0.313 |
| White weight | **-0.702** | -0.277 | 0.306 | **0.694** | 0.446 | -0.164 |

**Bold**: Given the sample size of 194, a criterion of ≥|0.39| was considered for variable loadings to be significant (Hair et al., 2010).

For the model that did not include pH values for yolk and white, the first, second and third significant canonical correlations produced Wilk's Lambda values that were found to be highly significant through the use of a chi-square test that yielded $p < 0.001$. The fourth canonical correlation also proved significant at the $p < 0.05$ level. However, only the first and second canonical correlations were highly significant (p

< 0.001) and the third canonical correlation was significant ($p < 0.05$) when yolk and white pH were not considered. All other canonical correlations were found to be non-significant.

When we did not consider yolk and white pH, the first, second, third and fourth canonical correlations produced an r ($R_c$) of 0.936 which indicates that the four variates have a shared variance ($r^2$ or $R_c^2$) of 87.6%, respectively (Table 3 and Supplementary Table S9). The literature proposes three methods to determine the relative importance of each original variable in each function: (1) canonical weights (standardized coefficients), (2) canonical loadings (structural correlations) and (3) canonical cross-loadings. As the canonical weights, are vulnerable to multicollinearity, the use of canonical loadings or cross-loadings is recommended (Table 4 and Supplementary Table S10).

**Table 4.** Correlations between the variables and related canonical variables (canonical loadings) and between the variables and the other set of canonical variables (canonical cross-loadings) for internal and external egg quality-related traits, excluding yolk and white pH, in Utrerana hens compared to laying lineage (n = 194).

| Variable | $U_1$ | $U_2$ | $U_3$ | $U_4$ | $U_5$ | $U_6$ | $V_1$ | $V_2$ | $V_3$ | $V_4$ | $V_5$ | $V_6$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Egg weight | **-0,996** | **-0,014** | **0,003** | **0,070** | **0,046** | **0,032** | -0,932 | -0,009 | 0,001 | 0,022 | 0,012 | 0,002 |
| Major diameter | **-0,745** | **-0,211** | **-0,222** | **0,448** | **0,168** | **-0,349** | -0,697 | -0,130 | -0,088 | 0,141 | 0,044 | -0,024 |
| Minor diameter | **-0,742** | **0,060** | **0,347** | **-0,497** | **0,114** | **-0,255** | -0,695 | 0,037 | 0,136 | -0,156 | 0,030 | -0,017 |
| Shell$^{L*}$ | **-0,087** | **0,817** | **-0,471** | **-0,018** | **0,319** | **0,039** | -0,081 | 0,503 | -0,185 | -0,006 | 0,083 | 0,003 |
| Shell$^{a*}$ | **0,013** | **-0,351** | **-0,292** | **-0,362** | **-0,753** | **-0,305** | 0,012 | -0,216 | -0,115 | -0,114 | -0,196 | -0,021 |
| Shell$^{b*}$ | **0,044** | **-0,934** | **-0,029** | **-0,293** | **-0,194** | **0,031** | 0,041 | -0,575 | -0,011 | -0,092 | -0,050 | 0,002 |
| White height | -0,364 | 0,177 | 0,063 | -0,088 | -0,010 | 0,023 | **-0,389** | **0,288** | **0,160** | **-0,281** | **-0,038** | **0,344** |
| Yolk colour | -0,039 | -0,260 | -0,132 | -0,122 | 0,016 | -0,034 | **-0,042** | **-0,422** | **-0,335** | **-0,389** | **0,062** | **-0,505** |
| Yolk$^{L*}$ | -0,011 | 0,372 | -0,157 | 0,066 | 0,126 | -0,006 | **-0,011** | **0,604** | **-0,399** | **0,209** | **0,486** | **-0,093** |
| Yolk$^{a*}$ | 0,038 | -0,243 | -0,091 | 0,034 | -0,149 | -0,010 | **0,040** | **-0,394** | **-0,232** | **0,109** | **-0,573** | **-0,145** |
| Yolk$^{b*}$ | 0,201 | -0,249 | 0,022 | 0,134 | -0,189 | -0,011 | **0,215** | **-0,405** | **0,057** | **0,426** | **-0,728** | **-0,156** |
| Yolk diameter | -0,288 | -0,079 | -0,028 | -0,027 | -0,050 | 0,003 | **-0,308** | **-0,129** | **-0,072** | **-0,087** | **-0,193** | **0,048** |
| Shell weight | -0,582 | 0,340 | 0,025 | -0,079 | -0,100 | -0,009 | **-0,622** | **0,553** | **0,064** | **-0,250** | **-0,384** | **-0,132** |
| Yolk weight | -0,390 | -0,313 | -0,194 | -0,044 | -0,037 | 0,003 | **-0,417** | **-0,508** | **-0,492** | **-0,139** | **-0,143** | **0,051** |
| White weight | -0,800 | -0,015 | 0,102 | 0,082 | 0,039 | -0,001 | **-0,855** | **-0,024** | **0,260** | **0,262** | **0,151** | **-0,010** |

$U_1$, $U_2$, $U_3$, $U_4$, $U_5$, $U_6$: canonical variate containing external quality related traits; $V_1$, $V_2$, $V_3$, $V_4$, $V_5$, $V_6$: canonical variate containing internal quality related traits. Bold: canonical loadings; Regular: canonical cross-loadings.

Significance was determined by using factor loading guidelines commonly found in the literature considering the sample sizes of 194 (when yolk and white pH were not considered) and 97 (when yolk and white pH had been included) (Thompson, 1991; Liu et al., 2009; Hair, et al., 2010; Hester, 2016). We used both loadings and cross-loadings; however, there is no established cutoff. There is a rule of thumb that if any variable loading is ≥|0.30|, then it can be considered to be an important contributing variable in the function. However, this is only for explanatory studies. Hair, et al. (2010) discuss the ideal case for each factor loading, i.e., the common variance should be greater than the unique one (Wilk's Lambda ≥0.72 in order to have a variance ≥0.50), but mainly for the average; that is the reason why we use the average variance attracted (AVE ≥ 0.50). In some cases, especially a new measure, lambda ≥0.5 (AVE > 0.25) can be considered to be acceptable (but we have to address the limitation of this low AVE measure). In our case, loadings ≥|0.53| were used considering the sample size (n = 97) that included yolk and white pH among the variables, while the greater sample when both variables were excluded (n = 194) permitted considering loadings ≥|0.39| following Hair, et al. (2010) criteria.

Egg weight showed a strong negative loading on the first canonical variate of external quality-related traits. Hence the first canonical variate was given the title "external lightness", reflecting upon the negative values associated with the pool of internal quality-related variables.

In addition, only white and yolk weight were found to have significant negative loadings on the second canonical variate of internal quality-related traits. Because of this, the first canonical variate on internal quality-related traits was given the title of "internal lightness".

A negative loading means that eggs scoring high on the canonical variate will tend to score low on the variable, and vice versa. Hence, the heavier the egg, white and yolk weight is, the lower the score it will receive on external and internal lightness canonical covariates, respectively.

Shell$^{b*}$ (Shell b*, shell yellow/blue coordinate) showed a strong negative loading on the second canonical variate of external quality-related traits. Hence the second canonical variate was given the title "external yellow/blue coordinate absence",

reflecting upon the negative and positive values associated with the pool of internal quality-related variables.

Interestingly, while yolk lightness ($Yolk^{L*}$, Yolk L*) and shell weight were found to have significant positive loadings on the second canonical variate of internal quality-related traits, yolk weight loading, significantly scored negatively. Because of this, the second canonical variate on internal quality-related traits was given the title of "internal brightness". This means those eggs presenting a high internal brightness present high yolk lightness and shell weight values and low yolk weight.

The minor diameter showed a moderate positive loading on the third canonical variate of external quality-related traits. By contrast, $shell^{b*}$ (Shell b*, shell yellow/blue coordinate) showed a strong negative loading on the third canonical variate of external quality-related traits. Hence the third canonical variate was given the title "egg wideness", reflecting upon the negative and positive values associated with the pool of internal quality-related variables. Eggs with a higher wideness presented lower values for the shell yellow/blue coordinate.

$Yolk^{L*}$ and $Yolk^{a*}$ color decompositions (Yolk L* or lightness and Yolk a* or red/green coordinate, respectively) and yolk weight were found to have significant moderate negative loadings on the third canonical variate of internal quality-related traits. However, $yolk^{b*}$ (Yolk b* or yellow/blue coordinate) significantly scored positively. Due to this, the third canonical variate on internal quality-related traits was given the title of "yolk dullness and yellow dominance". This means that those eggs presenting a high yolk lightness and yellowness present high values for the yolk yellow/blue coordinate and low values for the yolk lightness color coordinate, yolk red/green coordinate and yolk weight.

The minor diameter, $Shell^{L*}$ (Shell L*, shell lightness) and $Shell^{b*}$ (Shell yellow/blue coordinate) showed a high negative loading on the fourth canonical variate of external quality-related traits. By contrast, the major diameter showed a moderate positive loading on the fourth canonical variate of external quality-related traits. Hence, the fourth canonical variate was given the title "egg length and external dullness", reflecting upon the negative and positive values associated with the pool of internal quality-related variables. This means that the eggs which were longer

were also duller and reported lower values for the shell yellow/blue coordinate, thus they were orangish.

The $Yolk^{L*}$, $Yolk^{a*}$ and $Yolk^{b*}$ color lightness and coordinates (Yolk L* or lightness, Yolk a* or red/green coordinate, and Yolk b* or yellow/blue coordinate, respectively) and yolk weight were found to have significant moderate positive loadings on the fourth canonical variate of internal quality-related traits with $Yolk^{L*}$ or Yolk lightness reporting the highest loading (0.670). However, Shell weight and yolk color measured with the DSM Yolk Color Fan (formerly Roche Yolk Color Fan) significantly scored negatively. Due to this, the fourth canonical variate on internal quality-related traits was given the title of "yolk yellowness and lightness, color coordinates balance, shell lightness". This means those eggs presenting high yolk lightness and scoring low in the DSM Yolk Color Fan (yellowish) present balanced values for the yolk yellow/blue and yolk red/green coordinates and low shell weights.

Table 5 and Supplementary Table S11 show the imbalance between the proportion of variance explained by each of the canonical variates of the two sets of variables (external and internal quality-related traits) and their opposites. The proportion of the variance of external quality-related variables explained by its own canonical variate (38.2% to 35.1%, when yolk and white pH were included and excluded respectively) was slightly different to the proportion of variance of internal quality-related variables explained by opposite canonical variate (35.5% to 28.5% when yolk and white pH were included and excluded respectively). By contrast, the proportion of variance of external quality-related variables explained by its own canonical variate (15.6% to 8.0%, when yolk and white pH were included and excluded respectively) was similar to the proportion of variance of internal quality-related variables explained by opposite canonical variate (14.5% to 11.2% when yolk and white pH were included and excluded respectively).

**Table 5.** Proportion of explained variance, eigenvalues and percentages of explained common variance associated with each factor of internal and external egg quality-related trait, excluding yolk and white pH, in Utrerana hens compared to laying lineage (n = 194).

| Canonical Variable | 1st | 2nd | 3rd | 4th | 5th | 6th |
|---|---|---|---|---|---|---|
| Eigenvalue | 7.056 | 0.610 | 0.184 | 0.110 | 0.072 | 0.05 |
| Wilk's Λ Statistic | 0.054 | 0.439 | 0.707 | 0.836 | 0.928 | 0.995 |
| Proportion of variance of external quality related variables explained by its own canonical variate ($U_e^2$) | 0.351 | 0.308 | 0.176 | 0.154 | 0.351 | 0.308 |
| Proportion of variance of external quality related variables explained by opposite canonical variate ($V_e^2$) | 0.285 | 0.108 | 0.169 | 0.064 | 0.285 | 0.108 |
| Proportion of variance of internal quality related variables explained by its own canonical variate ($U_i^2$) | 0.080 | 0.012 | 0.075 | 0.012 | 0.080 | 0.012 |
| Proportion of variance of external quality related variables explained by opposite canonical variate ($V_i^2$) | 0.112 | 0.011 | 0.069 | 0.007 | 0.112 | 0.011 |

## 4. Discussion

The demand for products deriving from non-industrial production systems has triggered and increased the interest in more sustainable farming practices, enabling the introduction of products stemming from native breeds in the common production systems and commercial chains (Lordelo, et al., 2016). This context lays the basis for the characterization of the quality of differentiated products linked to sustainable production involving autochthonous breeds. The differences in the values obtained in this study for egg quality-related parameters may promote the definition of products depending on which and at which level egg components are present across the different varieties and breeds studied. For instance, eggs with greater egg yolk proportions may make for richer and softer baked final products and better quality pasta, while egg whites provide the resulting products with lighter and airier textures and are richer in lysozyme (Hester, 2016), which is currently, the only lysozyme industrially applied for food applications.

Among external egg quality-related traits, the Leghorn hen breed's eggs were heavier than those from the Utrerana hen breed, due to the higher weight of their shell and white. By contrast, although the Franciscan variety presented eggs with

lower weight, the eggs of the Utrerana breed generally presented similar or heavier weights than other Spanish breeds (Villalba et al., 2007; Rivera et al., 2009; Cajal and Francesch, 2014).

The eggs of laying pullets presented a significantly lower weight than the eggs of laying hens. In addition, egg weight was observed to increase with the age of the flock hens (in consecutive months), except for March, when a higher weight of eggs was observed in the flock than that reported in April or May. The fact that first laying hens had not yet started laying eggs in March may be one of the main reasons for this finding. These results are supported by other studies in which hens of different laying periods were compared (Saatci et al., 2005; Samiullah et al., 2016; Iqbal et al., 2017). Some authors report a simultaneous increase in egg weight while there is a decrease in shell weight, which may be attributable to such parameters being conditioned by the weight of egg components (yolk and albumen). Simultaneously, egg weight has been reported to increase as the age of hens increases, while eggshell quality deteriorates, which translates in greater quality larger chicks (Iqbal, et al., 2017).

The major diameter of the eggs was often related to the weight of the eggs. As results showed, the Leghorn eggs had significantly longer major diameters and minor diameters. However, the partridge variety reached the same major diameter as the Leghorn eggs. In addition, as previously described by Saatci et al. (2005), a smaller size of the eggs of the first laying hens was observed. Variety or plumage color has been reported to significantly affect egg weight in other local bird breeds such as in Native Turkish Geese ($p < 0.05$). However, such differences were not observed regarding shape index ($p > 0.05$), or length or breadth (parameters involved in the calculation of shape index) as opposed to our results (Iqbal, et al., 2017).

Another important characteristic of the commercialization of the product is the eggshell color profile that represents an important trait for consumer's perception. Almost equal numbers of brown and white eggs are sold in the markets of some countries such as Spain, Germany, and Holland (Arthur and O'Sullivan, 2005). In the present study, a significant increase in lightness ($Shell^{L*}$ values) on Leghorn eggs regarding Utrerana breed was observed. However, in terms of redness ($Shell^{a*}$ values) and yellowness ($Shell^{b*}$ values), the Utrerana breed showed higher values.

These results could be due to a large amount of genetic variation for eggshell characteristics (Hocking et al., 2003).

An increased value of shell$^{a*}$ was observed in the Franciscan variety, suggesting the hybridization with the Plymouth Rock breed (a breed with barred feathers and brown eggs), which was carried out while aiming at defining the barred feather characteristic in the Utrerana, also added to the appearance of the undesirable characteristic of darker shell eggs. No significant differences were observed to shell color between the white variety of Utrerana and Leghorn. When Shell$^{L*}$ value was considered to measure for eggshell lightness (Aygun, 2014), both the white Utrerana variety and Leghorn breed reported the brightest shell tone of all remaining varieties studies.

According to other authors, the month of laying did not have a significant effect on shell weight; although egg size increases with the hen's age, the shell weight mantains values around the same range (Nys, 1986; Saatci, et al., 2005; Sirri et al., 2018a). Heat stress reduces the shell thickness and the shell quality in laying hens (Emery et al., 1984; Usayran et al., 2001). However, the Utrerana eggshell weight showed no significant differences in all the studied months. It is well known that the south of Spain is influenced by Mediterranean weather - maximum temperatures of 40 °C were reached in June 2018 in Cordoba, as reported by the State Meteorological Agency (AEMET) from Spain, with very high temperatures since late spring and summer. Taking into account that this study occurred during this period, these results suggest that the Utrerana breed tolerates high temperature-induced stress, so this might be an interesting alternative to commercial production systems with fewer adapted animals.

The Utrerana breed showed a lower eggshell weight in comparison with the Leghorn breed. Modern commercial birds showed clear differences in terms of shell weights in regard to traditional breeds (Curtis et al., 1985; Hocking, et al., 2003). Nevertheless, the selection of breeds for one characteristic such as egg weight can affect others such as the quality of the eggshell (Poggenpoel et al., 1996). By contrast, Sreenivas et al. (2018) suggested that Leghorn eggshell contributes a lower proportion to overall egg weight when compared to native poultry.

Some authors have reported the characteristics of the egg white to be conditioned by the strain of bird and genetic selection (Tharrington et al., 1999; Toussant and Latshaw, 1999; Silversides and Scott, 2001). In this study, the Leghorn white weight was significantly heavier than those of the Utrerana varieties. In the Franciscan variety, the white weight was significantly lower than in the rest of the varieties. This could explain the lower weight of the eggs of this variety. No significant differences were observed in white weight in all the studied months, neither between the laying pullets, nor the laying hens. However, there were significant differences between June and the rest of the months, which suggests that the white height reduces as age increases, supported by the findings of Renden et al. (1984).

Although the yolk diameter did not differ between breeds, the yolk weight was significantly higher in the Utrerana breed. The selection of the modern lines of laying hens induced an increase in egg weight, which translated into a simultaneous decrease in the energy content of the egg as a direct consequence of a decrease in the percentage of egg yolk. The egg white contains a larger amount of water than the yolk which results in heavier eggs. This greater contribution to egg weight is produced at a lower energetic cost as its synthesis is energetically more efficient, on a weight for weight basis, than deposition of yolk which contains proportionally 0.5 of solids with equal proportions of fat and protein (Arthur and O'Sullivan, 2005; Sirri, et al., 2018b).

At the same time, as consumers begin to demand and consider egg energy as a quality criterion, egg selection for a higher percentage of yolk will be necessary (Hartmann et al., 2000). The yolk weight of the partridge and Franciscan varieties was significantly higher than the rest of the varieties or even the Leghorn breed, which may make them profitable alternatives.

An important aspect for the commercialization of the eggs is yolk color as European consumers tend to prefer darker ones, given the psychological misattribution of a healthier origin (Lordelo, et al., 2016). The strain of the laying hen has been suggested to determine egg pigmentation (Sirri et al., 2007). In addition, the yolk darkness is determined by the Yolk$^{a*}$ value (Aygun, 2014). In the present study, yolk$^{a*}$ and yolk$^{b*}$ values were observed to be higher in Utrerana eggs than in Leghorn ones. On the other hand, in April and May, the yolk$^{a*}$ value decreased, in comparison

to what happened in March and June. This finding suggests that yolk$^{a*}$ value, which accounts for the darker yolk in Utrerana, significantly decreased when the laying of the hen was higher. This suggests that the reduction in the yolk$^{a*}$ color coordinate could be a consequence of the dilution effect originated by the increase in egg production (Hocking, et al., 2003).

A higher Yolk$^{a*}$ value was found for the Franciscan variety, which could be linked to the higher Shell$^{a*}$ value observed for the same variety. However, Aygun (2014) reported that there was no significant correlation between eggshell color and that of the yolk. Besides, no significant differences were detected between the color of the eggshell and yolk color between white variety and Leghorn, suggesting that there was a great resemblance in the egg color of the two breeds when their plumage was white.

White quality is affected by the age of the laying hens, the strain of the birds and the storage time of the eggs (Silversides and Scott, 2001). The Leghorn hen's eggs showed higher values of white height than the Utrerana breed's eggs. The Leghorn breed laid heavier eggs with a higher proportion of white too. These results could suggest that the white height is correlated with the percentage of white, in accordance with similar observations in earlier reports (Sreenivas, et al., 2018). When months were compared, the white height presented lower values in June, when the temperature increased. During the storage of eggs, a decrease in white height at higher temperatures has been reported by Keener et al. (2000).

The Utrerana breed's eggs reported higher white pH values in comparison to the Leghorn breed's eggs. The white pH has been suggested to increase as $CO_2$ decreases inside the egg. Factors related to this loss of $CO_2$ such as the time of storage and high temperatures have been suggested to promote such a pH increase and a subsequent decrease in white viscosity and flavor, hence directly depreciating egg quality (Samiullah, et al., 2016).

According to our results for the canonical correlation analysis (Table 5 and Supplementary Table S11), the higher the value an egg scores on the external lightness variable of egg weight, the more likely this egg will also score a higher value on internal lightness variables such as white and yolk weight, as it was also reported for the same phenotypical correlations in Japanese quails, exotic Isa brown

layers and naked neck, normal and dwarf strains of Tswana chickens (Olawumi and Ogunlade, 2008; Kgwatalala et al., 2016; Olawumi and Christiana, 2017).

Similarly, those scoring a low value on the external yellow/blue coordinate are more likely to report higher values for internal brightness. Those eggs presenting high egg wideness presented higher yolk dullness and yolk yellow dominance. By contrast, the longer and duller the egg was externally, the more yellowish and lighter it was, the more balanced their color coordinates and the less heavy their shells. These results contradict some previous studies in which a weaker statistical analysis is performed (Yang et al., 2009; Aygun, 2014), and in which it is stated that the external color of the egg is not related to its internal color. Given our results, the Utrerana breed eggs allow consumers to associate the external appearance of the egg with their internal characteristics. The relationship between the outer shape of the egg and the internal color of the egg may suggest that there could be a dilution effect of pigments depending on the shape and size of the egg as reported by other studies (Hocking, et al., 2003).

The moderately high values for the proportion of variance of external quality-related variables explained by its own and opposite canonical variable, which doubles the explanatory power of variance of the internal quality-related traits set, suggest external quality-related traits may have a remarkably 2-fold higher predictive power of internal quality-related traits than vice versa. Interestingly, the reduction in the proportion of variance of internal quality-related variables, explained by its own canonical variate from 15.6 to 8.0% when pH was excluded, suggests these variables (yolk and white pH) may be relevant traits to consider for the determination of internal egg quality. Furthermore, external egg quality-related traits may act as better predictors of internal quality-related traits, which is desirable as it permits not having to break the eggs to classify them, relying on their internal quality to enable the implementation of an effective noninvasive method for internal quality determination.

## 5. Conclusions

Involving autochthonous breeds in common production systems and commercial chains seeking the characterization of the quality of differentiated products could be the key to future poultry sustainable productions. Leghorn eggs

are heavier than those from Utrerana hens; however, these generally presented similar or heavier weights than other Spanish breeds. There is a simultaneous increase in egg weight and a decrease in shell weight, which may be conditioned by the weight of egg components (yolk and albumen). Egg weight increases with age, while eggshell quality deteriorates. The variety or plumage color affects egg weight and egg length or breadth. Utrerana hybridization with the Plymouth Rock breed (a breed with barred feathers and brown eggs) added to the appearance of the undesirable characteristic of darker shell eggs, while the possible hybridization between the white Utrerana variety and Leghorn breed may account for the increased values for shell brightness reported. The Utrerana breed may tolerate high temperature-induced stress better than the Leghorn breed, so this might be an interesting alternative to commercial production systems with fewer adapted animals. The Leghorn breed's white weight was significantly heavier than those of the Utrerana varieties. The white height reduces as age increases. The modern line selection of laying hens has induced an increase in egg weight, which translates into a simultaneous decrease in the energy content of the egg, as a direct consequence of a decrease in the percentage of egg yolk. As white pH increases, $CO_2$ content decreases inside the egg. Simultaneous to this decrease, there is a subsequent decrease in white viscosity and flavor, which directly depreciates egg quality. The canonical correlation analysis addresses the possibility to develop a tool comprising external indicators that may indirectly report information on certain determinants of the internal quality of these eggs. This could mean a great advancement in the identification and typification of specific products, which may cover the currently increasing demand from markets for non-conventional quality products linked to specific breeds or production systems, or even settle new commercialization niches linked to local defined traceable products.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2076-2615/9/4/153/s1. Table S1: Summary of the significant ($p < 0.05$) pairwise differences (Green) obtained after Dunn's test for the levels of the factors of month, order, period, and variety and Mann-Whitney U Test ($p < 0.05$) differences between groups (Green) on internal and external egg quality-related traits, excluding yolk and white pH, in Utrerana hens compared to laying lineage (n = 194); Table S2; Summary of the significant ($p < 0.05$) pairwise differences (Green) obtained after Dunn's test for the levels of the factors of month, order, period, and variety and Mann-Whitney U Test ($p < 0.05$) differences between groups (Green) on internal and external egg quality-related traits including yolk and white pH in Utrerana hens compared to laying lineage (n = 97); Table S3: Summary of the results for the Kruskal-Wallis H test and the determinative coefficient through r or partial eta squared ($\eta p^2$), for fixed effects for internal and external egg quality traits from the model including yolk and white

pH in Utrerana hens (n = 97); Table S4: Summary of the results of the independent sample median test of the factors month, order, period, laying age, variety and breed on internal and external egg quality-related traits, excluding yolk and white pH, in Utrerana hens compared to laying lineage (n = 194); Table S5: Summary of the results of the independent sample median test of the factors month, order, period, laying age, variety and breed on internal and external egg quality-related traits including yolk and white pH in Utrerana hens compared to laying lineage (n = 97); Table S6: Descriptive statistics for external and internal egg quality-related traits in Utrerana hens compared to laying lineage in two models, including (n = 97) and excluding (n = 194) yolk and white pH; Table S7: Median for external and internal egg quality-related traits in Utrerana hens compared to laying lineage in two models, including (n = 97) and excluding (n = 194) yolk and white pH for each of the levels of the factors of month of laying, laying order, controlled period, laying age within study, variety and breed (the greener the higher, the redder the lower); Table S8: Pearson product-moment correlation coefficient between external and internal egg quality-related traits in Utrerana hens compared to laying lineage in two models, including (n = 97) and excluding (n = 194) yolk and white pH; Table S9: Standardized canonical coefficients of variables, canonical correlations between two sets of variables (r), squared canonical correlation ($r^2$) and their probabilities (F) for internal and external egg quality-related traits including yolk and white pH in Utrerana hens compared to laying lineage (n = 97); Table S10: Correlations between the variables and related canonical variables (canonical loadings) and between the variables and the other set of canonical variables (canonical cross-loadings) for internal and external egg quality-related traits including yolk and white pH in Utrerana hens compared to laying lineage (n = 97); Table S11: Proportion of explained variance, eigenvalues and percentages of explained common variance associated with each factor of internal and external egg quality-related traits including yolk and white pH in Utrerana hens compared to laying lineage (n = 97).

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# Egg Quality-related Data Mining based Discriminant Analysis Tool for Native Hen Breed Productive Characterization

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**Abstract:** Despite Spanish wide biodiversity of zootechnical interest avian species, projects aiming at characterizing these genotypes and their products is necessary. External and internal egg quality traits were measured in 819 eggs laid by hens of 10 different genotypes: White, Franciscan, Black and Partridge varieties of Utrerana, Blue Andalusian, Spanish White-Faced, Andalusian Tufted White and Black varieties, Araucana; and Leghorn Lohmann LSL-Classic lineage (commercial hybrid line) hen breeds. After multicollinearity analysis of egg quality-related traits was performed (VIF≤4), major diameter, minor diameter, egg weight, and albumen height were deemed redundant explanatory variables and discarded. A stepwise discriminant canonical analysis was developed to cluster eggs across hen genotypes considering egg quality attributes. Shell a* and b* variables reported the highest discriminant power (Wilks' Lambda: 0.699 and 0.729, respectively). The first two discriminant functions captured 60.48% of the variance across groups (F1: 39.36%; F2: 21.12%). Araucana eggs quality was reported to be the most distant from the rest. Clear quality differentiation signs are evidenced for Mediterranean native breeds' eggs when compared to Leghorn's. Consequently, these evidences of egg quality differentiation may favour the standardization of breed and variety linked distinctive products, which may open new market opportunities based on the existence of a wide spectrum of diet or culinary applications.

**Keywords:** Local breeds; hen genetic resources; biostatistical tool; external quality traits; internal quality traits.

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## 1. Introduction

In recent years, consumers have shown increasing interest for animal products that are obtained through sustainable production systems. The purpose of sustainable systems is to obtain differentiated food, with a low impact on environment and human health and considering animal welfare (Ureña et al., 2021). Most of the eggs consumed worldwide are laid by hens from commercial hybrid lines (Castillo et al., 2021). However, a new market niche is emerging for products with special characteristics, closely linked to native breeds and traditional breeding systems (Busse et al., 2019). As a result, the existence of local breeds may eventually lead to the parallel development of alternative production systems and the fixation of population in rural areas, which in turn may contribute to the prevention of biodiversity loss and the disappearance of animal genetic resources (Toalombo et al., 2019).

The acceptability of specific products by consumers has been reported to depend on quality traits related to the eggshell, the albumen, and the yolk (González Ariza et al., 2019a). Depending on the need to break the egg to measure quality features, these can be classified into external or internal quality traits (González Ariza et al., 2019b). Previous research reported that quality of hen egg can be influenced by genetic and non-genetic components, such as age of the hen, feed intake and environmental and meteorological factors (Sokołowicz et al., 2018; Zheng et al., 2020; González Ariza et al., 2021b). Egg parameters has been reported to influence fertility, embryo development, hatchability, and chicken viability (Abioja et al., 2020).

Spanish Atlantic and Mediterranean trunks cluster together all the hen breeds that spread across the territory. Hens of Atlantic trunk are generally semi-heavy birds, with red earlobes and brown-shelled eggs. On the other hand, the Mediterranean population comprises light individuals, with white earlobes and white-shelled eggs (Orozco, 1989). Egg production under alternative poultry systems promotes and

sets its basis on the use of local hen breeds, which are able to efficiently produce differentiated products under adverse weather conditions (Miguel et al., 2007). Contextually, Andalusia (southern Spain), is influenced by Mediterranean climate, with very high temperatures from May to October, hence, only certain autochthonous laying hen genotypes (Utrerana, Blue Andalusian, Spanish White-Faced and Andalusian Tufted) are adapted enough as to thrive when kept in the traditional backyard and extensive conditions of the area (González Ariza et al., 2019b).

Several studies have focused on disentangling the existing genetic, productive and reproductive differences within the varieties of Utrerana avian breed and across Andalusian autochthonous breeds (Dávila et al., 2015; González Ariza et al., 2019a; González Ariza et al., 2019b; Macrì et al., 2019; González Ariza et al., 2021b). In these regards, discriminant canonical analysis approaches have been suggested as a validation tool for Utrerana egg commercial quality classification depending on internal and external quality-related traits (González Ariza et al., 2021a).

To this aim, the present study seeks to determine the differential clustering patterns of egg quality-related traits from the eggs laid by four Spanish native breeds (white-shelled eggs layers) and their varieties: Utrerana (Franciscan, White, Black and Partridge), Blue Andalusian, Spanish White-Faced and Andalusian Tufted breeds (White and Black) in comparison to Araucana breed as a foreign native breed outgroup (American continent) and a control flock of a commercial laying lineage. The outcomes of the present study may support the characterization and typification of the entity of the products derived from Spanish laying breeds, as a strategy to plan potential marketing and commercialization alternatives to support the sustainability of the breeding program of those endangered genotypes.

## 2. Material and methods

### *2.1. Institutional Animal Care and Use Committee Statement*

The present research was conducted under the scope of the European Union legislation (2010/63/EU, from the 22 September 2010) and its transposition to the Spanish law document (Royal Decree Law 53/2013). Animals received humane care in compliance with the national guide for the care and the use of laboratory and farm animals in research. As recommended by Royal Decree Law 53/2013, the study

protocol was submitted to the legally constituted Ethics Committee of Animal Experimentation of the University of Córdoba, Spain which deemed the study to be exempt from review.

*2.2. Layer Flock and Environmental Conditions*

The experiment took place at the Agropecuary Provincial Center of Diputación of Córdoba, in the south of Spain (37°54'50.9"N-4°42'40.4"W), for 1 year (from February 2019 to February 2020). The eggs used in the present study were obtained from a flock of layers (40.44 ± 19.20 weeks old) comprising animals belonging to different breeds distributed as described in Table 1. The birds, from which the eggs were collected were placed in pens, with a stocking density of 1 animal per each $m^2$ and were fed on the same commercial feed (Chemical composition: 15.20% crude protein, 4.60% crude fats and oil, 3.20% crude fiber, 14.00% crude ashes, 4.10% calcium, 0.66% phosphorus, 0.19% sodium, 0.31% methionine, 0.72% lysine). Water and feed were provided ad libitum.

**Table 1.** Number of individuals (N) used in each studied breed and variety.

| Breed and variety | n |
|---|---|
| White Utrerana | 15 |
| Franciscan Utrerana | 15 |
| Black Utrerana | 15 |
| Partridge Utrerana | 15 |
| Blue Andalusian | 10 |
| Spanish White-Faced | 8 |
| White Andalusian Tufted | 8 |
| Black Andalusian Tufted | 8 |
| Araucana | 4 |
| Leghorn | 10 |

*2.3. Work Sample*

A total of 819 eggs comprised the egg sample. Eggs were laid during a complete laying cycle. Table 2 shows the classification of eggs depending on the laying hen genotype. The same information registration protocol was performed individually for all the eggs of the sample.

**Table 2.** Number of observations (n) used in each studied breed and variety.

| Breed and variety | n |
|---|---|
| White Utrerana | 98 |
| Franciscan Utrerana | 109 |
| Black Utrerana | 95 |
| Partridge Utrerana | 77 |
| Blue Andalusian | 45 |
| Spanish White-Faced | 47 |
| White Andalusian Tufted | 73 |
| Black Andalusian Tufted | 84 |
| Araucana | 21 |
| Leghorn | 170 |
| **Total** | 819 |

### *2.4. Measurements of External and Internal Quality-Related Traits*

External quality-related traits were measured following non-invasive methods, that is, without breaking the eggshell. The following external egg quality traits measures were evaluated: major and minor diameters of egg; egg weight; eggshell colour lightness, redness-greenness and yellowness-blueness coordinates (shell L*, shell a*, and shell b*), and shape index.

On the other hand, when the egg had to be broken to be evaluated, the scored internal egg quality-related traits were as follow: eggshell weight; eggshell thickness; eggshell resistance, composed by eggshell strength and area under the force-displacement curve (area); albumen height; Haugh units; albumen weight; albumen pH; yolk pH; yolk color fan; yolk lightness, redness, and yellowness variables (yolk L*, yolk a*, and yolk b*), yolk diameter; yolk weight and the presence or absence of visual defects in yolk and/or albumen. Haugh units and shape index (Table 3) were calculated following the premises established by Eisen et al. (1962) and Anderson et al. (2004).

**Table 3.** Mathematical description of the egg quality-related indices.

| Trait | Mathematical expression | |
|---|---|---|
| Shape index | $SI = (ØM/Øm) * 100$ | where SI: shape index; ØM: major diameter; Øm: minor diameter |
| Haugh units | $HU = 100 * \log(h - 1.7w^{0.37} + 7.6)$ | where HU: Haugh units; h: albumen height (mm); w: egg weight (g) |

The egg quality evaluation was measured within 24 h after oviposition, every 15 days for one year. Room temperature was 22±1 °C at the time of the egg quality evaluation. Further information regarding the data collection protocol used can be consulted in González Ariza et al. (2021a).

*2.5. Canonical Discriminant Analysis*

Canonical Discriminant analyses (CDA) were performed to design a tool that enables the classification of egg while it determines whether linear combination of measures of internal and external egg quality-related traits describe within and between population groups clustering patterns. The explanatory variables used for the present analyses were: major diameter, minor diameter, egg weight, shell L*, shell a*, shell b*, shape index, eggshell weight, eggshell thickness, eggshell strength, area, albumen height, Haugh units, albumen weight, albumen pH, yolk pH, yolk color fan, yolk L*, yolk a*, yolk b*, yolk diameter, yolk weight, and visual defects. The genotype of the laying hen was considered as the clustering criterion.

Canonical relationships with traits were plotted to depict the group differences into an easily interpretable territorial map. Regularized forward stepwise multinomial logistic regression algorithms were used to perform the variable selection. Priors were regularized according to the group sizes calculated using the prior probability of a commercial software (SPSS Version 26.0 for Windows, SPSS, Inc., Chicago, IL) instead of considering them the same, to avoid that groups with different sample sizes affect the quality of the classification (Marín Navas et al., 2021).

Same sample size contexts to those used in this study across groups have been reported be robust. In these regards, some authors have reported a minimum sample size of at least 20 observations for every 4 or 5 predictors, and the maximum number of independent variables should be n-2, where n is the sample size, in order to palliate possible distortion effects (Poulsen and French, 2008; Marín Navas et al., 2021).

Consequently, the present study used a 4 or 5 times higher ratio between observations and independent variables than those described above which renders makes discriminant approaches efficient. Multicollinearity analysis were run to ensure the independency and strong linear relationship across predictor. Variables chosen by the forward or backward stepwise selection methods were the same.

Finally, the progressive forward selection method was performed since it requires less time than the backward selection method.

Discriminant routine of the Classify package of the SPSS version 26.0 software and the Canonical Discriminant Analysis routine of the Analyzing Data package of XLSTAT software (Addinsoft Pearson Edition 2014, Addinsoft, Paris, France) were used to perform the canonical discriminant analysis.

2.5.1. Multicollinearity Preliminary Testing.

Multicollinearity assumption must be tested before running a discriminant canonical analysis, to ensure that redundancies in the variables considered do not overinflate the variance explanatory potential. The variance inflation factor (VIF) is the most common indicator used in detecting multicollinearity. A recommended VIF value of 4 was used in the study (Pan and Jackson, 2008). VIF was computed according to the following formula as a subroutine of the Canonical Discriminant Analysis routine of the Analyzing Data package of XLSTAT software (Addinsoft Pearson Edition 2014, Addinsoft, Paris, France):

$$VIF = 1/(1 - R^2),$$

where $R^2$ was the coefficient of determination of the regression equation.

2.5.2. Canonical Correlation Dimension Determination.

The maximum number of canonical correlations between two sets of variables is the number of variables in the smaller set. First canonical correlation usually explains most of the relationships between different sets. In any case, attention should be paid to all canonical correlations, despite reporting of only the first dimension being common in previous research (Toalombo Vargas et al., 2020). When canonical correlation values are 0.30 or higher, they correspond to about 10% of variance explained.

2.5.3. Canonical Discriminant Analysis Efficiency.

Wilks' Lambda test evaluates which variables may significantly contribute to the discriminant function. When Wilks' Lambda approximates to 0, contribution of that variable to the discriminant function increases. $\chi^2$ tests the Wilks' Lambda

significance. If significance is below 0.05, the function can be concluded to explain the group adscription well (Anuthama et al., 2011).

2.5.4. Canonical Discriminant Analysis Model Reliability.

Pillai's trace criterion, as the only acceptable test to be used in cases of unequal sample sizes, was used to test the assumption of equal covariance matrices in the discriminant function analysis (Zhang et al., 2020). Pillai's trace criterion was computed as a subroutine of the Canonical Discriminant Analysis routine of the Analyzing Data package of XLSTAT software (Addinsoft Pearson Edition 2014, Addinsoft, Paris, France). A significance of ≤ 0.05 is indicative of the set of predictors considered in the discriminant model being statistically significant. Pillai's trace criterion is argued to be the most robust statistic for general protection against departures from the multivariate residuals' normality and homogeneity of variance. The higher the observed value for Pillai's trace is, the stronger the evidence that the set of predictors has a statistically significant effect on the values of the response variable. That is, the Pillai trace criterion evidences potential linear differences in the combined internal and external egg quality traits across hen genotype clustering groups (Pieruccini-Faria et al., 2021).

2.5.5. Canonical Coefficients and Loading Interpretation and Spatial Representation.

When CDA is implemented, a preliminary principal component analysis is used to reduce the overall variables into few meaningful variables that contributed most to variations between eggs from different genotypes. The use of the CDA determined the percentage assignment of eggs within its own group. Variables with a discriminant loading of ≥|0.40|, were considered substantive, indicating substantive discriminating variables. By the use of stepwise procedures technique, non-significant variables were prevented from entering the function. Coefficients with large absolute values correspond to variables with greater discriminating ability. Data were standardized following procedures reported by Manly and Alberto (2016). Then, Squared Mahalanobis distances and principal component analysis were computed, using the following formula:

$$D_{ij}^2 = (\bar{Y}_i - \bar{Y}_j)\, COV^{-1} (\bar{Y}_i - \bar{Y}_j)\,,$$

where $D_{ij}^2$: distance between population i and j; $COV^{-1}$: inverse of the covariance matrix of measured variable x; $\bar{Y}_i$ and $\bar{Y}_j$: means of variable x in the ith and jth populations, respectively.

The Squared Mahalanobis distance matrix was converted into a Euclidean distances matrix and a dendrogram was built using the underweighted pair-group method arithmetic averages (UPGMA; Rovira i Virgili University, Tarragona, Spain), and the Phylogeny procedure of MEGA X 10.0.5 (Institute of Molecular Evolutionary Genetics, The Pennsylvania State University, State College, PA, USA).

2.5.6. Discriminant Function Cross Validation.

Afterwards, to determine the probability that an egg of an unknown background belongs to a particular classification group (Hair et al., 2010), the hit ratio parameter was computed. For this, the relative distance of the problem observation to the centroid of its closest group was used. The hit ratio is the percentage of correctly classified eggs, that is correctly ascribing to the hen genotype that originally laid them. The leave-one-out cross-validation procedure is use as a form of significance, to consider if the discriminant functions can be validated. Classification accuracy is achieved when the classification rate is at least 25% higher than obtained by chance.

Press' Q statistic can support these results, since it can be used to compare the discriminating power of the cross-validated function, as follows:

$$Press\ Q' = \frac{[n - (n'K)]^2}{n(K-1)},$$

where n: number of observations in the sample; n': number of observations correctly classified; K: number of groups.

The value of Press' Q statistic must be compared with the critical value of 6.63 for $\chi^2$ with a degree of freedom in a significance of 0.01. When Press' Q exceeds the critical value of $\chi^2 = 6.63$, the cross-validated classification can be regarded as significantly better than chance.

2.5.7. Data Mining CHAID Decision Tree

Chi-Squared Automatic Interaction Detection (CHAID) decision tree (DT) data mining method was used for classification, prediction, interpretation, and discrete

categorized data manipulation. CHAID based algorithm decision support tool includes a root node, branches, and leaf nodes. For each internal node to be built around an egg quality trait (input variables), a Chi-square test significance split criterion ($P<0.05$) must be fulfilled (pre-pruning). According to Breiman et al. (1984) pruning (either pre or post) processes must be implemented to prevent trees from presenting a large amount of branches and to prevent them from failing fail to pursue branches which can add significantly to the overall fit. After computing a tree exhaustively depicting the significant relationship across independent variables detected, nodes which do not contribute to the overall prediction are discarded. Furthermore, CHAID adds an element of penalization as an indirect cost derived from model complexity. In these regards, Bonferroni inequality significant adjustment for significance levels was used. Breiman's method resembles forward stepwise regression with a cutting back on the final number of steps using Chi squared tests instead of F-to-enter based tests. Each branch represents an outcome of the test (in a number of two or more), and each leaf node (or terminal node) represents a category level of the target variable (hen genotype). The top most node in a tree is the root node. The decisions are made at each node and each records of data continues through the tree along a path until the record reaches a leaf or terminal node of the tree (Ceylan et al., 2018).

Afterwards, cross validation was performed to validate the set of predictors considered measuring the differences between the prediction error for a tree applied to a new sample and a training sample. Cross validation of decision tree was performed using the 'Complexity Parameter' and cross validated error to estimate how accurately the model generalizes for unseen data, i.e., how well it performs/predicts. Ten-fold cross validation (Baykara, 2015) was performed keeping every sample record in either training sample and study data. Resubstitution Error Rate measures the proportion of original observations that were misclassified by various subsets of the original tree. The aim is to determine the shortest tree collecting the highest number of significant relationships. However, the lowest resubstitution rate is not always the optimal choice, as this tree will have a bias. In the same manner, large trees will put random variation in the predictions as they overfit outliers. For these reasons, instead of selecting a tree based on the resubstitution error rate, X-fold cross-validation is used to obtain a

cross-validated error rate, from which the optimal tree is selected. The X-fold cross-validation involves creating X-random subsets of the original data, setting one portion aside as a test set, constructing a tree for the remaining X-1 portions, and evaluating the tree using the test portion. This is repeated for all portions, and an estimate of the error is evaluated. Adding up the error across the X portions represents the cross-validated error rate. The tree yielding the lowest cross-validated error rate is selected as the tree that best fits the data.

## 3. Results

### *3.1. Descriptive Statistics*

Mean, standard deviation, maximum, minimum and percentiles for each egg quality-related trait of the study are shown in Supplementary Table S1.

**Table 4.** Multicollinearity analysis of quality-related traits of eggs.

| Statistics/Parameters | Tolerance (1 - $R^2$) | VIF |
|---|---|---|
| Yolk weight | 0.4579 | 2.1839 |
| Shell b* | 0.4595 | 2.1764 |
| Shell L* | 0.4811 | 2.0786 |
| Yolk diameter | 0.4827 | 2.0717 |
| Eggshell weight | 0.5329 | 1.8766 |
| Eggshell strength | 0.5384 | 1.8575 |
| Yolk color fan | 0.5838 | 1.7128 |
| Resistance area | 0.5868 | 1.7040 |
| Albumen weight | 0.6666 | 1.5002 |
| Yolk a* | 0.6891 | 1.4512 |
| Eggshell thickness | 0.6895 | 1.4502 |
| Yolk L* | 0.7429 | 1.3461 |
| Yolk b* | 0.7818 | 1.2791 |
| Albumen pH | 0.8041 | 1.2436 |
| Haugh units | 0.8149 | 1.2272 |
| Shell a* | 0.8307 | 1.2038 |
| Shape index | 0.8649 | 1.1562 |
| Yolk pH | 0.8853 | 1.1296 |
| Visual defects | 0.9579 | 1.0439 |

Interpretation thumb rule: VIF = 1 (Not correlated); 1 < VIF < 4 (Moderately correlated); VIF ≥ 4 (Highly correlated).

### *3.2. Canonical Discriminant Analysis Model Reliability*

Major diameter, minor diameter, egg weight, and albumen height were discarded from the analyses due to them presenting VIF values over 4 (Table 4). Significant Pillai´s trace criterion (Value: 1.8923; df1: 180; df2: 7173; P<0.0001) determined discriminant canonical analysis was feasible. As reported in Table 5, out of the nine discriminant functions designed after the analyses, seven presented a significant discriminant ability. The discriminatory power of the function F1 was high (eigenvalue of 1.23; Figure 1) with 60.48% of the variance being explained by F1 and F2.

**Table 5.** Canonical Discriminant analysis efficiency parameters to determine the significance of each canonical discriminant function.

| Test of Function(s) | Wilks' Lambda | Chi-square | df | Sig. |
|---|---|---|---|---|
| 1 through 9 | 0.082 | 1214.592 | 171 | 0.000 |
| 2 through 9 | 0.199 | 786.456 | 144 | 0.000 |
| 3 through 9 | 0.381 | 468.933 | 119 | 0.000 |
| 4 through 9 | 0.583 | 262.279 | 96 | 0.000 |
| 5 through 9 | 0.734 | 150.466 | 75 | 0.000 |
| 6 through 9 | 0.817 | 98.074 | 56 | 0.000 |
| 7 through 9 | 0.884 | 60.241 | 39 | 0.016 |

df: degrees of freedom.

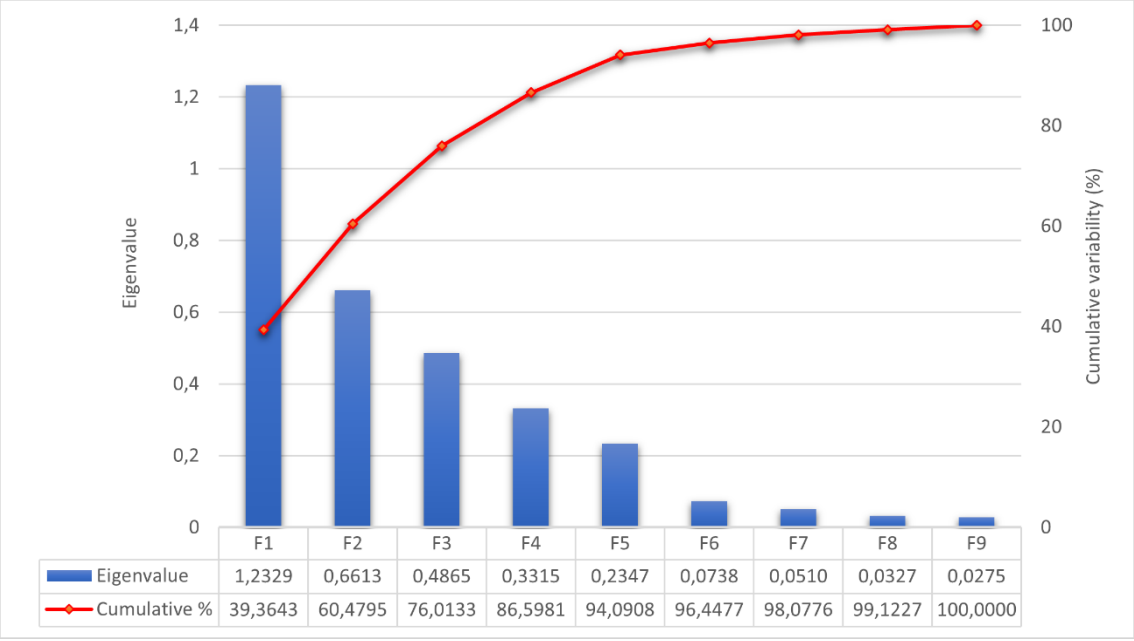


**Figure 1.** Canonical variable functions and percentages of self-explained and cumulative variance.

### 3.2.1. Coefficients, Loading Interpretation, and Spatial Representation

Variables were ranked depending on their discriminating properties. For this, a test of equality of group means across egg quality classification was used (Table 6). Lower values of Wilks' Lambda and greater values of F indicate a better discriminating power, which translates into a better position in the rank. The analyses revealed yolk and white pH not to significantly contribute ($P<0.05$) to the discriminant ability of significant discriminant functions.

**Table 6.** Results for the tests of equality of group means to test for difference in the means across egg groups once redundant variables have been removed.

| Variable | Wilks' Lambda | F | df1 | df2 | *p*-value | Rank |
|---|---|---|---|---|---|---|
| Shell a* | 0.699 | 38.633 | 9 | 808 | < 0.0001 | 1 |
| Shell b* | 0.729 | 33.442 | 9 | 808 | < 0.0001 | 2 |
| Albumen weight | 0.768 | 27.158 | 9 | 808 | < 0.0001 | 3 |
| Shape index | 0.812 | 20.770 | 9 | 808 | < 0.0001 | 4 |
| Haugh units | 0.820 | 19.736 | 9 | 808 | < 0.0001 | 5 |
| Yolk weight | 0.858 | 14.821 | 9 | 808 | < 0.0001 | 6 |
| Eggshell weight | 0.859 | 14.756 | 9 | 808 | < 0.0001 | 7 |
| Yolk diameter | 0.880 | 12.250 | 9 | 808 | < 0.0001 | 8 |
| Shell L* | 0.881 | 12.095 | 9 | 808 | < 0.0001 | 9 |
| Yolk b* | 0.892 | 10.916 | 9 | 808 | < 0.0001 | 10 |
| Area | 0.940 | 5.739 | 9 | 808 | < 0.0001 | 11 |
| Yolk color fan | 0.940 | 5.729 | 9 | 808 | < 0.0001 | 12 |
| Eggshell strength | 0.960 | 3.743 | 9 | 808 | 0.0001 | 13 |
| Visual defects | 0.963 | 3.444 | 9 | 808 | 0.0004 | 14 |
| Eggshell thickness | 0.964 | 3.381 | 9 | 808 | 0.0004 | 15 |
| Yolk a* | 0.973 | 2.538 | 9 | 808 | 0.0071 | 16 |
| Yolk L* | 0.976 | 2.178 | 9 | 808 | 0.0216 | 17 |
| Albumen pH | 0.982 | 1.655 | 9 | 808 | 0.0958 | 18 |
| Yolk pH | 0.987 | 1.181 | 9 | 808 | 0.3036 | 19 |

Standardized discriminant coefficients measure the relative weight of each egg quality trait across the discriminant functions (Figure 2 and 3). Out of the seven significant discriminant functions (Table 5), only the two most relevant were used to build a standardized discriminant coefficients biplot, capturing the highest fraction of variance (Figure 3). In these regards, those variables whose vector extends further apart from the origin, most relevantly contributed to the first (F1) and second (F2) discriminant functions. Figure 4 suggests clear differentiation across eggs laid by the hens belonging to the different genotypes considered in the

analyses. The relative position of centroids was determined through the substitution of mean value for observation sin each term of the first two discriminant functions (F1 and F2). The larger the distance between centroids, the better the predictive power of the canonical discriminant function in classifying observations. Supplementary Tables S2 and S3 report the results obtained in the classification and leave-one-out cross-validation. A Press' Q value of 1939.49 (N=819; n=460; K=10) was obtained. So, it can be considered that predictions were significantly better than chance at 95% (Chan, 2005).

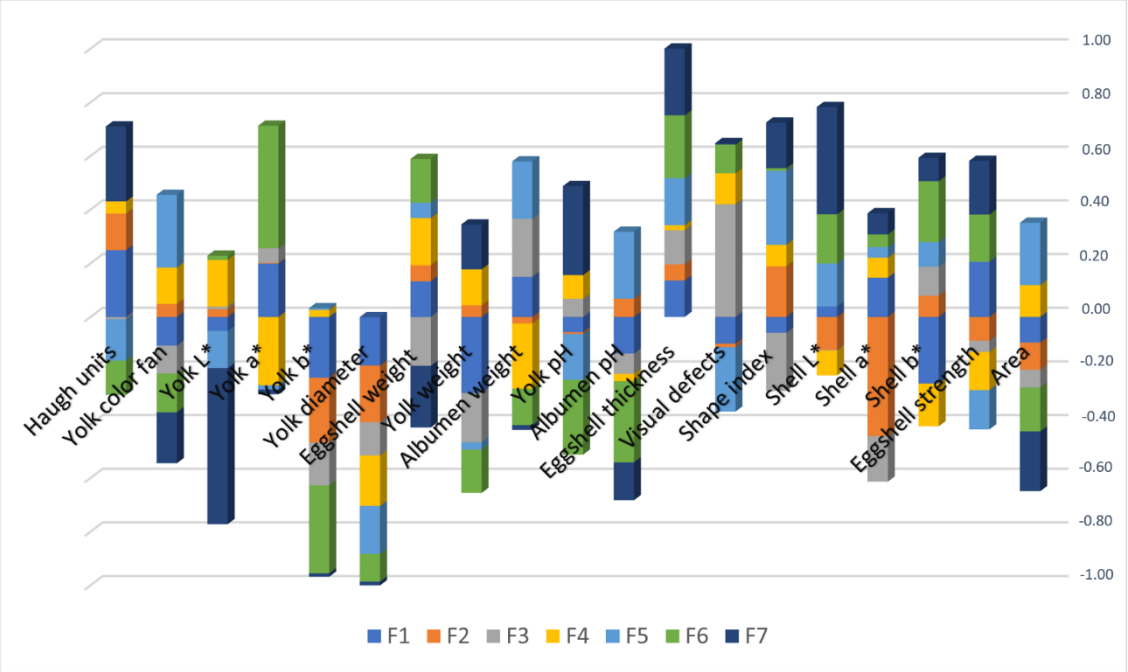


**Figure 2.** Discriminant loadings for external and internal quality-related traits determining the relative weight of each trait on each canonical discriminant function.

Additionally, in order to evaluate the proximity between hen genotype clusters, Mahalanobis distances were represented (Figure 5). Araucana hens were those most distantly located in respect to the rest of hen genotypes, with Andalusian Tufted black and white varieties' eggs clustering together and further away from them than the rest of eggs. A certain connection is evidenced between black tufted, blue Andalusian and black Utrerana's eggs. Still, a central Utrerana egg cluster revealed a closer relative relationship between black Utrerana and Franciscan and Partridge varieties eggs. White Utrerana eggs were closely related to eggs laid by the rest of Utrerana varieties but also a certain close connection is reported with White-faced and Leghorn's eggs.

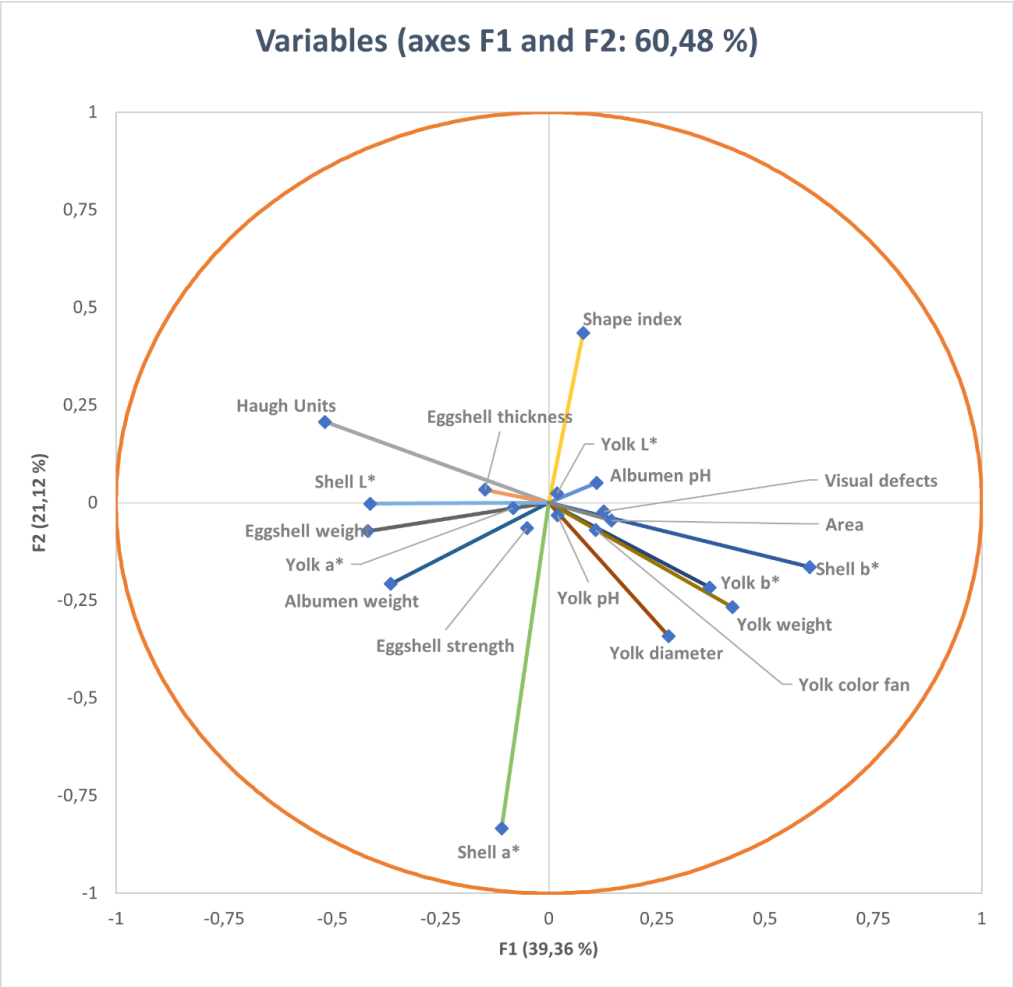


**Figure 3.** Vector plot for discriminant loadings for egg quality-related traits.

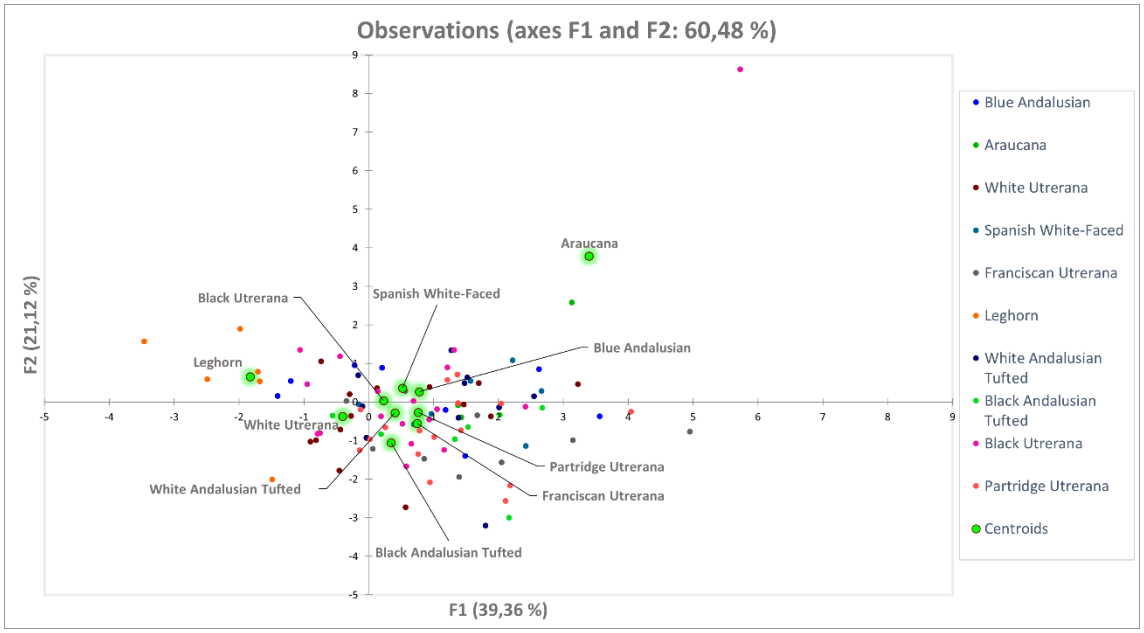


**Figure 4.** Territorial map depicting the eggs considered in the canonical discriminant analysis sorted across laying hen genotypes.

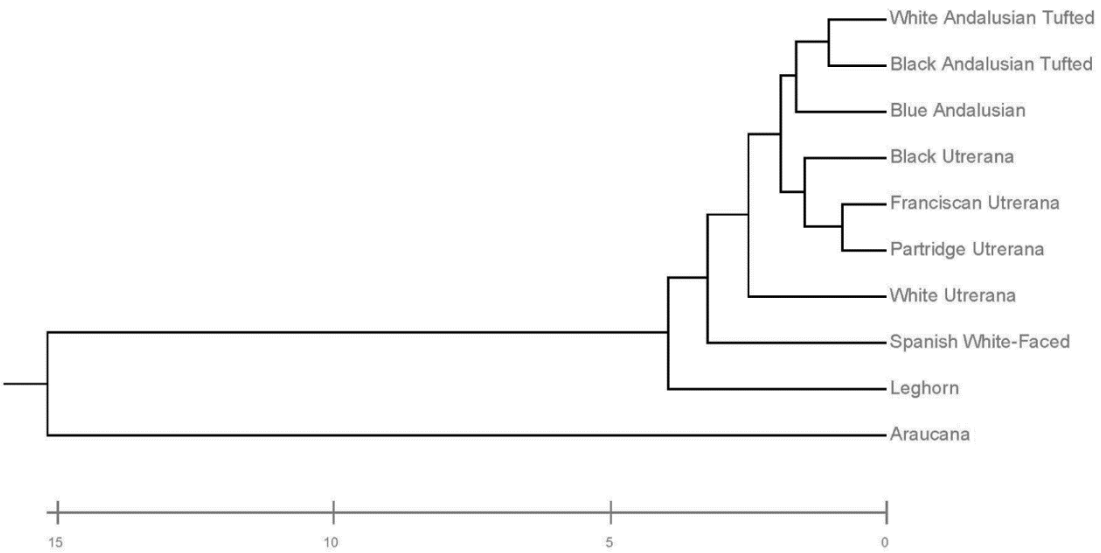


**Figure 5.** Cladogram constructed from Mahalanobis's distances between laying hen genotypes.

The underlying basis for this classification patterns was found after the evaluation of the Data Mining CHAID Decision Tree obtained of chi-square dissimilarity matrix. In thee regards, Chi squared bases branch and node distribution suggested eggs significantly ($P<0.05$) differed, thus, were classified in five subgroups depending on their values of shell b* (≤1.49; 1.49-2.09; 2.09-2.91; 2.91-7.51; ≥7.51). Leghorn eggs predominate when shell b* values are lower than 2.09. Eggs with values of ≤1.49 and 2.91-7.51 for shell b* were classified by values of shape index; eggs with values of 2.09-2.91 for shell b* were classified depending on the yolk weight; and eggs with values of ≥7.51 for shell b* were classified according to egg weight. At the same time, eggs with values of 2.09-2.91 for shell b* and a yolk weighing more than 19.03 g were classified by area. This last distinction allowed the definition of mostly the eggs of the genotypes Blue Andalusian, White Andalusian Tufted and Franciscan Utrerana from those produced by Partridge Utrerana. Another subgroup was defined by eggs with values of ≥7.51 for shell b* and total egg weight of >60.04 g. In this case, the shape index was quite important and separated Black Andalusian Tufted eggs (44.40% of eggs that showed values of >7.51 for shell b*, >60.40 g of total egg weight and ≤73.06 for shape index proceeded from Black Andalusian Tufted).

10-fold cross validation reported closely similar resubstitution and cross-validation error rate estimates of 0.623 and 0.665, for which standard error was 0.17 and 0.16,

respectively, which supported the robustness of the results obtained and the validity of the conclusions drawn from them.

## 4. Discussion

High correlations between major diameter and minor diameter with shape index can be explained by the fact that mathematical expression for Haugh units calculation (retained in the analyses) comprises the aforementioned parameters. Additionally, egg weight, which was also deemed redundant, hence discarded, can be calculated from the sum of yolk, albumen and eggshell weights. These findings are supported by a previous research (González Ariza et al., 2021a), in which same redundancies were detected.

The pH-related traits showed the lowest non-significant discriminating power between different groups of eggs (Table 6). The low contribution of PH related traits to discriminating function may derive from the low variability in egg pH found. pH can be taken as a measure of egg freshness: over time, there is a loss of CO2 and H2O inside the egg, accompanied by detrimental effects for egg quality, like a decrease in flavor and albumen viscosity (Lakins et al., 2009; Samiullah et al., 2016; Nematinia and Abdanan Mehdizadeh, 2018). Although egg pH has been reported to be conditioned by the hen strain (Lordelo et al., 2020), in the present research, pH measurements were taken within 24 hours of oviposition. Therefore, these results may suggest eggs durability may be condition by hen strains but at later stages, hence the eggs in the present could be considered fresh enough as not to obtain large variations in pH values between different breeds and varieties of hens. This explains the low variability in pH values across groups. Furthermore, and considering egg albumen and yolk pH values are correlated with embryo development (Ipek and Sozcu, 2017), and in light of the potential existence of differences at later stages, additional studies considering the evolution of pH along storage time of eggs must be developed in order to determine the breeds with a higher egg durability and to reinforce breeding strategies through conservation programs for those endangered breeds for which egg durability could be more easily compromised.

High values of Wilks' Lambda and low values for F Yolk color, measured by the L*a*b* color space and the yolk color fan systems also suggested the limited discriminating potential of these traits. In these regards, while L* measures the

degree of lightness, a* and b* parameters measure chromaticity: redness-greenness and yellowness-blueness, respectively. Photometric determination by spectrophotometer has been reported to be more precise than the yolk color fan (Dvořák et al., 2009), with a similar discriminating power being reported for both parameters in the current study. Values for yolk b* were the more determinant yolk color-related parameter in the classification of eggs from different genotypes. These results are supported by Dvořák et al. (2009), who not only reported a significant negative correlation with other quality related traits such as total egg weight (r = −0.919) and white weight (r = −0.918), but also reported broader distribution for absolute frequency values of yolk b* (from 22.00 to 47.99) parameter when compared with yolk L* and yolk a*. Additionally, a strong mutual relation between yolk colouration parameters L∗ and b∗ (r = 0.927), hence the deposition of yellow pigment in egg yolk could be presumed to be affected by the current metabolism capability of the hen, which has been reported to be a source for variability across breeds on which adaptability to the environment often relies (Kim et al., 2021). Parallelly, the lowest discriminant relevance of a∗ parameter may be supported by the Dvořák et al. (2009), who elicited a* parameter to define red colour spectrum component, with increasing egg yolk weight values being linked to decreased proportion of orange colour which is preferred by consumers but, which however, has been reported to be independent from cholesterol concentration, thus egg internal quality (Ingr and Simeonova, 1983).

The variables area, eggshell strength and eggshell thickness occupied the 11$^{st}$, 13$^{rd}$ and 15$^{th}$ in the rank. However, eggshell weight is the best positioned shell-quality related trait, in the first half of the ranking. Previous studies have reported egg weight values are not directly proportionally related to eggshell resistance (Knaga et al., 2019). Concentration of Mg, Na and K in eggshell may be responsible for the eggshell's strength. High concentrations of these micronutrients in eggshell translates into increased egg fragility and softness (Orłowski et al., 2019).

Leghorn eggshells has been reported to have greater concentration of these micronutrients when are compared with local breeds (González Ariza et al., 2021b). For instance, Iqbal et al. (2017) observed that eggshell weight and eggshell thickness were positively correlated and significantly conditioned egg size. Consequently,

multicollinearity problems may derive from the strong relationship between eggshell thickness and weight, the reason due to which eggshell thickness may have been penalized (values of 0.964 for Wilks' Lambda and 3.381 for F).

Visual defects parameter showed to have low discriminating power. Blood and meat spots produce defects in yolk and albumen of eggs, that cause rejection by egg consumers (Brant et al., 1953; Sokołowicz et al., 2018). The rupture of an ovarian follicle at a different position from the stigma during ovulation and synthesis of different egg components, could produce these visual defects (Rizzi, 2020). Chromaticity of yolk can be altered by the presence of these spots, which could lead to high correlations between visual defects, yolk a*, and yolk b* (González Ariza et al., 2021a).

Yolk size-related parameters showed high discriminating power (6$^{th}$ and 8$^{th}$ positions for yolk weight and yolk diameter in the rank). These findings support the fact that hen genotype causes significant differences in the percentage of yolk. Several authors concluded that native breeds lay smaller eggs with higher percentage of yolk that commercial hybrid strains (Zanon et al., 2006; Rizzi and Marangon, 2012; González Ariza et al., 2019b). The greater contribution of commercial lines of laying hens to annual number of eggs and the egg weight is produced at a more energetically efficient cost, laying eggs with a larger amount of albumen and therefore, of water (Sirri et al., 2018).

Albumen represents approximately 57-71% of the egg weight (Galli et al., 2018; Sun et al., 2019; Ianni et al., 2021). For this, albumen weight ranked the first between the weight-related traits at the test for equality of group means. Leghorn egg has shown to have the heaviest eggshell (Supplementary Table S1). Hybrid strains has been subjected to high selective pressures in terms of eggshell quality due to its commercial and transport purposes (Knaga et al., 2019). However, in previous research, local genotypes has shown to have a stronger and stiffer eggshell when are compared to Leghorn's ones, despite these may present a lower eggshell weight than that of Leghorn's (González Ariza et al., 2021b).

Haugh units ranked at the fifth position at the tests of equality of group means. It is used as an indicator of albumen quality. Haugh units values are conditioned by storage conditions and time of storage (Yimenu et al., 2017), but also have been

reported to remarkably depend on hen genotype. When Haugh units are contrasted with yolk and white pH values, the differences may suggest that even if eggs durability has been reported to strongly vary across hen strains, variability occurs at later stages (with some breeds showing longer durability periods than others), with reduced variability being found right after laying.

Results obtained in the present study are in accordance with previous research (Franco et al., 2020; Lordelo et al., 2020), since they reported high values for Haugh units in selected lines of laying hens in comparison with native breeds. However, percentage of albumen are directly correlated with the albumen height (Sreenivas et al., 2013). Hence, the fact that commercial hybrid strains had high percentage of albumen, could provide certain advantage to these genotypes in terms of more desirable Haugh units values.

Shape index allow to classify eggs as round eggs (shape index > 76), standard eggs (shape index = 72-76), and sharp eggs (shape index < 72) (Galic et al., 2019). The high discriminating power reported by the trait evidences a great variability across the eggs of different genotypes used in the present study. While Araucana reported to have round eggs (shape index = 76.84), White Utrerana eggs showed sharp shape (shape index = 71.32).

Chromaticity parameters of eggshell reported the highest discriminating power. Although the most of the genotypes used in the present study laid white-shelled eggs, Araucana breed is distinguished by the laying of green-blue eggs (Castelló, 1921). Thus, shell a* and shell b* occupied the first positions in the rank in the test of equality of group means. Chromaticity parameters were responsible for Araucana breed clustering in a different group (Figure 5). Still, even if the rest of genotypes used in the study laid white-shelled egg, shell b* allowed to classify different breeds and varieties (Supplementary Figure S1). For instance, Leghorn breed showed values for shell b* close to 0. It has been suggested that high values for shell L* cause a decrease in values for shell b* (Odabaşi et al., 2007). In this context, according to Aygun (2014), if the eggshell L* value decreases (eggshell darkness increases), the Haugh unit value also decreases, but the shell strength increases. Hence, reporting the L* value on egg cartons could serve as a quick predictor of the durability of egg storage and as a trace of differential breed quality mark.

Figure 5 reports the clear diversification of breeds depending on internal and external egg quality traits. In this regards, Araucana's egg group differed from the rest of Mediterranean and hybrid lines. Araucana geographic isolation may not only have promoted a genetic and phenotypic distancing of this breed from the rest, but also caused a clear differentiation of its product (Carvalho et al., 2020; Mehlhorn and Petow, 2020).

The separation of Spanish White-Faced and White Utrerana eggs in different clusters from the rest of autochthonous genotypes and their approach, in terms of egg quality to the commercial hybrid line, suggests that breeders could have crossed individuals with Leghorn hen, in an attempt to decrease consanguinity in Spanish White-Faced and White Utrerana, which account with the smallest number of animals and face a high endangerment risk. Still, the diversification of Leghorn eggs differed from the rest of native Spanish breeds' eggs suggests the aforementioned native breeds could constitute an alternative to eggs from other breeds that have traditionally been sold in the market (Rondoni et al., 2020).

Similarities between egg quality-related traits of Partridge and Franciscan Utrerana were expected, since both varieties showed a higher proportion of yolk than the rest of genotypes. On the other hand, Supplementary Figure S1 suggests that Blue Andalusian eggs have, at the same time, similar characteristics with Black Utrerana and the two varieties of Andalusian Tufted breed. 81% of eggs with values of >7.51 for shell b*, >60.04 g for egg weight and >73.06 for shape index are laid by Blue Andalusian, Black Utrerana or Andalusian Tufted genotypes. Among the varieties of Utrerana hen, black variety shares a high morphological resemblance with individuals of black plumage from Blue Andalusian breed (Campo, 2007). Therefore, phenotypic similarities between these two genotypes, both morphological and productive, may evidence reminiscences of hybridization.

The closeness in the territorial map between eggs from the two varieties of Andalusian Tufted breed suggest a lack of reproductive management and a crossbreeding between both varieties, due to the low availability of animals belonging to the breed and the endangered situation the breed is facing. In addition, the absence of an official recognition and a breeding program of certain local breeds

can lead to a deterioration of the phenotypic and genotypic identity of their individuals (Wang et al., 2017).

The present study develops a tool that allows to efficiently classify eggs from 10 different genotypes based on quality-related traits. For this, some variables like shell a*, shell b*, albumen weight, shape index and Haugh units play an important role in the determination of external and internal quality of eggs. 91.18 and 61.90% of eggs of Leghorn and Araucana eggs, respectively, were correctly classified. However, 15.58% of Partridge Utrerana eggs were classified as Franciscan Utrerana eggs and 20.48% of Black Andalusian Tufted eggs were classified as White Andalusian Tufted eggs.

The present study suggests the combination between discriminant canonical analysis and data mining CHAID decision trees may constitute an efficient classification tool to sort eggs from different genotypes considering quality egg traits, which may permit to reveal the existence of hybridization or potential mixing across breeds.

Product differentiation and the productive characterization of breeds may play a pivotal role to ensure the sustainability of some populations and maintaining the biodiversity of specific areas. Being able to ask market demands through the supply of products derived from autochthonous breeds offers the possibility to cover a wide spectrum of consumer necessities may enable long-term maintenance of these genotypes. It has been observed that some external characteristics, like chromaticity of eggshell and egg shape index, easily measurable without the need to break the eggshell can provide us a large amount of information that allow us to classify correctly eggs from different genotypes.

Among the different internal quality-related traits, albumen characteristics, like Haugh units and albumen weight, play a pivotal role in the determination of differences between the egg groups. Results evidenced great differential quality properties when native breeds in Spain were compared to those of commercial hybrid lines or other foreign native breeds such as the Araucana hen. However, some native varieties (white and black varieties) still display evidences of a certain degree on hybridization with both commercial strains but also other native breeds sharing the same area (Leghorn and Spanish Withe-Faced or White Utrerana hens and Black

Utrerana and Andalusian Blue) with which has affected the quality of the eggs. In these regards, the similar proportions of the different parts of the egg (albumen, yolk, and shell) between the Franciscan and Partridge varieties of the Utrerana breed could lead an egg classification confusion. These results contrast those in the Andalusian Tufted breed, for which the lack of proper reproductive management may have produced low product differentiation. Conclusively, the proper management and application of reproductive plans and the preservation and differentiation of products derived from local breeds is feasible and may enable covering a wider spectrum on market demands.

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**Supplementary Table S1.** Summary of descriptive statistics for egg quality-related traits in each studied group.

| | | | Albumen height | Haugh Units | Yolk Color fan | Yolk L* | Yolk a* | Yolk b* | Yolk diameter | Eggshell weight | Yolk weight | Albumen weight | Yolk pH | Albumen pH | Eggshell thickness | Egg weight | Major diameter | Minor diameter | Shape index | Shell L* | Shell a* | Shell b* | Eggshell strength | Area |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Andalusian Blue | Mean | | 7.59 | 84.78 | 12.79 | 50.76 | 8.16 | 37.24 | 43.98 | 8.67 | 19.94 | 34.64 | 6.24 | 8.52 | 0.38 | 64.82 | 58.89 | 44.46 | 75.60 | 85.98 | -0.35 | 7.86 | 4.32 | 0.43 |
| | Std. Deviation | | 1.59 | 10.61 | 1.36 | 4.18 | 3.65 | 9.16 | 1.88 | 1.61 | 1.80 | 4.30 | 0.17 | 0.33 | 0.07 | 4.86 | 2.47 | 1.10 | 3.27 | 8.77 | 0.94 | 4.11 | 1.06 | 0.11 |
| | Minimum | | 3.51 | 49.50 | 11.00 | 42.41 | 2.36 | 25.87 | 40.15 | 5.74 | 17.20 | 25.39 | 5.95 | 7.94 | 0.24 | 54.84 | 54.49 | 42.01 | 69.90 | 61.37 | -1.17 | 2.59 | 1.24 | 0.10 |
| | Maximum | | 10.88 | 101.98 | 16.00 | 60.63 | 17.26 | 55.82 | 47.99 | 13.57 | 23.60 | 42.79 | 6.62 | 9.67 | 0.60 | 76.40 | 64.28 | 46.49 | 82.53 | 93.27 | 2.78 | 16.27 | 5.85 | 0.61 |
| | | 25% | 6.49 | 79.25 | 12.00 | 47.91 | 4.88 | 29.19 | 42.72 | 7.45 | 18.56 | 32.26 | 6.12 | 8.24 | 0.34 | 61.45 | 56.88 | 43.92 | 73.27 | 85.53 | -0.88 | 3.97 | 3.62 | 0.40 |
| | Percentiles | 50% | 7.67 | 85.65 | 13.00 | 50.36 | 7.90 | 35.72 | 43.80 | 8.57 | 19.47 | 34.77 | 6.22 | 8.60 | 0.38 | 65.66 | 59.03 | 44.56 | 74.92 | 88.70 | -0.68 | 6.59 | 4.64 | 0.45 |
| | | 75% | 9.12 | 93.44 | 14.00 | 54.20 | 10.31 | 46.05 | 45.01 | 9.43 | 21.42 | 38.13 | 6.35 | 8.71 | 0.42 | 67.88 | 60.67 | 45.30 | 78.47 | 91.28 | -0.27 | 10.30 | 5.00 | 0.50 |
| Araucanian | Mean | | 6.37 | 80.74 | 11.83 | 52.32 | 7.88 | 32.98 | 42.23 | 6.37 | 18.63 | 27.55 | 6.22 | 8.58 | 0.36 | 54.24 | 54.65 | 41.97 | 76.84 | 81.47 | -4.45 | 12.20 | 3.13 | 0.36 |
| | Std. Deviation | | 1.19 | 8.50 | 0.99 | 3.32 | 3.10 | 6.26 | 1.60 | 0.50 | 1.10 | 4.10 | 0.14 | 0.32 | 0.09 | 4.20 | 1.94 | 1.05 | 1.81 | 4.44 | 2.46 | 4.80 | 0.91 | 0.06 |
| | Minimum | | 3.87 | 57.66 | 10.00 | 45.32 | 1.90 | 24.00 | 40.36 | 5.01 | 17.21 | 21.73 | 6.05 | 7.90 | 0.22 | 47.73 | 51.63 | 40.55 | 73.96 | 73.68 | -7.79 | 2.14 | 1.90 | 0.25 |
| | Maximum | | 8.27 | 93.29 | 13.00 | 56.57 | 11.36 | 47.64 | 45.64 | 7.03 | 20.61 | 35.14 | 6.70 | 9.02 | 0.55 | 61.34 | 57.69 | 43.98 | 80.59 | 90.56 | 0.49 | 20.46 | 4.85 | 0.50 |
| | | 25% | 5.41 | 75.27 | 11.00 | 50.01 | 5.46 | 28.58 | 40.66 | 6.09 | 17.66 | 24.22 | 6.14 | 8.37 | 0.30 | 50.72 | 52.82 | 41.06 | 75.67 | 77.88 | -6.56 | 9.89 | 2.50 | 0.32 |
| | Percentiles | 50% | 6.36 | 82.86 | 12.00 | 52.13 | 9.24 | 32.75 | 41.86 | 6.46 | 18.53 | 26.18 | 6.20 | 8.64 | 0.34 | 53.21 | 54.45 | 41.98 | 76.55 | 81.32 | -4.69 | 11.22 | 2.80 | 0.37 |
| | | 75% | 7.35 | 86.38 | 13.00 | 54.96 | 10.09 | 35.28 | 43.63 | 6.73 | 19.62 | 30.74 | 6.28 | 8.84 | 0.42 | 58.44 | 56.64 | 42.88 | 78.15 | 83.83 | -3.28 | 17.51 | 3.86 | 0.40 |
| Black Utrerana | Mean | | 7.45 | 84.78 | 12.85 | 50.76 | 7.70 | 36.01 | 42.60 | 8.38 | 18.50 | 34.61 | 6.27 | 8.45 | 0.40 | 62.88 | 58.22 | 43.94 | 75.56 | 88.25 | -0.17 | 6.89 | 4.14 | 0.45 |
| | Std. Deviation | | 1.44 | 8.90 | 1.29 | 4.44 | 3.81 | 8.99 | 2.23 | 1.35 | 1.88 | 5.43 | 0.16 | 0.36 | 0.07 | 7.42 | 2.92 | 1.70 | 2.84 | 6.30 | 1.40 | 4.15 | 1.27 | 0.12 |
| | Minimum | | 3.50 | 57.83 | 8.00 | 42.01 | -1.66 | 19.87 | 36.46 | 5.13 | 11.58 | 20.20 | 5.95 | 7.70 | 0.25 | 45.41 | 50.79 | 39.23 | 68.22 | 51.57 | -8.24 | 0.72 | 1.50 | 0.10 |
| | Maximum | | 10.68 | 102.34 | 15.00 | 62.47 | 14.79 | 65.70 | 47.85 | 11.99 | 23.30 | 47.50 | 6.88 | 9.13 | 0.62 | 81.43 | 66.34 | 48.15 | 81.81 | 94.31 | 2.60 | 18.00 | 6.60 | 0.71 |
| | | 25% | 6.73 | 80.78 | 12.00 | 47.66 | 5.17 | 29.51 | 41.20 | 7.35 | 17.43 | 30.86 | 6.17 | 8.17 | 0.36 | 57.31 | 56.22 | 42.78 | 73.87 | 87.34 | -0.66 | 3.23 | 3.29 | 0.39 |
| | Percentiles | 50% | 7.43 | 85.77 | 13.00 | 49.99 | 7.83 | 34.12 | 42.61 | 8.49 | 18.47 | 34.23 | 6.25 | 8.43 | 0.39 | 61.84 | 58.23 | 43.93 | 75.59 | 89.06 | -0.36 | 6.51 | 4.18 | 0.46 |
| | | 75% | 8.37 | 89.61 | 14.00 | 52.86 | 10.68 | 42.54 | 44.41 | 9.39 | 19.36 | 38.78 | 6.36 | 8.79 | 0.46 | 68.32 | 60.34 | 45.14 | 77.17 | 91.46 | 0.42 | 8.74 | 5.31 | 0.51 |
| Franciscan Utrerana | Mean | | 6.89 | 82.17 | 12.25 | 52.98 | 8.37 | 36.33 | 43.62 | 8.15 | 19.10 | 30.96 | 6.22 | 8.43 | 0.38 | 59.13 | 58.34 | 42.59 | 73.09 | 87.84 | 0.20 | 6.95 | 3.98 | 0.40 |
| | Std. Deviation | | 1.49 | 9.71 | 1.59 | 4.05 | 3.71 | 7.91 | 2.17 | 1.05 | 1.95 | 4.15 | 0.15 | 0.31 | 0.07 | 5.71 | 2.38 | 1.42 | 2.95 | 6.53 | 1.24 | 4.56 | 1.21 | 0.13 |
| | Minimum | | 2.53 | 48.79 | 8.00 | 43.90 | -1.64 | 23.28 | 38.52 | 4.80 | 14.91 | 20.02 | 5.75 | 7.15 | 0.20 | 38.48 | 47.71 | 38.20 | 66.56 | 54.74 | -1.30 | 0.63 | 1.20 | 0.06 |
| | Maximum | | 11.44 | 104.50 | 16.00 | 62.94 | 16.32 | 60.58 | 50.83 | 11.46 | 25.30 | 43.31 | 6.70 | 9.06 | 0.57 | 73.83 | 65.91 | 45.80 | 82.75 | 95.08 | 5.72 | 24.48 | 6.39 | 0.80 |
| | | 25% | 6.27 | 77.71 | 11.00 | 50.10 | 6.07 | 30.36 | 42.19 | 7.46 | 17.87 | 28.35 | 6.12 | 8.24 | 0.33 | 13.61 | 6.06 | 3.43 | 6.63 | 16.88 | 8.28 | 18.32 | 2.95 | 0.25 |
| | Percentiles | 50% | 6.93 | 83.53 | 12.00 | 52.91 | 8.27 | 35.33 | 43.59 | 7.96 | 18.96 | 30.66 | 6.22 | 8.46 | 0.37 | 36.02 | 15.55 | 8.92 | 13.58 | 42.74 | 10.84 | 17.28 | 5.10 | 0.61 |
| | | 75% | 7.67 | 87.71 | 13.00 | 55.72 | 10.32 | 40.16 | 45.03 | 8.77 | 20.05 | 33.52 | 6.31 | 8.67 | 0.42 | 62.66 | 59.95 | 43.60 | 74.72 | 91.87 | 0.70 | 10.81 | 4.82 | 0.48 |
| Leghorn | Mean | | 9.13 | 94.50 | 11.95 | 51.74 | 8.73 | 27.89 | 42.05 | 8.89 | 16.83 | 35.43 | 6.24 | 8.42 | 0.40 | 62.68 | 58.82 | 43.61 | 74.27 | 92.30 | -0.65 | 1.66 | 3.99 | 0.36 |
| | Std. Deviation | | 1.07 | 5.50 | 1.35 | 4.45 | 2.93 | 6.49 | 2.81 | 1.26 | 2.81 | 4.57 | 0.28 | 0.35 | 0.06 | 7.19 | 3.17 | 1.70 | 3.55 | 2.96 | 0.36 | 1.93 | 1.32 | 0.15 |
| | Minimum | | 6.46 | 78.43 | 7.00 | 33.60 | 1.17 | 14.77 | 33.08 | 6.33 | 9.86 | 23.79 | 5.11 | 7.05 | 0.19 | 40.23 | 47.26 | 37.57 | 65.04 | 69.17 | -1.43 | -0.60 | 1.00 | 0.02 |
| | Maximum | | 11.56 | 104.92 | 15.00 | 63.18 | 18.02 | 56.29 | 48.33 | 11.94 | 30.78 | 50.92 | 6.90 | 9.10 | 0.57 | 93.38 | 69.06 | 50.04 | 89.57 | 95.43 | 2.67 | 12.67 | 7.37 | 0.80 |
| | | 25% | 8.45 | 91.50 | 11.00 | 49.00 | 6.71 | 24.40 | 40.28 | 7.94 | 15.05 | 32.89 | 6.07 | 8.17 | 0.36 | 57.31 | 56.22 | 42.78 | 73.87 | 87.34 | -0.66 | 3.23 | 3.29 | 0.39 |
| | Percentiles | 50% | 9.22 | 95.60 | 12.00 | 52.09 | 8.65 | 26.98 | 42.14 | 8.63 | 16.89 | 35.23 | 6.21 | 8.48 | 0.40 | 62.27 | 58.53 | 43.63 | 74.35 | 92.81 | -0.66 | 1.16 | 4.07 | 0.40 |
| | | 75% | 9.89 | 98.05 | 13.00 | 55.20 | 10.61 | 29.91 | 43.94 | 9.97 | 18.62 | 38.22 | 6.37 | 8.69 | 0.44 | 66.85 | 60.35 | 44.66 | 76.66 | 94.34 | -0.59 | 1.98 | 4.87 | 0.46 |

| | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Partridge Utrerarna | Mean | | 7.68 | 86.45 | 12.32 | 51.50 | 6.96 | 36.74 | 43.82 | 7.74 | 19.46 | 33.09 | 6.30 | 8.45 | 0.38 | 61.87 | 59.21 | 43.17 | 73.03 | 86.95 | -0.34 | 7.01 | 3.87 | 0.41 |
| | Std. Deviation | | 1.40 | 8.69 | 1.18 | 3.91 | 3.44 | 10.22 | 2.30 | 1.05 | 2.53 | 4.31 | 0.18 | 0.30 | 0.07 | 6.48 | 2.96 | 1.54 | 3.43 | 8.04 | 0.72 | 3.98 | 0.82 | 0.08 |
| | Minimum | | 4.15 | 57.16 | 8.00 | 43.20 | -0.97 | 18.90 | 38.21 | 4.84 | 14.02 | 20.00 | 6.00 | 7.88 | 0.14 | 44.63 | 52.73 | 39.85 | 59.95 | 55.60 | -1.50 | 1.53 | 2.21 | 0.20 |
| | Maximum | | 11.64 | 107.72 | 15.00 | 60.46 | 16.36 | 62.62 | 48.10 | 10.31 | 23.89 | 44.15 | 6.85 | 9.02 | 0.63 | 77.42 | 67.87 | 46.10 | 81.45 | 94.62 | 1.38 | 20.81 | 6.11 | 0.60 |
| | | 25% | 6.85 | 82.52 | 12.00 | 49.52 | 5.20 | 28.51 | 42.22 | 6.94 | 17.35 | 30.12 | 6.18 | 8.20 | 0.34 | 57.04 | 56.58 | 42.11 | 70.94 | 86.28 | -0.85 | 3.90 | 3.39 | 0.35 |
| | Percentiles | 50% | 7.60 | 86.81 | 12.00 | 51.81 | 7.38 | 36.10 | 43.88 | 7.68 | 19.48 | 33.54 | 6.29 | 8.44 | 0.38 | 62.04 | 59.19 | 43.30 | 72.92 | 89.58 | -0.54 | 6.95 | 3.86 | 0.41 |
| | | 75% | 8.54 | 92.18 | 13.00 | 53.40 | 8.68 | 45.44 | 45.81 | 8.40 | 21.49 | 35.42 | 6.36 | 8.73 | 0.41 | 65.89 | 61.24 | 44.33 | 75.27 | 92.05 | -0.11 | 9.93 | 4.43 | 0.47 |
| Black And. Tufted | Mean | | 7.63 | 85.34 | 12.14 | 50.54 | 9.06 | 34.32 | 44.57 | 8.13 | 19.42 | 36.19 | 6.25 | 8.46 | 0.38 | 64.68 | 60.42 | 43.91 | 72.77 | 88.21 | 0.86 | 8.44 | 3.97 | 0.38 |
| | Std. Deviation | | 1.47 | 9.21 | 1.11 | 4.78 | 2.74 | 9.77 | 2.11 | 1.31 | 2.70 | 4.21 | 0.25 | 0.36 | 0.08 | 6.47 | 2.93 | 1.12 | 2.39 | 3.20 | 1.41 | 3.31 | 1.11 | 0.13 |
| | Minimum | | 4.00 | 54.06 | 10.00 | 36.95 | 1.31 | 18.93 | 39.25 | 5.61 | 15.65 | 26.28 | 5.58 | 7.27 | 0.19 | 54.59 | 55.22 | 41.54 | 67.09 | 81.99 | -1.59 | 2.47 | 1.00 | 0.04 |
| | Maximum | | 10.59 | 100.43 | 15.00 | 63.56 | 13.51 | 58.54 | 49.62 | 12.55 | 36.30 | 46.26 | 6.93 | 9.11 | 0.65 | 84.26 | 67.70 | 47.15 | 78.00 | 94.40 | 4.19 | 15.13 | 6.11 | 0.60 |
| | | 25% | 6.71 | 81.77 | 11.00 | 47.52 | 7.35 | 27.93 | 43.12 | 7.17 | 17.77 | 32.99 | 6.14 | 8.26 | 0.33 | 60.28 | 58.29 | 43.12 | 70.96 | 85.59 | -0.22 | 5.90 | 3.43 | 0.35 |
| | Percentiles | 50% | 7.66 | 86.40 | 12.00 | 50.26 | 9.00 | 31.97 | 44.42 | 7.94 | 19.36 | 35.73 | 6.22 | 8.48 | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 | 0.41 | 0.41 | 0.41 | 0.37 | 0.37 |
| | | 75% | 8.69 | 91.42 | 13.00 | 53.14 | 11.41 | 37.76 | 46.26 | 8.70 | 20.69 | 38.59 | 6.31 | 8.75 | 0.43 | 68.14 | 61.93 | 44.50 | 74.29 | 90.32 | 1.99 | 11.05 | 4.77 | 0.45 |
| White And. Tufted | Mean | | 7.29 | 83.81 | 12.09 | 53.11 | 8.36 | 35.93 | 44.19 | 7.98 | 19.05 | 34.72 | 6.24 | 8.55 | 0.37 | 62.62 | 59.33 | 43.38 | 73.23 | 90.31 | -0.31 | 4.82 | 3.69 | 0.41 |
| | Std. Deviation | | 1.59 | 9.56 | 1.30 | 4.15 | 3.53 | 7.56 | 2.25 | 1.39 | 2.31 | 4.96 | 0.19 | 0.32 | 0.07 | 7.91 | 3.09 | 1.76 | 3.29 | 2.84 | 0.77 | 3.38 | 1.20 | 0.14 |
| | Minimum | | 3.81 | 58.16 | 7.00 | 44.79 | -0.66 | 24.40 | 39.43 | 5.81 | 13.84 | 24.57 | 6.00 | 7.84 | 0.19 | 54.59 | 55.22 | 41.54 | 67.09 | 81.99 | -1.59 | 2.47 | 1.00 | 0.04 |
| | Maximum | | 11.72 | 103.69 | 15.00 | 61.69 | 14.10 | 59.06 | 49.39 | 11.92 | 23.96 | 47.57 | 6.83 | 9.00 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.57 | 0.57 | 0.57 | 0.52 | 0.52 |
| | | 25% | 6.18 | 77.79 | 11.50 | 49.22 | 5.20 | 30.69 | 42.32 | 6.77 | 17.64 | 30.56 | 6.11 | 8.27 | 0.32 | 57.42 | 56.98 | 42.44 | 70.93 | 88.24 | -0.63 | 2.71 | 2.76 | 0.35 |
| | Percentiles | 50% | 7.26 | 85.14 | 12.00 | 53.41 | 9.08 | 34.06 | 44.59 | 7.80 | 19.02 | 34.74 | 6.17 | 8.65 | 0.37 | 36.02 | 15.55 | 8.92 | 13.58 | 42.74 | 10.84 | 17.28 | 5.10 | 0.61 |
| | | 75% | 8.24 | 90.51 | 13.00 | 56.25 | 11.17 | 39.51 | 46.07 | 8.85 | 20.47 | 38.93 | 6.31 | 8.83 | 0.41 | 67.40 | 61.33 | 44.49 | 75.71 | 92.86 | -0.20 | 6.55 | 4.41 | 0.51 |
| White Utrerana | Mean | | 7.85 | 86.89 | 11.89 | 52.06 | 7.42 | 32.85 | 42.68 | 8.00 | 18.01 | 35.93 | 6.27 | 8.41 | 0.37 | 63.46 | 60.66 | 43.20 | 71.32 | 88.74 | -0.18 | 5.54 | 3.46 | 0.33 |
| | Std. Deviation | | 1.47 | 9.65 | 1.34 | 4.49 | 3.41 | 9.34 | 2.17 | 1.02 | 2.32 | 3.69 | 0.19 | 0.32 | 0.06 | 5.45 | 2.82 | 1.15 | 2.90 | 7.19 | 0.75 | 4.44 | 1.12 | 0.12 |
| | Minimum | | 4.07 | 53.87 | 7.00 | 41.28 | -1.42 | 19.28 | 35.19 | 5.58 | 13.43 | 26.91 | 5.90 | 7.65 | 0.24 | 48.09 | 53.02 | 39.36 | 62.93 | 53.45 | -1.76 | -0.11 | 0.70 | 0.04 |
| | Maximum | | 10.83 | 102.32 | 15.00 | 64.47 | 15.50 | 63.90 | 48.17 | 10.36 | 31.23 | 46.59 | 6.85 | 9.06 | 0.54 | 76.37 | 68.60 | 46.40 | 78.47 | 99.48 | 2.62 | 19.46 | 6.46 | 0.60 |
| | | 25% | 7.08 | 84.21 | 11.00 | 49.14 | 5.47 | 26.42 | 41.41 | 7.21 | 16.71 | 33.36 | 6.13 | 8.17 | 0.33 | 60.14 | 58.60 | 42.57 | 69.20 | 86.89 | -0.64 | 2.05 | 2.71 | 0.28 |
| | Percentiles | 50% | 8.06 | 89.66 | 12.00 | 52.31 | 7.35 | 30.74 | 42.85 | 8.00 | 17.82 | 36.06 | 6.24 | 8.39 | 0.37 | 63.01 | 60.58 | 43.37 | 71.42 | 89.89 | -0.36 | 3.98 | 3.48 | 0.35 |
| | | 75% | 9.01 | 93.88 | 13.00 | 54.48 | 9.45 | 35.80 | 43.97 | 8.77 | 19.26 | 38.74 | 6.36 | 8.68 | 0.41 | 66.92 | 62.50 | 44.02 | 73.37 | 93.36 | 0.22 | 9.09 | 4.37 | 0.42 |
| Spanish White-Faced | Mean | | 7.52 | 86.76 | 11.78 | 52.06 | 7.60 | 37.58 | 42.95 | 7.86 | 18.45 | 27.09 | 6.26 | 8.50 | 0.36 | 54.67 | 55.56 | 42.30 | 76.16 | 91.29 | -0.59 | 2.80 | 4.00 | 0.38 |
| | Std. Deviation | | 2.03 | 12.49 | 0.84 | 4.42 | 2.02 | 10.06 | 2.06 | 0.90 | 2.44 | 3.35 | 0.14 | 0.28 | 0.06 | 4.69 | 1.75 | 1.24 | 2.12 | 2.64 | 0.31 | 2.87 | 0.89 | 0.08 |
| | Minimum | | 3.13 | 52.52 | 10.00 | 40.35 | 1.93 | 21.53 | 38.47 | 6.07 | 14.21 | 19.02 | 6.01 | 7.99 | 0.16 | 44.90 | 51.89 | 39.47 | 72.08 | 82.38 | -1.69 | 0.27 | 1.30 | 0.10 |
| | Maximum | | 12.79 | 112.16 | 14.00 | 60.46 | 10.96 | 67.30 | 47.78 | 10.64 | 30.60 | 33.46 | 6.66 | 9.39 | 0.50 | 63.51 | 59.03 | 44.81 | 81.42 | 94.51 | -0.04 | 12.21 | 5.56 | 0.51 |
| | | 25% | 6.03 | 78.26 | 11.00 | 50.86 | 6.57 | 31.44 | 41.13 | 7.28 | 17.32 | 24.95 | 6.16 | 8.24 | 0.32 | 50.69 | 54.34 | 41.47 | 74.70 | 90.46 | -0.73 | 0.84 | 3.55 | 0.35 |
| | Percentiles | 50% | 7.29 | 87.26 | 12.00 | 51.88 | 7.57 | 35.23 | 43.43 | 7.87 | 18.53 | 27.06 | 6.26 | 8.47 | 0.35 | 54.71 | 55.43 | 42.40 | 76.32 | 91.90 | -0.55 | 1.56 | 4.16 | 0.39 |
| | | 75% | 8.75 | 96.13 | 12.00 | 54.93 | 9.35 | 44.52 | 44.39 | 8.44 | 19.30 | 29.48 | 6.33 | 8.73 | 0.39 | 58.53 | 56.70 | 43.16 | 77.33 | 93.14 | -0.43 | 4.00 | 4.54 | 0.42 |

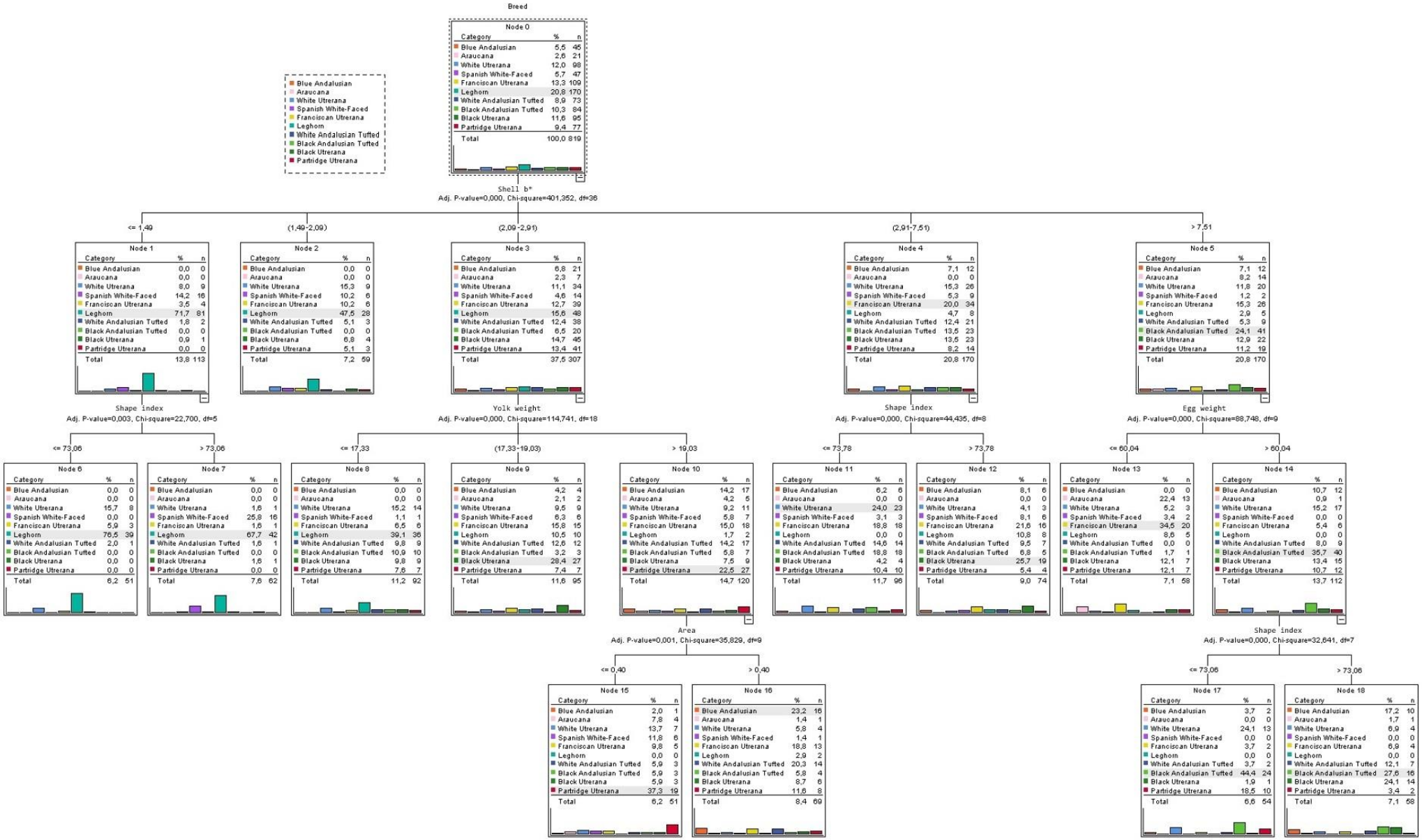
Albumen height, yolk diameter, eggshell thickness, major diameter, and minor diameter measurements are expressed in millimeters; eggshell weight, yolk weight, albumen weight, and egg weight measurements are expressed in grams; eggshell strength measurements are expressed in kilograms; are measurements are expressed in kilograms/seconds.

**Supplementary Table S2.** Appropriately classified eggs according to the genotype of the laying hen.

| from \ to | Blue Andalusian | Araucana | White Utrerana | Spanish White-Faced | Franciscan Utrerana | Leghorn | White Andalusian Tufted | Black Andalusian Tufted | Black Utrerana | Partridge Utrerana | Total | % correct |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Blue Andalusian** | 18 | 0 | 1 | 4 | 4 | 4 | 8 | 3 | 2 | 1 | 45 | 40.00 |
| **Araucana** | 0 | 13 | 0 | 2 | 1 | 0 | 2 | 1 | 0 | 2 | 21 | 61.90 |
| **White Utrerana** | 1 | 0 | 55 | 0 | 3 | 17 | 6 | 5 | 8 | 3 | 98 | 56.12 |
| **Spanish White-Faced** | 0 | 0 | 0 | 28 | 8 | 5 | 4 | 0 | 1 | 1 | 47 | 59.57 |
| **Franciscan Utrerana** | 3 | 0 | 5 | 6 | 53 | 8 | 13 | 8 | 6 | 7 | 109 | 48.62 |
| **Leghorn** | 2 | 0 | 2 | 1 | 4 | 155 | 2 | 0 | 4 | 0 | 170 | 91.18 |
| **White Andalusian Tufted** | 6 | 0 | 7 | 3 | 6 | 4 | 30 | 11 | 4 | 2 | 73 | 41.10 |
| **Black Andalusian Tufted** | 4 | 0 | 2 | 1 | 9 | 8 | 17 | 41 | 1 | 0 | 83 | 49.40 |
| **Black Utrerana** | 6 | 1 | 8 | 3 | 11 | 7 | 7 | 4 | 42 | 6 | 95 | 44.21 |
| **Partridge Utrerana** | 4 | 0 | 7 | 3 | 12 | 4 | 10 | 3 | 9 | 25 | 77 | 32.47 |
| **Total** | 44 | 14 | 87 | 51 | 111 | 212 | 99 | 76 | 77 | 47 | 818 | 56.23 |

**Supplementary Table S3.** Leave-one-out cross-validation of eggs according to the genotype of the laying hen.

| from \ to | Blue Andalusian | Araucana | White Utrerana | Spanish White-Faced | Franciscan Utrerana | Leghorn | White Andalusian Tufted | Black Andalusian Tufted | Black Utrerana | Partridge Utrerana | Total | % correct |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Blue Andalusian** | 13 | 0 | 1 | 5 | 5 | 5 | 8 | 4 | 3 | 1 | 45 | 28.89 |
| **Araucana** | 0 | 13 | 0 | 2 | 1 | 0 | 2 | 1 | 0 | 2 | 21 | 61.90 |
| **White Utrerana** | 1 | 0 | 48 | 0 | 3 | 17 | 7 | 6 | 10 | 6 | 98 | 48.98 |
| **Spanish White-Faced** | 0 | 0 | 0 | 25 | 9 | 7 | 4 | 0 | 1 | 1 | 47 | 53.19 |
| **Franciscan Utrerana** | 4 | 0 | 5 | 7 | 42 | 8 | 15 | 11 | 8 | 9 | 109 | 38.53 |
| **Leghorn** | 2 | 0 | 3 | 1 | 4 | 152 | 4 | 0 | 4 | 0 | 170 | 89.41 |
| **White Andalusian Tufted** | 6 | 0 | 10 | 3 | 6 | 4 | 26 | 11 | 5 | 2 | 73 | 35.62 |
| **Black Andalusian Tufted** | 4 | 0 | 3 | 1 | 10 | 9 | 17 | 38 | 1 | 0 | 83 | 45.78 |
| **Black Utrerana** | 7 | 1 | 8 | 3 | 12 | 8 | 7 | 4 | 38 | 7 | 95 | 40.00 |
| **Partridge Utrerana** | 4 | 0 | 9 | 3 | 14 | 4 | 13 | 3 | 10 | 17 | 77 | 22.08 |
| **Total** | 41 | 14 | 87 | 50 | 106 | 214 | 103 | 78 | 80 | 45 | 818 | 50.37 |




**Supplementary Figure S1.**

# Hen breed and variety factors as a source of variability for the chemical composition of eggs

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**Abstract:** Eggshell, white and yolk chemical composition was compared across Utrerana breed varieties (black, partridge and franciscan) and Leghorn Lohmann LSL-Classic lineage. An Utrerana hens flock (n = 51) and a control group of Leghorn hens (n = 17) were housed individually allowing egg identification and quality characteristics assessment. Eggshell, yolk and white macroelements and microelements, carbohydrates, moisture, ashes, protein, fat (polyunsaturated and saturated), sugars, cholesterol, and α-tocopherol contents were analyzed. Simultaneously, itemized yolk fatty acids composition was evaluated. Calcium contents were higher in Utrerana eggshell (358.53 g/kg vs. 337.01 g/kg) and white (593.75 mg/kg vs. 584.31 mg/kg), while protein contents were higher in Utrerana yolk (17.40 % vs. 16.90 %) and white (10.60 % vs 10.30 %). Utrerana yolks reported higher α-tocopherol (102.00 mg vs. 88.00 mg), total polyunsaturated fatty acids (19.80 % vs. 16.60 %), and some monounsaturated fatty acids content (C18:1 n9: 42.68 % vs. 41.31 %; C16:1 n9: 0.60 % vs. 0.50 %). Black variety and Leghorns reported higher linoleic acid contents (13.72 % and 13.27 %, respectively) than the rest of the Utrerana varieties. Conclusively, detailed knowledge on differentiated properties of eggs depending on the animals originating them enables a correctly approaching market needs to improve the profitability and find a competitive niche for local products.

**Keywords**: Micronutrients; macronutrients; moisture; polyunsaturated and monounsaturated fatty acids (PUFA and MUFA); local breed; commercial lineage

## 1. Introduction

Since its creation during the first half of the 20th century, Utrerana avian breed has been productively oriented towards a laying aptitude. Utrerana's eggs weight around 64 g and present a characteristic white eggshell color. The breed's annual egg output ranges from 120 to 180 eggs. Despite its traditional link to egg production, the use of surplus males for meat production has also provided the breed with a collateral dual purpose nature (Campo, 2007). This breed is characterized by a high environmental adaptability to the conditions of the extensive family farms found in the Andalusian countryside from southern Spain (Campo, 2007).

Not only the four varieties of Utrerana breed present particular features in regards the colour of their feathers, tarsus, and skin (namely, partridge, franciscan, black and white), but also, the eggs from Utrerana physically and organoleptically differ among varieties and from those of commercial laying lineages (González Ariza et al., 2019a, c).

Avian eggs represent an important source of nutrients which contains all the proteins, lipids, minerals, and vitamins that enable the development of the embryo. Some of these constituents, such as enzymes or immune proteins, may have multibiological functions (Nowaczewski et al., 2013). As a result, egg is present in a great fraction of the human diet across the different cultures in the world. This added value of eggs is supplemented by its wide cuisine applicability as a product. They can be used for breakfast or home meal preparations, baking and as an ingredient in many culinary recipes.

In this context, the emergence of commercial hybrid lines of laying hens, brought about an increase in the productive capacity of egg and meal from the middle of the last century. As a result, local genotypes were replaced and with them the genetic diversity that such resources implied (Cardellino, 2003). Commercial hybrid genotypes present homogeneous product characteristics that may no longer fulfil market demands considering the need of customers for new products. Under this framework, some authors have addressed that differences among native breeds and varieties may promote the increase and diversification of egg cuisine opportunities,

at the same time that they open new commercial niches, as it has been reported for the Utrerana breed (González Ariza et al., 2019a).

Despite the dual-purpose of some of these native breeds, which decreases their productive performance in comparison to egg or meat specialized breeds (Castellini et al., 2010), they are usually more resistant to pathologies, thermal stress, environmental conditions and have a great ability to search for food in the wild (Palacios et al., 2016; Lordelo et al., 2017). Additionally, the comparatively reduced productivity of these animals may be counteracted by the greater quality of their products, which helps widening the variety of foods offered to the market.

As a way to better respond to the necessities of the market, recent research has focused on scientifically proving the superior organoleptic and sensorial attributes of Utrerana breed eggs when compared to the eggs of a laying lineage among cuisine professionals (González Ariza et al., 2019a). Such attributes may base on the difference in the chemical composition between eggs produced by different strains.

Conservation of animal genetic resources cannot be understood without considering these animals as productive units within a sustainable framework (Lordelo et al., 2020). With this in mind, biodiversity conservation plays a pivotal role as it may allow the conservation of animal genetic resources and the maintenance of local populations in the rural areas where they originated through the promotion of their products (Barba et al., 2016). Farm animal sustainability and welfare are intertwined concepts which increasingly concern a greater part of society every day. Therefore, alternative forms of livestock, such as free range and organic farms become necessary (Taylor et al., 2017).

Seeking an increased productivity, commercial genotypes are usually produced under free range and organic production systems, as barn systems. However, these genotypes might have a lower ability to adapt to these organic and free-range systems than local breeds, which in turn translates into a decrease in profitability and lower survival and resistance to animal diseases (Alderson, 2018). Oppositely, local breeds, and among them, the Utrerana hen, are perfectly adapted to thrive under the conditions of these aforementioned production systems.

The conditioning effect of nutrition has been reported to be more influential than genetics in regards to the chemical composition of eggs. However, there are some

relevant pieces of evidence for a heredity influence on some aspects of egg composition; namely, relative proportion of yolk and white, white quality, qualitative protein polymorphism, total protein content, contents of cholesterol, vitamin A, thiamine, riboflavin, fatty acids, enzymes, and deposition of metabolic products in the eggs (Washburn, 1979).

Another important aspect regarding the chemical composition of the egg of laying hens is the composition of heavy metals in eggs, which usually derives from the fishmeal that is supplied to animals in their diets (Farahani et al., 2015). Feed provided to laying hens may often be contaminated with mercury or arsenic, among others. However, the ability of these animals to metabolize such substances, and the heritable component of this ability may become relevant, as they may translate into the reduction of the accumulation of residues in the final products that reach the market, which minimizes the potential harms to humans (Ding et al., 2019).

Egg components are not only quantitatively conditioned by genetic factors, but can also be qualitatively affected. Certain egg quality parameters, such as the proportion of yolk and albumen and albumen quality have been previously studied in Utrerana hens given their implications with the final value of the products (González Ariza et al., 2019c). Contextually, chemical composition concerning the metabolism and concentration of diverse elements of nutritive interest in laying hens, like fatty acid composition have been reported to be affected by other factors such as age, genotype and environmental (Rizzi and Chiericato, 2010). Furthermore, apart from the influence of genetic factors themselves, an interaction between strain and environmental conditions has been reported in literature and has been suggested to condition the absorption and use of dietary components by laying hens. This genetically dependent interaction may play a decisive role in the determination of the ability to incorporate some diet nutrients like fatty acids to the products to which they give origin (Rizzi and Chiericato, 2010).

Among other important nutritional resources that can be conditioned by heritable components, albumen quality widely reflects the variation in protein polymorphism, which have been shown to present high heritability values (Washburn, 1979). This high heritabilities contribute to the greater possibilities to

perform effective selection strategies based on a wider genetic variability among individuals.

To this aim, the first goal of this study was to compare the chemical composition (carbohydrates, moisture, raw ashes, crude protein, crude fat, polyunsaturated fatty acid (PUFA), saturated fatty acid (SFA), sugars, cholesterol, and vitamin E) of the yolk and the white of eggs produced by hybrid commercial strain hens to those of Utrerana hen breed reared under the same production system. Secondly, we assessed the ability of these hen breeds to metabolize heavy metals and to accumulate them in different parts of the egg (yolk, white and eggshell). Finally, we determined the concentration of fatty acids that deposits in the yolk of the three varieties of the Utrerana avian breed (black, partridge, and franciscan) in comparison to those from the eggs produced by a Leghorn Lohmann LSL-Classic lineage (Leghorn further on the text).

## 2. Materials and Methods

### *2.1. Institutional animal care and use committee statement*

Animals were housed following specific codes of good practices and therefore, the receiving humane care in compliance with the national guide for the care and use of laboratory and farm animals in research. The study was conducted in accordance with the Declaration of Helsinki. The Spanish Ministry of Economy and Competitivity through the Royal Decree Law 53/2013 and its credited institution the Ethics Committee of Animal Experimentation from the University of Córdoba permitted the application of the protocols present in this study as cited in the 5$^{th}$ section of its 2$^{nd}$ article provided the animals considered in the study were used for credited zootechnical use. This national Decree follows the European Union Directive 2010/63/UE, from the 22nd of September of 2010.

### *2.2. Layer flock and environmental conditions*

The experiment was carried out in the public hatchery located at the Agropecuary Provincial Centre of Diputación of Córdoba, Spain (37° 54’50.9"N-4° 42’40.4"W). The eggs used in the study were obtained from a layer flock of 51 autochthonous Utrerana hens and 17 Leghorn Lohmann LSL-Classic lineage hens at

70 weeks of age. The breeding flock comprised 17 hens of each Utrerana variety (black, franciscan and partridge).

The layer flock, from which the eggs were collected, was reared in individual cages (50 × 62 × 41 cm) following Council Directive 1999/ 74/EC of 19 July 1999, laying down minimum standards for the protection of laying hens at the Agropecuary Provincial Centre of the Council Office of Córdoba (Spain), for 6 months (January to June 2018). All the animals were fed on the same commercial feed (15.2 % crude protein, 4.1 % calcium, 0.66 % available phosphorus) for the whole experimental period. Table S1 describes the composition of the feed supplied to the animals. Feed and water were available ad libitum. The birds were subjected to the same prophylaxis procedures (Marek's disease, Newcastle disease, Gumboro disease, infectious bronchitis, coccidiosis, and fowl pox vaccines, Table S2) and rearing conditions (temperature and photoperiod) until the end of the experimental period.

All the birds were reared according to the regulations of the European Union (2010/63/EU) in their transposition to the Spanish law (RD 53/2013). Further information regarding the rearing system used can be consulted in González Ariza et al. (2019c).

*2.3. Egg pool*

A total of 5090 eggs (3678 egg from Utrerana hens and 1412 from Leghorn Lohmann LSL-Classic hens) were collected in three different phases of the hen laying cycle (at the beginning (25-36 weeks of hen age), in the middle (47-52 weeks) and at the end (70-73 weeks), respectively). This was performed following the premises described in Campo (2007), with the aim to perform a complete sampling of the different stages of the laying curve as maturity in this breed is reached around 25 weeks of age.

Whole eggs and shell, albumen and yolk were individually weighted. Albumen height was measured as referred in González Ariza et al. (2019c). All eggs collected were analyzed and the outputs derived from chemical analyses were considered for the statistical analysis. At 52 weeks of age, a total of 48 eggs (36 egg from Utrerana hens; 12 per variety and 12 from Leghorn Lohmann LSL-Classic hens) were collected per group during the production period going from March to June. Defective eggs (double yolk and/or abnormal shell) were excluded.

The yolk was manually separated from the albumen 3 times (by means of a yolk/white separator composed of the top cup designed to retain the yolk while letting the albumen slide to the bottom cup) to obtain 12 samples per Utrerana variety and 12 from Leghorn Lohmann LSL-Classic. Samples were frozen at -20 °C up to 20 days for further laboratory analyses as described in Rizzi and Chiericato (2010).

*2.4. Basic chemical composition*

The moisture, ash, carbohydrates, sugars, protein, lipid and α-tocopherol contents in albumen and yolk were quantified. Egg samples were separately homogenized by a laboratory blender (Moulinex A327, France). Carbohydrate content was computed from total sum of moisture, fat, protein, ash percentage and subtracted from 100. Moisture was determined by drying the samples in a conventional oven at 98 °C for 24 h according to AOAC (2000). The ash content was analyzed by ashing the samples using a muffle furnace oven at 525 °C for 12 h (Hui and Sherkat, 2005). The total fat content in the albumen and yolk was assessed using $CO_2$ in the supercritical state (TFE-2000 analyzer, LECO, USA) (Domagała et al., 2010). In order to assess protein content, the nitrogen amount was determined by the Dumas method, by means of the TruSpec N LECO Company Analyzer. Values of N% were multiplied by 6.25 to calculate the protein percentage.

The sugar composition in eggs was measured using Megazyme assay kits (Megazyme International Ireland Ltd, Wicklow, Ireland). Albumen and yolk were homogeneized separately (Vitamix, Olmsted Township, Ohio). The amount of individual sugars in the egg samples was determined by reading the absorbance of sample solutions on a 96-well plate at 340 nm using a microplate reader (SpectraMax 190; Molecular Devices, Sunnyvale, California), and calculation of their concentration was performed using formulas described in the kit procedures. Samples were weighed and values normalized per 100 g weight. α-tocopherol analysis was conducted by saponification and liquid extraction of organic solvents with HPLC-Fluorescence detection.

As part of the quality control of the above methods, Certified Reference Materials were tested. The laboratory competences in all analytical methods were confirmed in interlaboratory/international test.

*2.5. Multielemental composition*

The elements analyzed were aluminum (Al), arsenic (As), boron (B), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Farahani et al.), iodine (I), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), selenium (Se) and zinc (Zn). Approximately 0.2-0.5 g of sample were digested in screw-cap Teflon bombs with concentrated high purity nitric acid. Bombs were heated from 2 to 8 h and opened three times to release $CO_2$ buildup. Most elements in the digestate were analyzed with a Perkin-Elmer, model ELAN 5000, inductively coupled plasma spectrometer. As, Se, Pb, and Cd were analyzed with a Varian VGA-76 hydride generation accessory mounted to an atomic absorption spectrophotometer, AA Perkin-Elmer, model 3030. Hg was analyzed by the standard cold vapor atomic absorption method. The lowest detection limits were calculated by a standard procedure which is based on the analysis of seven samples of the matrix with the analyte. Percent recoveries of spiked samples and certified reference materials were above 90 % in most cases. Mean relative percent differences between duplicates were <10 %.

*2.6. Fatty acid composition*

Total egg yolk lipids were extracted using the FAT Extractor TFE 2000 Leco, St. Joshep USA, with liquid carbon dioxide as a solvent. After the extraction of fat, the lipids were esterified according to the method described by De Man (1964). 0.1 mL of extracted fat was placed in the glass test tube of 2 mL capacity, and 0.5 mL of 0.025 M solution of sodium methylate was added. The mixture was heated in a closed tube at 60 °C until the mixture was clear. The analysis of fatty acids was carried out using gas chromatograph Trace GC Ultra (Thermo Electron Corporation, USA) with a Supelcowax 10 column (dimensions 30 m x 0.25 mm x 0.25 mm). Helium as a gaseous phase was applied with the flow rate of 5 mL/min. The feeder was set at the temperature of 220 °C. The temperature of the column was kept for 3 min at 60 °C, then increased at a rate of 7 ºC/min up to 200 °C and held at this temperature for 20 min. The detector had the temperature of 250 °C and the split flow was 10 mL/min. (Kostogrys et al., 2017). The fatty acid percentage was calculated with respect to the total fatty acid mass expressed as total area. Each fatty acid was identified in the

form of a methyl ester by comparing the retention times with the standard sample (F.A.M.E. mix C4-C24, lipid standard, 18919-1AMP, Sigma-Aldrich, St. Louis, MO).

*2.7. Cholesterol content*

Cholesterol content in yolk was determined following the method described in Kostogrys et al. (2017). Cholesterol was extracted from the egg yolks by hexane after saponification by 60 % potassium hydroxide. The extracts were analyzed using a gas chromatograph equipped with a FID detector (Model GC-2010, Shimadzu Corporation, Kyoto, Japan FID: 300 °C). A non-polar column was used for the analysis of cholesterol (Zebron ZB-5, l = 30 m, I.D. = 0.25, df = 0.25). The injector and detector temperatures were set at 300 °C, split 1:10. The internal standard (5a-Cholestan) was used. Helium was used as a carrier gas at a flow rate of 1.9 mL/min, volume of sample 5 µL. Analysis time was 30 min.

*2.8. Statistical analysis: egg pool descriptive statistics*

For eggshell, the components analyzed were as follows, Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Ni, P, Pb, S, Se and Zn. Apart from the components that were previously analyzed for the eggshell, carbohydrates %, moisture (4 h a 103 °C) %, raw ashes (out of fresh matter) %, raw protein (out of fresh matter) %, fat raw (out of fresh matter) %, polyunsaturated fat (PUFA) %, saturated fats %, sugars mg/kg, cholesterol (mg) and vitamin E (α-tocopherol) (mg) were also determined for the yolk and white. Breed (Utrerana and Leghorn Lohmann LSL-Classic lineage) and varieties (black, franciscan and partridge) were considered independent categorical factors.

Although, separate pools for Utrerana varieties were not analyzed for general chemical composition (given the costs involved), fatty acid detailed profile was computed using pools for each variety as follows.

To determine and quantify the effect of the breed factor (Utrerana vs Leghorn) on the chemical composition of eggs through pooled sampling, we opted for the implementation of pooled-variance t procedures provided their robustness to violations of the normality assumption than their one-sample counterparts. Pooled variances were computed after squaring pooled standard deviations (sp) as follows (McNaught and Wilkinson, 1997):

$$S_p = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + \cdots + (n_k - 1)s_k^2}{n_1 + n_2 + \cdots + n_k - k}}$$

The subindexes 1, 2, …, k refer to the different series of measurements. In pooled sampling conditions, a single underlying standard deviation (σ) is assumed to exist across groups. In this context, pooled standard deviation sp is a better estimate than the individual calculated standard deviations $s_1$, $s_2$, …, $s_k$. If k sets of duplicate measurements are available, the above equation reduces to (McNaught and Wilkinson, 1997),

$$S_p = \sqrt{\frac{\sum(x_{i1} - x_{i2})}{2k}}$$

Afterward, Cohen's d effect size (Cohen, 1977) can be calculated as follows, $d = \frac{x_1 - x_2}{s_p}$. Cohen's d works best for larger sample sizes (> 50). A correction factor is available, which reduces effect sizes for small samples as follows, $d = \frac{x_1 - x_2}{s_p}\left(\frac{n-3}{n-2.25}\right)\sqrt{\frac{n-2}{n}}$. A d of 1 indicates the two groups differ by 1 standard deviation, a d of 2 indicates they differ by 2 standard deviations, and so on. Standard deviations are equivalent to z-scores (1 standard deviation = 1 z-score). To interpret Cohen's d, "rule of thumb" guidelines should be used cautiously. Ranges found in literature state that a value for Cohen's d of 0.20 to 0.25 should be considered a small effect, a value of 0.5 to 0.75 should be considered a medium effect and a value of 0.8 or above should be considered a large effect. By "small" effects we consider those effects which are difficult to see with the naked eye, "medium" effects may probably be big enough to be discerned with the naked eye, while effects that are "large" can definitely be seen with the naked eye (Cohen calls this "grossly perceptible and therefore large"). Oppositely, values for Cohen's d below 0.2 may denote trivial effects, even if statistically significant differences have been found. A "large" effect is not necessarily better than a "small" effect, especially in settings where small differences can have a major impact. Hence, prior research should be consulted for an idea of where our findings fit into the bigger context.

In small sample contexts, the bias occurring is slightly smaller for Hedges' g alternative method, which uses n-1 for each sample as follows $g = \frac{d}{\sqrt{\frac{n}{df}}}$, where; n is sample size and df are the degrees of freedom. Additionally, d could also be transformed into r correlation coefficient through the following formula $r = \frac{d}{\sqrt{d^2+4}}$. Remarkably, Cohen's d is not influenced by the ratio of $n_1$ to $n_2$, but rpb and eta-squared are, hence Cohen's d may be preferable. The magnitude of effect power should be considered bearing the seriousness of Type II to Type I errors in mind. For $P < 0.05$, r are frequently characterized as small (r = 0.10), medium (r = 0.30), and large (r = 0.50) as described by Cohen (1977). At a laboratory level, even small effects could be detected, as error terms are often attempted to be kept at very small levels. However, the common conditions simultaneously dealt with when a small effect is detected, such as the existence of a small sample, and a binary decisional $P < 0.05$ makes interpreting such small effects difficult. As stated above, Cohen (1977) recommended a value of 0.8 as a convention for the desirable level of power. With a "small" effect (i.e., r = 0.10, d = 0.20), a power of 0.8 would require us to employ a total N of approximately 1000 to detect various effects at p = 0.05, two-tailed (Cohen, 1977). With a "medium" effect (i.e., r - 0.30, d -0.63), it would mean a total N of approximately 115 sampling units, and with a "large" effect (i.e., r -0.50, d = 1.15) a total N of approximately 40 sampling units, to detect various effects at p = 0.05, two-tailed.

*2.9. Effect power of breed and variety factors on shell, yolk and white chemical composition*

The effect power of the factors of breed and variety were also computed following the premises developed by Onderdeel van Slim Academy. According to these guidelines, we must first find out the value of x under the wrong null hypothesis to figure out when H0 is rejected under the wrong null hypothesis. Then, according to the following equation, $z = \frac{\overline{x_1} - \mu}{\frac{\alpha}{\sqrt{n}}}$, we may possibly calculate the correct probability of rejecting H0 (Ha = μ2), as follows; $PHa = (\bar{x} > \overline{x_1}) = PHa =$

$\left(\frac{\bar{x}-\mu_2}{\frac{\alpha}{\sqrt{n}}}\right) > \left(\frac{\overline{x_1}-\mu_2}{\frac{\alpha}{\sqrt{n}}}\right)$, solving the equation considering the area under a normally distributed curve is 1, as follow $PHa = \left(Z > \left(\frac{\overline{x_1}-\mu_2}{\frac{\alpha}{\sqrt{n}}}\right)\right) = 1 - \alpha_Z$.

At this point, we may be able to determine rejection region for twotailed Z Test (H1: μ≠ μ0) with α = 0.05. The decision rule to make is, we either reject H0, if Z < -1.960 or Z > 1.960 and consider mean from first group to be higher than mean from second group, or we accept H0 when -1.960 ≤ Z ≤ 1.960, and determine both means are statistically equal.

*2.10. Effect power of breed factor on fatty acid detailed composition*

The second section of the study aimed at assessing the effects of breed and variety (Utrerana and Leghorn Lohmann LSL-Classic lineage) on fatty acid detailed composition profile. All the data available were tested to check whether data violated the assumptions for regular parametric tests to report valid results.

Those fatty acids in the profile that followed a normal distribution (P > 0.001) and were homoscedastic were subjected to one-way ANOVA as a completely randomized design, with breed (Utrerana and Leghorn Lohmann LSL-Classic lineage) and variety (black, franciscan and partridge) as the independent factors, using independent samples t-test and One-Way ANOVA tasks and Waller-Duncan posthoc test, respectively, from the Compare Means procedure of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016).

Contrastingly, when a certain fatty acid component did not fit to a normal distribution or were heteroscedastic (P < 0.001), we used a Mann-Whitney U test to compare differences between the two independent groups of the breed factor (Utrerana and Leghorn Lohmann LSL-Classic lineage). Then, a Kruskal-Wallis H test was performed to study the potentially existing differences across the three levels of the variety factor (black, franciscan and partridge). After conducting Mann-Whitney U test (for two groups) and Kruskal-Wallis H with three or more groups (k), we measured the strength of the effect of the factors of breed and variety on fatty acids profile using r and partial eta squared ($\eta p^2$) as quantification measures depending on whether Mann-Whitney U or Kruskal-Wallis H tests had been carried

out beforehand. Partialeta squared was computed following the methodology described and reported for non-standard evaluations in research (Li et al., 2019).

Cohen's guidelines for r are that a small effect is 0.1, a medium effect is 0.3, and a large effect is 0.5. Calculation of r, $r^2$, or $\eta^2$ from these z values is possible because partial eta-squared ($\eta p^2$) can be computed as the ratio of variance associated with an effect, plus that effect and its associated error variance. Crosstabs procedure from SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016) were used to calculate $\eta p^2$ and to measure the strength of association between the categorical independent factors of breed and variety and the fatty acids comprised in the profile considered in our study.

Cohen's f allows to analyze the relationship between a quantitative variable and a categorical variable in the case where the latter has more than two possible levels (k values). SPSS cannot calculate the Cohen's f directly, but they can be calculated using partial $eta^2$ ($\eta p^2$). Cohen analyzes the relationship between the d and f values of Cohen and partial Eta2, through $\eta^2 = \frac{f^2}{(1+f^2)}$ and $f = \sqrt{\frac{\eta^2}{(1-\eta^2)}}$, respectively, where $f^2$ is the square of the effect size, and $\eta^2$ is to $\eta p^2$ calculated by SPSS (Cohen, 1988).

Once effect power has been determined, we evaluated pairwise comparisons across the levels of the independent factors of breed and variety for which the Kruskal-Wallis test had resulted significant using the Dunn's test. Then, Bonferroni correction was applied to compensate for the increase in the likelihood of incorrectly rejecting the existence of statistically significant differences between two or more groups.

All nonparametric tests were carried out using the independent samples package from the non-parametrical task of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016).

## 3. Results

A summary of the results for pooled descriptive statistics (mean, mean difference and pooled standard deviation) and effect power (Cohen's d, Corrected d, Hedge's g, r correlation coefficient and Pha (Z)) for the breed factor on general chemical composition of the shell, yolk and white derived from pooled-variance t-test procedures is shown in Tables S3, S4 and S5, respectively.

Fatty acids data distribution properties are shown in Table S6 (Shapiro-Wilk's, $P < 0.001$ for significant results). Levene's test for equality of error variance reported that the error variance around predicted scores was not the same for all predicted values ($P < 0.001$), thus there was not homogeneity of variances for each combination of the levels of the independent factors, and the assumption of homoscedasticity was violated. A summary of descriptive statistics for fatty acids profile in egg yolk is shown in Table S7.

The existence of outliers could be a cause for the lack of normality of the variables studied in our population. Walsh's Outlier non-parametric test was used to detect multiple outliers in the data set. Although this test requires a large sample size ($n>220$ for a significance level $\alpha$ of 0.05), it may be used whenever the data are not normally distributed. However, no outlier was detected. We assessed multicollinearity through tolerance and variance inflation factor (VIF). If the VIF value lies between 1-10, then there is no multicollinearity. However, if the $VIF < 1$ or >10, then there is multicollinearity. Multicollinearity can also be detected with the help of tolerance, its reciprocal. A tolerance close to 1 means there is little multicollinearity, whereas a value close to 0 suggests that multicollinearity may be a threat. Hence, we used nonparametric tests to statistically assess the information recorded.

For nonnormally distributed fatty acids, a summary of the results for Mann-Whitney U test to assess for the effect of the breed factor on the detailed composition of fatty acids and for Kruskal-Wallis H and Dunn tests (nonnormally distributed fatty acids) to assess for the effect of the variety factor on the detailed composition of fatty acids is shown in Table 1. Afterward, Table 2 reports a summary of the results for median t-test for independent samples to detect differences in the medians for fatty acids composition between Utrerana and Leghorn breeds and across varieties of Utrerana and Leghorn's egg yolks.

Contrastingly for normally distributed fatty acids, Table 3 shows a summary of the results for one-way ANOVA test to detect differences in the mean across Utrerana varieties and Leghorn's egg yolk fatty acids profile. Then, Table 4 shows a summary of the results for one-way ANOVA to detect differences in the mean between Utrerana breed and Leghorn breed's egg yolk fatty acids profile.

After one-way ANOVA results have been presented in Tables 3, 4 and 5 reports a summary of the results for the Waller-Duncan posthoc test to detect differences across the mean for varieties of Utrerana and Leghorn's egg yolk fatty acids profile.

**Table 1.** Summary of the results for Mann Whitney's U to detect differences in fatty acids composition between Utrerana and Leghorn's eggs and for Kruskal-Wallis H to detect differences in fatty acids composition across eggs from the different Utrerana varieties (Black, Franciscan and Patridge) and Leghorns'.

| Factor | Breed (df=1) | | Variety (df=3) | |
|---|---|---|---|---|
| Parameter | Mann Whitney U | Asymp. Sig. | Kruskal-Wallis H | Asymp. Sig. |
| C14:0 | 2.687 | 0.101 | 7.004 | 0.072 |
| C16:1 n7 | 1.212 | 0.271 | 5.300 | 0.151 |
| C17:0 | 1.896 | 0.169 | 2.907 | 0.406 |
| C18:2 n6 | 5.650 | 0.017 | 8.506 | 0.037 |
| C18:3 n3 | 4.419 | 0.036 | 6.317 | 0.097 |
| C20:1 n9 | 1.105 | 0.293 | 1.602 | 0.659 |
| C20:2 n6 | 2.567 | 0.109 | 4.422 | 0.219 |
| C20:4 n6 | 3.712 | 0.054 | 5.019 | 0.170 |
| C22:4 n6 | 0.735 | 0.391 | 3.402 | 0.334 |
| C22:5 n3 | 0.063 | 0.802 | 0.134 | 0.987 |
| C22:6 n3 | 1.053 | 0.305 | 2.110 | 0.550 |

**Table 2.** Summary of the results for median t test for independent samples to detect differences in the means for fatty acids composition between Utrerana and Leghorn breeds and across varieties of Utrerana and Leghorn's egg yolks.

| **Factor** | **Breed (df=1)** | | | **Variety (df=3)** | | |
|---|---|---|---|---|---|---|
| Parameter | Median | Chi-Square | Asymp. Sig. | Median | Chi-Square | Asymp. Sig. |
| C14:0 | 0.282 | 2.310 | 0.129 | 0.282 | 7.527 | 0.057 |
| C16:1 n7 | 2.204 | 0.000 | 1.000 | 2.204 | 4.933 | 0.177 |
| C17:0 | 0.137 | 0.247 | 0.619 | 0.137 | 0.982 | 0.806 |
| C18:2 n6 | 12.605 | 4.059 | 0.044 | 12.605 | 7.267 | 0.064 |
| C18:3 n3 | 0.258 | 1.804 | 0.179 | 0.258 | 3.400 | 0.334 |
| C20:1 n9 | 0.279 | 0.451 | 0.502 | 0.279 | 1.400 | 0.706 |
| C20:2 n6 | 0.145 | 1.804 | 0.179 | 0.145 | 5.267 | 0.153 |
| C20:4 n6 | 2.151 | 0.451 | 0.502 | 2.151 | 1.400 | 0.706 |
| C22:4 n6 | 0.000 | 1.458 | 0.227 | 0.000 | 5.715 | 0.126 |
| C22:5 n3 | 0.169 | 0.031 | 0.861 | 0.169 | 0.247 | 0.970 |
| C22:6 n3 | 0.551 | 1.804 | 0.179 | 0.551 | 2.667 | 0.446 |

**Table 3.** Summary of results for One way ANOVA test to detect differences in the mean across Utrerana varieties and Leghorn's egg yolk fatty acids profile.

| Fatty acids profile | Parameters | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| C16:0 | Between Groups | 13.809 | 3 | 4.603 | 2.805 | 0.051 |
| C16:0 | Within Groups | 68.925 | 42 | 1.641 | | |
| C16:0 | Total | 82.734 | 45 | | | |
| C16:1 n9 | Between Groups | 0.205 | 3 | 0.068 | 5.345 | 0.003 |
| C16:1 n9 | Within Groups | 0.537 | 42 | 0.013 | | |
| C16:1 n9 | Total | 0.742 | 45 | | | |
| C17:1 | Between Groups | 0.001 | 3 | 0 | 1.183 | 0.328 |
| C17:1 | Within Groups | 0.018 | 42 | 0 | | |
| C17:1 | Total | 0.019 | 45 | | | |
| C18:0 | Between Groups | 6.214 | 3 | 2.071 | 1.95 | 0.136 |
| C18:0 | Within Groups | 44.61 | 42 | 1.062 | | |
| C18:0 | Total | 50.824 | 45 | | | |
| C18:1 n9 | Between Groups | 61.642 | 3 | 20.547 | 5.829 | 0.002 |
| C18:1 n9 | Within Groups | 148.056 | 42 | 3.525 | | |
| C18:1 n9 | Total | 209.698 | 45 | | | |
| C18:1 n7 | Between Groups | 0.177 | 3 | 0.059 | 2.244 | 0.097 |
| C18:1 n7 | Within Groups | 1.107 | 42 | 0.026 | | |
| C18:1 n7 | Total | 1.284 | 45 | | | |
| C20:3 n6 | Between Groups | 0.009 | 3 | 0.003 | 4.733 | 0.006 |
| C20:3 n6 | Within Groups | 0.026 | 42 | 0.001 | | |
| C20:3 n6 | Total | 0.035 | 45 | | | |
| C22:5 n6 | Between Groups | 0.074 | 3 | 0.025 | 0.998 | 0.403 |
| C22:5 n6 | Within Groups | 1.035 | 42 | 0.025 | | |
| C22:5 n6 | Total | 1.109 | 45 | | | |

## 4. Discussion

### *4.1. Study context and limitations*

The present study was developed in the context of a project financer with FEDER funds (Project PP.AVA.AVA201601.16) entitled "Conservation strategy of Utrerana hen: valorization of its products". Utrerana hen is an endangered breed whose population is comprised by a limited number of individuals (MAPA, 2020). According to Gonzalez Ariza et al., 2019b, although the fertility rate of the animals of this breed was 90.68 ± 0.72 %, the percentage of fertilized egg suffering embryonic death was 14.21 ± 0.98 % with the white feather color obtaining the lowest rates (5.88 ± 2.55 %). Therefore, Utrerana eggs must be destined for incubation to ensure the successful accomplishment of the breed's conservation strategies annually (González Ariza et al., 2019b). This situation is common to other endangered hen breeds, and limits the number of eggs used in research.

Several authors have identified reproductive, productive and genetic differences within the varieties of Utrerana hen and when compared to other breeds (Zofia et al., 2018; González Ariza et al., 2019b, c; Macrì et al., 2019). For this reason, and as it was also suggested by our statistical findings, the determination of the chemical basis on which to support the reproductive and productive characterization of the breed and its varieties can help managing and stabilizing new niches for its products.

Strategies based on standardizing the products of the different varieties can extend or diversify the application of the eggs laid by the hens of the different varieties. This in turn suggests the preservation for the breed diversity may be one of the motor elements to ensure the future survival of a breed, through the enhancement of its ability to cover a wider scope of market demands, hence to reach a broader public.

**Table 4.** Summary of results for One way ANOVA test to detect differences in the mean between Utrerana and Leghorn's egg yolk fatty acids profile.

| Fatty acids profile | Parameters | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| C16:0 | Between Groups | 5.774 | 1 | 5.774 | 3.301 | 0.076 |
| C16:0 | Within Groups | 76.96 | 44 | 1.749 | | |
| C16:0 | Total | 82.734 | 45 | | | |
| C16:1 n9 | Between Groups | 0.125 | 1 | 0.125 | 8.933 | 0.005 |
| C16:1 n9 | Within Groups | 0.617 | 44 | 0.014 | | |
| C16:1 n9 | Total | 0.742 | 45 | | | |
| C17:1 | Between Groups | 0.001 | 1 | 0.001 | 1.689 | 0.2 |
| C17:1 | Within Groups | 0.018 | 44 | 0 | | |
| C17:1 | Total | 0.019 | 45 | | | |
| C18:0 | Between Groups | 0.187 | 1 | 0.187 | 0.162 | 0.689 |
| C18:0 | Within Groups | 50.637 | 44 | 1.151 | | |
| C18:0 | Total | 50.824 | 45 | | | |
| C18:1 n9 | Between Groups | 39.689 | 1 | 39.689 | 10.272 | 0.003 |
| C18:1 n9 | Within Groups | 170.008 | 44 | 3.864 | | |
| C18:1 n9 | Total | 209.698 | 45 | | | |
| C18:1 n7 | Between Groups | 0.022 | 1 | 0.022 | 0.759 | 0.388 |
| C18:1 n7 | Within Groups | 1.263 | 44 | 0.029 | | |
| C18:1 n7 | Total | 1.284 | 45 | | | |
| C20:3 n6 | Between Groups | 0.005 | 1 | 0.005 | 7.78 | 0.008 |
| C20:3 n6 | Within Groups | 0.03 | 44 | 0.001 | | |
| C20:3 n6 | Total | 0.035 | 45 | | | |
| C22:5 n6 | Between Groups | 0.012 | 1 | 0.012 | 0.463 | 0.5 |
| C22:5 n6 | Within Groups | 1.097 | 44 | 0.025 | | |
| C22:5 n6 | Total | 1.109 | 45 | | | |

**Table 5.** Summary of the results for the Waller-Duncan test to detect differences across the mean for varieties of Utrerana and leghorn's egg yolk fatty acids profile after One-way ANOVA had been performed.

| Fatty acids profile | Subset for alpha = 0.05 | Leghorn | Black Utrerana | Franciscan Utrerana | Partridge Utrerana |
|---|---|---|---|---|---|
| C16:0 | 1 | N/A | 25.416 | 24.881 | 24.260 |
| C16:0 | 2 | 25.658 | 25.416 | 24.881 | N/A |
| C16:1 n9 | 1 | 0.497 | 0.555 | N/A | N/A |
| C16:1 n9 | 2 | N/A | 0.555 | 0.625 | 0.669 |
| C17:1 | 1 | N/A | N/A | N/A | N/A |
| C17:1 | 2 | N/A | N/A | N/A | N/A |
| C18:0 | 1 | N/A | N/A | N/A | N/A |
| C18:0 | 2 | N/A | N/A | N/A | N/A |
| C18:1 n7 | 1 | N/A | N/A | N/A | N/A |
| C18:1 n7 | 2 | N/A | N/A | N/A | N/A |
| C20:3 n6 | 1 | N/A | 0.157 | 0.160 | 0.137 |
| C20:3 n6 | 2 | 0.175 | 0.157 | 0.160 | N/A |
| C22:5 n6 | 1 | N/A | N/A | N/A | N/A |
| C22:5 n6 | 2 | N/A | N/A | N/A | N/A |

N/A: No significant differences in the mean were found.

### *4.2. Eggshell chemical composition*

Significant differences were found between Utrerana and Leghorn eggs in the concentrations of elements measured in eggshells, such as Ca, Cu, Fe, Mg, Mn, Na, P, Pb and S, in which Utrerana has a statistically significant higher mean than Leghorn, however K, Ni and Zn values were higher in Leghorn (Table S3), despite the fact of Küçükyılmaz et al. (2012) stated that eggshell composition is influenced by the quality of the feed and the production system. In any case, these results are in agreement with those obtained in different avian species by other authors (Dalbeck and Cusack, 2006; Dauphin et al., 2018). Higher Ca deposits were found for the eggshell in Utrerana avian breed. Sun et al. (2016) reported several genes could be involved on the avian eggshell calcification during the course of the egg through the uterus. However, for instance, some authors linked the eggshell strength with

calcification-related genes to determinate the eggshell strength (Ahmed et al., 2005). The CALB1 gene is associated with Ca transport, both in uterus and intestine. Hence, the CALB1 expression affects the strength of eggshell and could be variable between breeds or even varieties. In addition, other genes, such as the DMP4 gene, codes for a protein that is used to bind Ca to the eggshell (Wilson, 2017).

Mg is known to inhibit calcite nucleation, favoring the precipitation of aragonite (Rodriguez-Navarro et al., 2002). Therefore, it can be assumed that the Ca-Mg exchange equilibrium depends closely on changes in the concentration of either of these elements. According to our results, Utrerana hens could retain higher Mg and lower Ca in the shell gland mucosa and secrete less Mg and more Ca into the shell gland lumen for eggshell deposition, in comparison to Leghorn hens. The Mg content in Utrerana hens eggshells was much lower than that for Leghorn hens indicating that the eggshell Mg content might have an effect on eggshell quality (Shen and Chen, 2003).

Significant differences between the concentration of Na and Mg on eggshell from Utrerana and Leghorn hens were found. In general, hens' eggshells Mg concentration decreases after shell nucleation at the mammillary caps, showing a recovery in Mg concentration at termination of growth. The variability between breeds and strains may be attributed to organic distribution and crystal size. The same procedure occurs with Na deposits, which also shows a similar pattern between breeds with some exceptions. These differences may also be attributed to the same factors possibly influencing Mg distribution (Dalbeck and Cusack, 2006).

Several authors have suggested that some metals, primarily divalent cations, such as most trace elements, can replace Ca in both the eggshells and the inside of eggs (Van Dyke et al., 2013). In this context, calcium carbonate in an ionic form binds to membrane proteins on the albumen and begins to crystallize. Physiologically, this process continues until protective eggshell is formed, whereby the terminal cuticle is deposited and acts as a bacterial barrier (Wilson, 2017).

Metals could be harmful to humans, especially when they are taken in excessive quantities. Such toxic substances are eliminated by birds using several methods, including normal excretion, but also through their deposition in eggs and feathers (Burger, 1994). We found significant differences between the two breeds studied

with Leghorns showing higher deposits of some heavy metals like Cu, Ni and Zn in most egg structures than Utrerana varieties. In this case, this may be attributed to a greater excretion efficiency, which contrastingly may be detrimental if we consider the egg is the final product to be consumed by humans.

Moreover, the higher concentration found for Mg, Na and K may be responsible for the eggshell's strength. Eggshell with greater concentrations of these micronutrients are usually softer and fragile (Orłowski et al., 2019). According to this authors, Utrerana eggshells should be stronger and stiffer than Leghorn's ones, despite these present a lower eggshell weight than that of Leghorn's (González Ariza et al., 2019c).

In regards Pb concentrations, Patee (1984) reported even if concentrations of Pb were found in the eggshell of certain birds, little or no Pb was transferred to the egg contents (yolk and white). Our results partially agree those reported by Patee (1984) as no significant differences were found between the white of Leghorn's and Utrerana's eggs, even when significant differences had been found between the Pb concentrations in the eggshell of both breeds. The significant differences found between the egg yolks' of Utrerana and Leghorn breed, which contrarily were not reported between the white of both breeds may be supported on the considerably greater ability to capture dietary Pb of yolk in comparison to white when Pb concentrations are 30 mg/kg of diet (Yuan et al. 2013). As shown in Tables S3 to S5, the magnitude of the differences between Pb concentrations in yolk and egg for both breeds is the same. This suggests Pb diet concentrations may be in the limit for which such deposit start to significantly increase in egg white (Yuan et al. 2013).

As suggested by Šály et al. (2004), Pb addition in diet may promote a decrease in egg weight, strength and thickness of eggshell, Ca, Fe and Pb in blood and solidity of the tibiae of layers. This may support our previous results (González Ariza et al., 2019c), which suggested Utrerana's eggshells were significantly lighter ($P < 0.05$) that those of Leghorn's.

*4.3. Multielemental composition of yolk and white*

As for the macroelements in yolk eggs, an important fact is that Ca is lower in Utrerana breed (mean values of 1790.00 mg/kg) than in Leghorn ones (mean values of 2170.00 mg/kg) (Table S4). However, both breeds described higher Ca concentrations than other authors in previous studies (Rubio et al., 2017).

Contrastingly, Utrerana hens showed a significantly higher concentration for some toxic metals Pb, with mean values of 0.55 mg/kg, in comparison with Leghorn ones, with mean values of 0.52 mg/kg, that could produce reproductive and developmental issues when consumed in excess. Still, European legislation does not establish any limits on toxic metals in hens eggs, as it has been reported that the magnitude of these percentages in yolk and white does not imply a risk for health (Rubio et al., 2017).

*4.4. Basic chemical composition of yolk and white*

Utrerana hen breed eggs presented higher concentrations of raw proteins (17.40 %) and raw fats (30.50 %) in the yolk in comparison to Leghorn hens (16.90 and 25.60 % for raw proteins and raw fats, respectively) (Table S4), in agreement with other authors (Yin et al., 2008; Mori et al., 2020). The oil-water interface film forming function of egg yolk proteins decreases interfacial tension. In addition, this protein film acts as a mechanical barrier due to its viscoelastic properties which prevents disruption (Anton et al., 2003). According to others authors, yolk lipids and proteins are not influenced by the strain of hens (Ahn et al., 1997).

Moreover, similar values of crude protein and crude fat were observed in the Romagnola hen, a native Mediterranean avian breed (Sirri et al., 2018). Additionally, Utrerana egg yolks showed lower raw ash content than Leghorn's ones (3.20 vs 4.20 %). These results support those previously reported by other authors (Sirri et al., 2018), since lower ash contents have been reported for native breed yolks when compared to those from selected lines of laying hens.

Oppositely, no significant differences were observed for yolk cholesterol content between breeds and across varieties (Table S4). References have addressed the fact that cholesterol deposition in the egg yolk can be affected by nutrition. However, birds can produce 10 times more cholesterol per kg of liver than humans. Faitarone et al. (2013) reported that birds are able to maintain egg cholesterol at levels which are considered essential to ensure embryo development. For this reason, eggs have been historically blamed for causing coronary disease due to their high cholesterol content, which in turn even promoted its consumption to decrease. Contrastingly, clinical trials have demonstrated the absence of any link between egg intake and an

increase in serum cholesterol concentrations in humans which may oppose to traditional misconceptions (Djoussé and Gaziano, 2008).

Higher concentrations of polyunsaturated and saturated fats in Utrerana hens' egg yolks were found (19.80 and 10.70 % for polyunsaturated and saturated fats, respectively). This finding was also found for the yolk of some Italian hen breed, such as Ermellinata di Rovigo and Robusta maculate. These breeds showed a higher PUFA and SFA proportion when compared to that of a Hy-Line Brown (Rizzi and Marangon, 2012), suggesting possible similarities between fatty acid metabolism in the Utrerana hens and others unselected Mediterranean avian breeds.

Utrerana egg yolks showed significant higher values for vitamin E than Leghorn ones (102.00 vs 88.00 mg/kg). The mechanism of absorption, transportation, metabolism and deposition of maternal vitamin E in birds was linked to heritable influences (Müller et al., 2012). The major form of yolk vitamin E in domestic birds is α-tocopherol (Speake et al., 1999). The intake of grass by birds produces an increase in the levels of α-tocopherol, since the chloroplast membranes are rich in this substance (Speake et al., 1999; Bunea et al., 2017). In this experiment, both Utrerana and Leghorn breeds were fed on the same diets, hence, we can conclude a higher easiness of Utrerana hen to metabolize this substance may exist. In addition, a positive relationship between α-tocopherol contents and carotenoids concentration has been previously described for hens (Skřivan and Englmaierová, 2014). González Ariza et al. (2019c) reported Utrerana egg yolks presented a higher pigmentation than Leghorn ones. At a previous study, a greater difference was also observed between some hen breeds, with Utrerana hen reporting comparable results to those suggested for Rhode Island and Partridge Brahma breeds as far as α-tocopherol content is concerned (Bunea et al., 2017).

Carbohydrate levels found for Utrerana yolk (1.30 %) and white (1.10 %) (Tables S4 and S5) supported the data obtained by previous studies (Naderi et al., 2017; Réhault-Godbert et al., 2019). However, in the present study, carbohydrate content was higher for Leghorn yolks (7.10 %) and lower in Leghorn whites (0.01 %), when are compared to the levels found for the Utrerana breed. These results should be regarded as very positive, given Utrerana eggs may present more desirable features

for high-protein rich diets, especially indicated for people suffering certain dietary-linked or endocrinological conditions such as diabetes (Fuller et al., 2015).

A lower percentage of moisture (84.80 vs 89.70 %) and a higher concentration of carbohydrates (1.10 vs 0.01 %), raw protein (10.60 vs 10.30 %), fat raw (0.50 vs 0.30 %), and therefore, of raw ashes (3.00 vs 2.00 %) was observed in Utrerana whites, when these were compared to Leghorn ones (Table S5). Egg white characteristics have been suggested to be conditioned by the strain of bird and genetic selection (Silversides and Scott, 2001; Bílková et al., 2018). The selection of modern lines of laying hens may have induced an increase in egg weight, which translated in a larger amount of water, which mainly accumulates in the white of the egg. Hence, this greater contribution of water to egg weight, instead of protein, fat and carbohydrates is produced at a lower energetic cost thus translating into a more efficient energy synthesis (González Ariza et al., 2019c).

*4.5. Fatty acid composition of yolk*

Popiela-Pleban et al. (2013) suggested that the fatty acid composition of eggs depend on the composition of the diet provided to the individuals, the bird's digestive system, and the biosynthetic processes of the laying hens, while Goldberg et al. (2013) reported that diet is the main single determinant of fatty acid composition of yolk. Reductions in SFA levels may be considered beneficial for human nutrition as eggs have been criticized for their high SFA contents. This criticism mainly bases on the traditional association of high SFA dietary intake with the development of cardiovascular diseases (Tang et al., 2015). In the present study, the fatty acid composition obtained for both breeds was in concordance with the levels reported for previous studies (Bunea et al., 2017; Kostogrys et al., 2017).

Eggs are naturally poor in linoleic acid (C18:2 n6). However, a higher concentration of this essential fatty acid could be found in the yolk of the eggs of the Leghorn breed and black Utrerana variety in comparison with the rest of Utrerana varieties (franciscan and partridge). However, our results are opposed to those showed by Grobas et al. (2001), that did not observe significant differences in the linoleic acid deposition in the egg yolk of different lines of laying hens. Such an increase in the levels of this fatty acid would be nutritionally advantageous for humans, since we are unable to naturally produce it, hence, it must be supplied in the diet.

Furthermore, essential fatty acid are precursors of hormone-like eicosanoids, such as prostaglandins, leukotrienes, and thromboxanes. These substances are involved in the regulation of heart pressure, heart rate, vascular dilation, blood clotting, immune response, lipolysis, and the central nervous system in humans (Kostogrys et al., 2017).

The changes in the yolk fatty acid composition observed in the current study might be ascribed to differences in the variety metabolism, which translates into changes at several enzymatic processes related to the yolk sac membranes, lipoprotein transport and the activity of the stearoylcoenzime, a desaturase enzyme system in the liver (Latour et al., 1998). As a result, black variety and Leghorn breed eggs could be intended for special human diets with higher requirements of essential fatty acids, as for example in people with skin or cardiovascular diseases (Jandacek, 2017).

A significantly higher content for certain PUFA such as α-linolenic (C18:3 n3) and dihomo-gammalinolenic (C20:3 n6) was found in Leghorn hens. However, total PUFA was significantly higher in Utrerana breed yolks. Lordelo et al. (2020) partially agreed with the findings in this study, since a higher content in α-linolenic was observed in egg yolk of hybrid strain when are compared with those of indigenous chicken breed in Portugal, however, similar results in the content of dihomo-gammalinolenic were reported when compared the native breeds with the commercial strain. This type of fatty acids could be effective to prevent and treat chronic diseases, frequently occurring in Europe. As a result, many researchers have focused on enhancing the PUFA content in egg (Kostogrys et al., 2017). In this context, our results suggest Utrerana breed eggs could be considered functional foods, since they provide pieces of evidence that may support their physiological benefits on human health.

Additionally, the Utrerana hen yolks showed a significantly higher content in some monounsaturated fatty acid, specific, oleic acid (C18:1 n9) and 7-hexadecenoic acid (C16:1 n9). These results are in line with Lordelo et al. (2020), who reported differences for the levels of some monounsatured fatty acid, like oleic acid, between the egg yolk of eggs from commercial and native genotypes. The monounsaturated fatty acid (MUFA) percentage is directly dependent of the content of the diet

supplied to hens. Considering that all the animals in our study had the same diet, differences could be ascribed to other factors such as a potential inheritable genetic factor behind the metabolism of the MUFA (Bunea et al., 2017). Qian et al. (2016) reported that monounsaturated fatty acids, followed by PUFA are the most desirable fatty acids to be present in human diet. The reason for this, is that this type of fats produces a better mitochondrial functioning, which in turn prevents cardiovascular disease by lowering cholesterol and helps avoid complications associated with diabetes such as kidney damage (Abdullah et al., 2017; Qian et al., 2016).

## 5. Conclusions

The chemical characterization of Utrerana breed eggs, has revealed that these eggs may be quite beneficial for human consumption, mainly due to their high content in proteins, in some MUFA and in the total content of polyunsaturated fatty acids. Fatty acids profile may depend on certain genetic inherent characteristics linked to the differences across breeds but also varieties. However, the fact that no difference in regards fatty acid composition was found between black feathered Utrerana and Leghorn may suggest it may not be linked to colouration genetic factors, but others, as it has often been reported in literature. Standardizing this chemical profile may also evidence new strategies on how to cover the currently increasing demand for non-conventional quality products linked to particular breeds. Further studies are needed to determine the effect that this product would have on the health of people suffering from certain diseases. Conclusively, autochthonous breeds must be introduced in common production systems and commercial chains but always under the scope of sustainable production systems. For this, the principal product of each breed has to be characterized, seeking for a differentiated product focused at satisfying the need of specific sectors within the population.

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**Appendix A. Supplementary data:** Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.jfca.2020.103673.

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**Supplementary Table S1.** Recipe and chemical composition of the compound feed used for feeding the hen sets in the study.

| *RECIPE* | |
|---|---|
| Corn | |
| Wheat | |
| Shelled toasted soybeans flour | |
| Calcium carbonate | |
| Barley | |
| Monocalcium phosphate | |
| Soybean oil | |
| Sodium chloride | |
| Sodium bicarbonate | |
| *CHEMICAL COMPOSITION (%)* | |
| Crude protein | 15.7 |
| Crude fat and oils | 3.6 |
| Crude fiber | 2.4 |
| Crude ashes | 14 |
| Calcium | 4.1 |
| Phosphorus | 0.66 |
| Sodium | 0.15 |
| Methionine | 0.38 |
| Lysine | 0.79 |

**Supplementary Table 2.** Vaccination protocol.

| | **Marek** | **Newcastle + Bronquitis** | **Gumboro** | **Newcastle + Gumboro revaccination** |
|---|---|---|---|---|
| Vaccination age | 2 days | 15 days | 30 days | 6 weeks |
| Administration route | Subcutaneous | Drinking water | Drinking water | Drinking water |

**Supplementary Table S3.** Summary of pool descriptive statistics and effect power of breed and variety factors on shell chemical composition (n=48 eggs/Utrerana breed's eggs: 36 + Leghorn breed's eggs: 12).

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Al | mg/kg | 0.04 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.06 | 0.952 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.04 | 47 | | | | | | | | | |
| Utrerana | As | mg/kg | 0.09 | 47 | 0.01 | 0.05 | 0.20 | 0.19 | 0.19 | 0.10 | -0.29 | 0.772 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.10 | 47 | | | | | | | | | |
| Utrerana | B | mg/kg | 0.19 | 47 | 0.12 | 0.17 | 0.69 | 0.67 | 0.66 | 0.33 | -1.60 | 0.110 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.31 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Ca | g/kg | 358.53 | 47 | 21.52 | 2.32 | 9.28 | 8.91 | 8.81 | 0.98 | -2067.79 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 337.01 | 47 | | | | | | | | | |
| Utrerana | Cd | mg/kg | 0.03 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.12 | 0.904 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.03 | 47 | | | | | | | | | |
| Utrerana | Co | mg/kg | 0.02 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.18 | 0.857 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.02 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Cr | mg/kg | 0.12 | 47 | 0.08 | 0.14 | 0.57 | 0.54 | 0.54 | 0.27 | -0.93 | 0.352 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.20 | 47 | | | | | | | | | |
| Utrerana | Cu | mg/kg | 1.58 | 47 | 0.17 | 0.21 | 0.82 | 0.79 | 0.78 | 0.38 | -8.90 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 1.47 | 47 | | | | | | | | | |
| Utrerana | Fe | mg/kg | 6.64 | 47 | 1.69 | 0.65 | 2.60 | 2.50 | 2.47 | 0.79 | -39.30 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 4.95 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Hg | mg/kg | 0.24 | 47 | 0.05 | 0.11 | 0.45 | 0.43 | 0.42 | 0.22 | -1.13 | 0.258 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.19 | 47 | | | | | | | | | |
| Utrerana | I | mg/kg | 0.00 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.29 | 0.772 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.00 | 47 | | | | | | | | | |
| Utrerana | K | mg/kg | 718.47 | 47 | 127.15 | 5.64 | 22.55 | 21.66 | 21.42 | 1.00 | -4953.83 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 845.62 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Mg | mg/kg | 612.45 | 47 | 1.61 | 0.63 | 2.54 | 2.44 | 2.41 | 0.79 | -3497.31 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 610.84 | 47 | | | | | | | | | |
| Utrerana | Mn | mg/kg | 0.91 | 47 | 0.20 | 0.22 | 0.86 | 0.85 | -1.90 | 0.41 | -5.102 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 0.71 | 47 | | | | | | | | | |
| Utrerana | Na | mg/kg | 1860.00 | 47 | 0.20 | 2.24 | 8.94 | 8.59 | 8.49 | 0.98 | -10636.97 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 1840.00 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Ni | mg/kg | 0.45 | 47 | 0.13 | 0.18 | 0.72 | 0.69 | 0.68 | 0.34 | -2.41 | 0.016 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 0.32 | 47 | | | | | | | | | |
| Utrerana | P | mg/kg | 766.58 | 47 | 286.49 | 8.46 | 33.85 | 32.51 | 32.15 | 1.00 | -4661.99 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 480.09 | 47 | | | | | | | | | |
| Utrerana | Pb | mg/kg | 0.42 | 47 | 0.06 | 0.13 | 0.49 | 0.47 | 0.47 | 0.24 | -2.17 | 0.030 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 0.36 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | S | mg/kg | 6170.00 | 47 | 360.00 | 9.49 | 37.95 | 36.44 | 36.04 | 1.00 | -35579.33 | 0.000 | As $Z \leq -1.960$ we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 5810.00 | 47 | | | | | | | | | |
| Utrerana | Se | mg/kg | 0.10 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | -0.28 | 0.779 | As $-1.960 \leq Z \leq 1.960$ we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.10 | 47 | | | | | | | | | |
| Utrerana | Zn | mg/kg | 2.13 | 47 | 6.23 | 1.25 | 4.99 | 4.79 | 4.74 | 0.93 | -53.66 | 0.000 | As $Z \leq -1.960$ we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 8.36 | 47 | | | | | | | | | |

**Supplementary Table S4.** Summary of pool descriptive statistics and effect power of breed and variety factors on yolk chemical composition (n=48 eggs/Utrerana breed's eggs: 36 + Leghorn breed's eggs: 12).

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Al | mg/kg | 0.04 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.06 | 0.952 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.04 | 47 | | | | | | | | | |
| Utrerana | As | mg/kg | 0.08 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | -0.16 | 0.246 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.08 | 47 | | | | | | | | | |
| Utrerana | B | mg/kg | 8.28 | 47 | 8.22 | 2.06 | 5.73 | 5.51 | 5.45 | 0.94 | -55.19 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 0.06 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Ca | mg/kg | 1790.00 | 47 | 380.00 | 95.00 | 38.99 | 37.44 | 37.02 | 1.00 | -12766.51 | 0.000 | As $Z \leq -1.960$ we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 2170.00 | 47 | | | | | | | | | |
| Utrerana | Cd | mg/kg | 0.02 | 47 | 0.01 | 0.00 | 0.20 | 0.19 | 0.19 | 0.10 | 0.11 | 0.912 | As $-1.960 \leq Z \leq 1.960$ we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.03 | 47 | | | | | | | | | |
| Utrerana | Co | mg/kg | 0.02 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.18 | 0.857 | As $-1.960 \leq Z \leq 1.960$ we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.02 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Cr | mg/kg | 0.02 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.18 | 0.857 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.02 | 47 | | | | | | | | | |
| Utrerana | Cu | mg/kg | 3.58 | 47 | 0.35 | 0.09 | 1.18 | 1.14 | 1.12 | 0.51 | -22.49 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 3.93 | 47 | | | | | | | | | |
| Utrerana | Fe | mg/kg | 43.02 | 47 | 3.36 | 0.84 | 3.66 | 3.52 | 3.48 | 0.88 | -267.81 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 46.38 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Hg | mg/kg | 0.21 | 47 | 0.06 | 0.02 | 0.49 | 0.47 | 0.47 | 0.24 | -1.31 | 0.190 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 0.27 | 47 | | | | | | | | | |
| Utrerana | I | mg/kg | 533.85 | 47 | 33.08 | 8.27 | 11.50 | 11.05 | 10.92 | 0.99 | -3268.94 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 566.93 | 47 | | | | | | | | | |
| Utrerana | K | mg/kg | 748.62 | 47 | 13.62 | 3.41 | 7.38 | 7.09 | 7.01 | 0.97 | -4364.35 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 762.24 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Mg | mg/kg | 272.12 | 47 | 21.34 | 5.34 | 9.24 | 8.87 | 8.77 | 0.98 | -1574.36 | 0.000 | As $Z \leq -1.960$ we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 250.78 | 47 | | | | | | | | | |
| Utrerana | Mn | mg/kg | 0.00 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.29 | 0.772 | As $-1.960 \leq Z \leq 1.960$ we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.00 | 47 | | | | | | | | | |
| Utrerana | Na | mg/kg | 555.05 | 47 | 6.93 | 1.73 | 5.26 | 5.06 | 5.00 | 0.93 | -3214.53 | 0.000 | As $Z \leq -1.960$ we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 561.98 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Ni | mg/kg | 0.18 | 47 | 0.02 | 0.01 | 0.28 | 0.27 | 0.27 | 0.14 | -0.76 | 0.447 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.16 | 47 | | | | | | | | | |
| Utrerana | P | mg/kg | 5390.00 | 47 | 40.00 | 10.00 | 12.65 | 12.15 | 12.01 | 0.99 | -31035.26 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 5430.00 | 47 | | | | | | | | | |
| Utrerana | Pb | mg/kg | 0.55 | 47 | 0.03 | 0.01 | 0.35 | 0.33 | 0.33 | 0.17 | -2.88 | 0.004 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 0.52 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | S | mg/kg | 1249.10 | 47 | 27.79 | 6.95 | 10.54 | 10.13 | 10.01 | 0.98 | -7316.25 | 0.000 | As $Z \leq -1.960$ we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 1276.89 | 47 | | | | | | | | | |
| Utrerana | Se | mg/kg | 0.12 | 47 | 0.03 | 0.01 | 0.35 | 0.33 | 0.33 | 0.17 | -0.42 | 0.674 | As $-1.960 \leq Z \leq 1.960$ we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.09 | 47 | | | | | | | | | |
| Utrerana | Zn | mg/kg | 37.33 | 47 | 4.95 | 1.24 | 4.45 | 4.27 | 4.23 | 0.91 | -217.75 | 0.000 | As $Z \leq -1.960$ we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 32.38 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Carbohydrates | % | 1.30 | 47 | 5.80 | 1.45 | 4.82 | 4.63 | 4.57 | 0.92 | -46.04 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 7.10 | 47 | | | | | | | | | |
| Utrerana | Humidity (4h a 103°C) | % | 46.40 | 47 | 0.20 | 0.05 | 0.89 | 0.86 | 0.85 | 0.41 | -264.77 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 46.20 | 47 | | | | | | | | | |
| Utrerana | Raw Ashes (in fresh matter) | % | 3.20 | 47 | 1.00 | 0.25 | 2.00 | 1.92 | 1.90 | 0.71 | -24.68 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 4.20 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Raw Protein (in fresh matter) | % | 17.40 | 47 | 0.50 | 0.13 | 1.41 | 1.36 | 1.34 | 0.58 | -99.53 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 16.90 | 47 | | | | | | | | | |
| Utrerana | Fat Raw (in fresh matter) | % | 30.50 | 47 | 4.90 | 1.23 | 4.43 | 4.25 | 4.20 | 0.91 | -178.71 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 25.60 | 47 | | | | | | | | | |
| Utrerana | Polyunsaturated Fat | % | 19.80 | 47 | 3.20 | 0.80 | 3.58 | 3.44 | 3.40 | 0.87 | -115.93 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 16.60 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Saturated fats | % | 10.70 | 47 | 1.70 | 0.43 | 2.61 | 2.50 | 2.48 | 0.79 | -62.49 | 0.000 | As $Z \leq -1.960$ we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 9.00 | 47 | | | | | | | | | |
| Utrerana | Sugars | mg/kg | 0.01 | 47 | 0.05 | 0.01 | 0.45 | 0.43 | 0.42 | 0.22 | -0.10 | 0.920 | As $-1.960 \leq Z \leq 1.960$ we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.06 | 47 | | | | | | | | | |
| Utrerana | Cholesterol | mg/kg | 1085.00 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | -0.33 | 0.741 | As $-1.960 \leq Z \leq 1.960$ we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 1085.00 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value (IC 95%) | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana<br>Leghorn | Vitamin E (Alpha-Tocopherol) | mg/kg | 102.00<br>88.00 | 47<br>47 | 14.00 | 3.50 | 7.48 | 7.19 | 7.11 | 0.97 | -595.94 | 0.000 | As $Z \leq -1.960$ we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |

**Supplementary Table S5.** Summary of pool descriptive statistics and effect power of breed and variety factors on white chemical composition (n=48 eggs/Utrerana breed's eggs: 36 + Leghorn breed's eggs: 12).

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Al | mg/kg | 0.02 | 47 | 0.01 | 0.05 | 0.20 | 0.19 | 0.19 | 0.10 | 0.11 | 0.912 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.03 | 47 | | | | | | | | | |
| Utrerana | As | mg/kg | 0.18 | 47 | 0.01 | 0.05 | 0.20 | 0.19 | 0.19 | 0.10 | -0.75 | 0.453 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.17 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | B | mg/kg | 0.31 | 47 | 0.43 | 0.33 | 1.31 | 1.26 | 1.25 | 0.55 | -4.36 | 0.000 | As $Z \leq -1.960$ we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 0.74 | 47 | | | | | | | | | |
| Utrerana | Ca | mg/kg | 593.75 | 47 | 9.44 | 1.54 | 6.14 | 5.90 | 5.84 | 0.95 | -3398.39 | 0.000 | As $Z \leq -1.960$ we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 584.31 | 47 | | | | | | | | | |
| Utrerana | Cd | mg/kg | 0.02 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.18 | 0.857 | As $-1.960 \leq Z \leq 1.960$ we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.02 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Co | mg/kg | 0.01 | 47 | 0.01 | 0.05 | 0.20 | 0.19 | 0.19 | 0.10 | 0.17 | 0.865 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.02 | 47 | | | | | | | | | |
| Utrerana | Cr | mg/kg | 0.13 | 47 | 0.04 | 0.10 | 0.40 | 0.38 | 0.38 | 0.20 | -0.72 | 0.472 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.17 | 47 | | | | | | | | | |
| Utrerana | Cu | mg/kg | 1.69 | 47 | 0.56 | 0.37 | 1.50 | 1.44 | 1.42 | 0.60 | -13.11 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 2.25 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Fe | mg/kg | 5.34 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | -2.76 | 0.006 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 5.33 | 47 | | | | | | | | | |
| Utrerana | Hg | mg/kg | 0.01 | 47 | 0.01 | 0.05 | 0.20 | 0.19 | 0.19 | 0.10 | 0.17 | 0.865 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.02 | 47 | | | | | | | | | |
| Utrerana | I | mg/kg | 0.00 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.29 | 0.772 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.00 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | K | mg/kg | 1430.00 | 47 | 100.00 | 5.00 | 20.00 | 19.21 | 18.99 | 1.00 | -8833.26 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 1530.00 | 47 | | | | | | | | | |
| Utrerana | Mg | mg/kg | 270.55 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | -1.25 | 0.211 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 270.55 | 47 | | | | | | | | | |
| Utrerana | Mn | mg/kg | 1.55 | 47 | 0.38 | 0.31 | 1.23 | 1.18 | 1.17 | 0.52 | -11.10 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 1.93 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Na | mg/kg | 2010.00 | 47 | 250.00 | 7.91 | 31.62 | 30.37 | 30.03 | 1.00 | -13150.25 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 2260.00 | 47 | | | | | | | | | |
| Utrerana | Ni | mg/kg | 0.32 | 47 | 0.13 | 0.18 | 0.72 | 0.69 | 0.68 | 0.34 | -2.41 | 0.016 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 0.45 | 47 | | | | | | | | | |
| Utrerana | P | mg/kg | 369.37 | 47 | 62.36 | 3.95 | 15.79 | 15.17 | 15.00 | 0.99 | -2526.47 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 431.73 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Pb | mg/kg | 0.12 | 47 | 0.03 | 0.09 | 0.35 | 0.33 | 0.33 | 0.17 | -0.59 | 0.555 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.15 | 47 | | | | | | | | | |
| Utrerana | S | mg/kg | 1750.00 | 47 | 20.00 | 2.24 | 8.94 | 8.59 | 8.49 | 0.98 | -10123.23 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 1770.00 | 47 | | | | | | | | | |
| Utrerana | Se | mg/kg | 0.06 | 47 | 0.01 | 0.05 | 0.20 | 0.19 | 0.19 | 0.10 | -0.12 | 0.904 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.07 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Zn | mg/kg | 1.75 | 47 | 0.22 | 0.23 | 0.94 | 0.90 | 0.89 | 0.42 | -11.17 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 1.97 | 47 | | | | | | | | | |
| Utrerana | Carbohydrates | % | 1.10 | 47 | 1.09 | 0.52 | 2.09 | 2.01 | 1.98 | 0.72 | -7.08 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 0.01 | 47 | | | | | | | | | |
| Utrerana | Humidity (4h a 103°C) | % | 84.80 | 47 | 4.90 | 1.11 | 4.43 | 4.25 | 4.20 | 0.91 | -516.63 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Leghorn has a statistically significant higher mean than Utrerana |
| Leghorn | | | 89.70 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Raw Ashes (in fresh matter) | % | 3.00 | 47 | 0.70 | 0.42 | 1.67 | 1.61 | 1.59 | 0.64 | -17.53 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 2.30 | 47 | | | | | | | | | |
| Utrerana | Raw Protein (in fresh matter) | % | 10.60 | 47 | 0.30 | 0.27 | 1.10 | 1.05 | 1.04 | 0.48 | -60.51 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 10.30 | 47 | | | | | | | | | |
| Utrerana | Fat Raw (in fresh matter) | % | 0.50 | 47 | 0.20 | 0.22 | 0.89 | 0.86 | 0.85 | 0.41 | -2.76 | 0.000 | As Z ≤ -1.960 we reject H0 and can say Utrerana has a statistically significant higher mean than Leghorn |
| Leghorn | | | 0.30 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Polyunsaturated Fat | % | 0.00 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.29 | 0.772 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.00 | 47 | | | | | | | | | |
| Utrerana | Saturated Fats | % | 0.00 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.29 | 0.772 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.00 | 47 | | | | | | | | | |
| Utrerana | Sugars | mg/kg | 0.20 | 47 | 0.10 | 0.16 | 0.63 | 0.61 | 0.60 | 0.30 | -0.95 | 0.342 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.10 | 47 | | | | | | | | | |

| Levels | Variable | Units | Mean | df | Mean difference | Pooled standard deviation | Cohen's d | Corrected d | Hedge's g | r correlation coefficient | Pha (Z) | P-value | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Utrerana | Cholesterol | mg/kg | 0.00 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.29 | 0.772 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.00 | 47 | | | | | | | | | |
| Utrerana | Vitamin E (Alpha-Tocopherol) | mg/kg | 0.00 | 47 | 0.00 | 0.00 | N/A | N/A | N/A | N/A | 0.29 | 0.772 | As -1.960 ≤ Z ≤ 1.960 we accept H0 and can say Utrerana and Leghorn's means are statistically equal |
| Leghorn | | | 0.00 | 47 | | | | | | | | | |

**Supplementary Table S6.** Shapiro Wilks Francia normality testing on fatty acid profile of the egg yolk.

| Variable | W' | V' | z | Prob>z |
|---|---|---|---|---|
| C14:0 | 0.923 | 3.761 | 2.469 | 0.007[a] |
| C16:0 | 0.989 | 0.513 | -1.309 | 0.905[b] |
| C16:1 n9 | 0.976 | 1.188 | 0.331 | 0.370[b] |
| C16:1 n7 | 0.925 | 3.639 | 2.410 | 0.008[a] |
| C17:0 | 0.906 | 4.579 | 2.822 | 0.002[a] |
| C17:1 | 0.967 | 1.607 | 0.903 | 0.183[b] |
| C18:0 | 0.973 | 1.294 | 0.494 | 0.311[b] |
| C18:1 n9 | 0.972 | 1.346 | 0.569 | 0.285[b] |
| C18:1 n7 | 0.989 | 0.531 | -1.240 | 0.893[b] |
| C18:2 n6 | 0.920 | 3.909 | 2.539 | 0.006[a] |
| C18:3 n3 | 0.846 | 7.470 | 3.683 | 0.001[a] |
| C20:1 n9 | 0.706 | 14.274 | 4.789 | 0.001[a] |
| C20:2 n6 | 0.917 | 4.018 | 2.588 | 0.005[a] |
| C20:3 n6 | 0.986 | 0.691 | -0.720 | 0.764[b] |
| C20:4 n6 | 0.917 | 4.051 | 2.603 | 0.005[a] |
| C22:4 n6 | 0.614 | 18.768 | 5.246 | 0.001[a] |
| C22:5 n6 | 0.964 | 1.769 | 1.084 | 0.139[b] |
| C22:5 n3 | 0.807 | 9.385 | 4.077 | 0.001[a] |
| C22:6 n3 | 0.932 | 3.303 | 2.235 | 0.013[a] |

[a]P<0.05: significant, nonparametric approach suggested.
[b]P>0.05: non significant, parameteric approach suggested.

**Supplementary Table S7.** Summary of descriptive statistics for fatty acids profile in egg yolk.

| Fatty acids Profile in yolk | Mean | Median (percentile 50) | Mode | Std. Deviation | Variance | Skewness | Std. Error of Skewness | Kurtosis | Std. Error of Kurtosis | Minimum | Maximum | Percentile 25 | Percentile 75 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C14:0 | 0.29 | 0.28 | 0.25 | 0.04 | 0.00 | 0.95 | 0.35 | 0.93 | 0.69 | 0.21 | 0.41 | 0.27 | 0.31 |
| C16:0 | 25.06 | 25.10 | 22.88 | 1.36 | 1.84 | -0.06 | 0.35 | -0.28 | 0.69 | 21.66 | 27.62 | 24.22 | 26.00 |
| C16:1 n9 | 0.58 | 0.58 | 0.69 | 0.13 | 0.02 | 0.06 | 0.35 | -0.99 | 0.69 | 0.35 | 0.81 | 0.48 | 0.69 |
| C16:1 n7 | 2.27 | 2.20 | 1.76 | 0.42 | 0.18 | 0.84 | 0.35 | 0.02 | 0.69 | 1.76 | 3.34 | 1.90 | 2.46 |
| C17:0 | 0.14 | 0.14 | 0.12 | 0.03 | 0.00 | -1.00 | 0.35 | 3.40 | 0.69 | 0.02 | 0.19 | 0.12 | 0.16 |
| C17:1 | 0.10 | 0.10 | 0.09 | 0.02 | 0.00 | 0.50 | 0.35 | -0.26 | 0.69 | 0.07 | 0.15 | 0.09 | 0.12 |
| C18:0 | 9.68 | 9.76 | 8.10 | 1.06 | 1.13 | -0.52 | 0.35 | 0.10 | 0.69 | 6.81 | 11.60 | 9.04 | 10.51 |
| C18:1 n9 | 43.04 | 42.85 | 39.62 | 2.16 | 4.66 | 0.07 | 0.35 | -1.15 | 0.69 | 39.62 | 46.70 | 41.14 | 44.93 |
| C18:1 n7 | 1.46 | 1.44 | 1.25 | 0.17 | 0.03 | 0.09 | 0.35 | -0.60 | 0.69 | 1.12 | 1.83 | 1.34 | 1.58 |
| C18:2 n6 | 12.86 | 12.60 | 9.52 | 2.06 | 4.23 | 0.92 | 0.35 | 0.65 | 0.69 | 9.52 | 17.80 | 11.31 | 13.84 |
| C18:3 n3 | 0.28 | 0.26 | 0.24 | 0.11 | 0.01 | 1.54 | 0.35 | 2.27 | 0.69 | 0.14 | 0.62 | 0.20 | 0.31 |
| C20:1 n9 | 0.27 | 0.28 | 0.30 | 0.06 | 0.00 | -2.70 | 0.35 | 9.73 | 0.69 | 0.04 | 0.35 | 0.25 | 0.30 |
| C20:2 n6 | 0.15 | 0.14 | 0.13 | 0.03 | 0.00 | 1.04 | 0.35 | 1.40 | 0.69 | 0.10 | 0.24 | 0.13 | 0.16 |
| C20:3 n6 | 0.16 | 0.16 | 0.15 | 0.03 | 0.00 | 0.00 | 0.35 | -0.72 | 0.69 | 0.11 | 0.22 | 0.13 | 0.18 |
| C20:4 n6 | 2.18 | 2.15 | 2.08 | 0.29 | 0.09 | 0.99 | 0.35 | 2.09 | 0.69 | 1.55 | 3.03 | 2.00 | 2.31 |
| C22:4 n6 | 0.01 | 0.00 | 0.00 | 0.03 | 0.00 | 3.24 | 0.35 | 11.25 | 0.69 | 0.00 | 0.17 | 0.00 | 0.01 |
| C22:5 n6 | 0.66 | 0.69 | 0.49 | 0.16 | 0.03 | -0.21 | 0.35 | 0.07 | 0.69 | 0.25 | 1.03 | 0.53 | 0.75 |
| C22:5 n3 | 0.18 | 0.17 | 0.18 | 0.06 | 0.00 | 2.02 | 0.35 | 5.84 | 0.69 | 0.10 | 0.42 | 0.13 | 0.20 |
| C22:6 n3 | 0.57 | 0.55 | 0.60 | 0.14 | 0.02 | 0.91 | 0.35 | 1.00 | 0.69 | 0.35 | 0.94 | 0.48 | 0.63 |

# Chapter 4.

## Analysis of the potential commercial of Utrerana egg.

- *González Ariza A., A. Arando Arbulu, F. J. Navas González, J. M. León Jurado, C. J. Barba Capote, and M. E. Camacho Vallejo.* ***Sensory Preference and Professional Profile Affinity Definition of Endangered Native Breed Eggs Compared to Commercial Laying Lineages' Eggs.*** *Animals 2019, 9, 920.*
- *González Ariza A., A. Arando Arbulu, F. J. Navas González, J. V. Delgado Bermejo, and M. E. Camacho Vallejo.* ***Discriminant Canonical Analysis as a Validation Tool for Multivariety Native Breed Egg Commercial Quality Classification.*** *Foods 2021, 10, 632.*

# Sensory Preference and Professional Profile Affinity Definition of Endangered Native Breed Eggs Compared to Commercial Laying Lineages' Eggs

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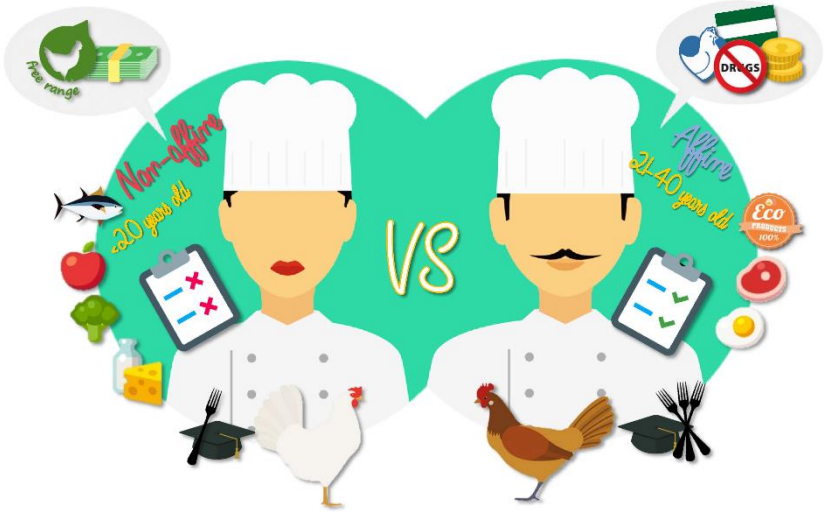
**Simple Summary:** A local breed's particularities may provide eggs with sensory properties which may overcome laying lineage, regardless of their production system characteristics. Hence, methods clarifying what the appreciation of a certain product is like can outline the actions required to improve the market value of that product. Affine and non-affine profiles were defined based on the information provided by sixty-four professionally-instructed panelists on sensory attributes, diet habits, production context awareness, product consciousness, cuisine applicability and panelist attributes. Egg consumption was lower in non-affine profile professionals, as were the scores provided to sensory attributes. The higher the knowledge about Utrerana breed, the greater the importance provided to the ecological and autochthonous nature of the products. The level of study, gender and age are crucial factors to consider when approaching the commercialization of Utrerana hen eggs. Conclusively, defining consumer profiles among professionals of the cuisine sector may improve the profitability of Utrerana eggs and may help educating non-affine profiles, something key to the success in product appreciation.

**Abstract:** This study aimed to compare Utrerana native hen eggs' sensory properties to Leghorn Lohmann LSL-Classic lineage's commercial and ecological eggs through free-choice profiling. Second, affine and non-affine profiles were defined using the information provided by professionally-instructed panelists on six sets (sensory attributes, diet habits, production context awareness, product consciousness, cuisine applicability and panelist attributes) using nonlinear canonical correlation analysis. Sixty-four instructed professional panelists rated 96 eggs on 39 variables comprising the above-mentioned sets. Observers reported a significantly higher appreciation ($p > 0.05$) towards yolk color, odor, flavor, texture, overall score, and whole and on plate broken egg visual value when Utrerana eggs were compared to the rest of categories. Professional Profile A (PPA), or egg non-affine profile, consumed less eggs and provided lower scores to sensory attributes than Professional Profile B (PPB), or affine profile. Additionally, PPB accounted for higher knowledge about the Utrerana breed and provided greater importance to a product's ecological and autochthonous nature. PPA was generally characterized by women under 20 years old with no higher studies, while PPB comprised 21-40 years old men with secondary studies. In conclusion, defining professional profiles enables correctly approaching market needs to improve the profitability of Utrerana eggs, meeting professional demands and educating non-affine profiles.

**Graphical abstract**:




**Keywords**: Egg quality; color coordinate decomposition; internal quality traits; external quality traits; DSM color fan

## 1. Introduction

The Utrerana hen is a rustic medium-sized Spanish endangered breed with four feather varieties (partridge, Franciscan, black, and white). Initially regarded as a white egg laying hen (120-180 eggs/year, mean ~64 g/egg for the entire laying period) raised on family farms, a traditional meat/egg aptitude has been addressed in literature and is reappearing in the market scene (Orozco, 1989).

In 2017, around 92% of eggs were produced by laying hens. The annual growth rate of egg production was approximately 0.6 million tons per year from 2000 to 2016, with a total of 1416 trillion tons of eggs, equivalent to 80 million metric tons (FAOSTAT, 2019). However, consumer tastes constantly evolve towards the obtainment of quality food products with special properties, aimed at a specialized market and obtained through sustainable production systems (Blokhuis et al., 2003). In this context, Utrerana eggs have shown to have differentiated internal and external characteristics from commercial line eggs (González Ariza et al., 2019). Hence, the need for animal genetic resources proper conservation has greatly increased, since these resources present economic, scientific and cultural interests (Alderson, 2018).

Modern livestock production high specialization increasingly threatens animal genetic resources diversity (Alderson, 2018). For instance, commercial herds are based on exploiting few genetically selected breeds for intensive production. However, specialized breeds do not guarantee a genetic reserve for the future. After long periods of natural selection and evolution, variability loss compromises diversity and characteristics such as adverse conditions adaptation, which conforms an invaluable protein rich source (Henchion et al., 2017).

Although nutrition has been suggested to be more influential than genetics in egg chemical composition, Washburn (1979) found evidence for a heredity component. Egg quality is directly related to characteristics determining consumer acceptability. For instance, some traits, such as egg weight and dense white height, are highly valued by consumers (Hanusova et al., 2015). However, there are a number of egg sensory attributes that are more difficult to assess, for which taster panels are used.

Food chemical composition is appreciated through the taste sense. Still, some caution should be taken to transform taste sensations into reliable measures, given

the existence of interpanelist variability sources that cannot be eliminated even after training (Williams and Arnold, 1985).

Panelists have been suggested to be unable to routinely describe the sensory attributes they perceive; hence, profiling methods must be homogenized (same sensory lexicon, among others). To prevent this, techniques such as hedonic scale measurements are used (Bárcenas et al., 2001).

Developing sensory tools to define potential consumers profiles and attitudes towards products has always concerned food scientists. Tests are diverse and range from those analyzing the process of standardization for food evaluation and perception derived from product/consumer interaction to panelists sensation elaboration and verbalization (Stone et al., 2008).

Empirically determining panelists discriminating capacity for organoleptic characteristics commonly involves the implementation of linear or logistic regression models used in preference surveys and social epidemiology (Frie and Janssen, 2009). However, in the case of multivariate analysis, non-linear canonical correlation analysis more appropriately allows to map a series of explanatory factors in correlation to different sensory attributes and eggs' cuisine applicability (Van der Burg et al., 1994).

First, we aimed to determine the ability of panelists to discriminate organoleptic characteristics across egg type categories basing on hedonic scales. Second, we inferred different professional profiles regarding their affinity to eggs and their personal context, as a strategy to plan potential marketing strategies to reinforce affine professionals and to attract non-affine ones, to promote autochthonous breed conservation strategies relying on the improvement of their profitability.

## 2. Materials and Methods

### *2.1. Free-Choice Profiling (FCP)*

#### 2.1.1. Professional Panel Description

Sixty-four professional background instructed panelists (students and teachers of the Hospitality School of Córdoba and Granada), with ages ranging between less than 20 to over 50 years old and a minimum formation of 30 h/week during a two-module professional expertise course (Royal Decree Law 687/2010), were

recruited to participate in a short-term sensory evaluation study. The number of panelists included in the hedonic test complies with recommendations for best practice in sensory and consumer science proposed by Hough et al. (2006) for primary and processed food.

The recruitment was performed after a filter questionnaire, which included demographic and socio-economic information (gender, age and academic level), food products consumption frequency and egg cuisine applicability willingness. No panelist was removed given the lack of missing values.

2.1.2. Sampling

Ecological and commercial eggs (A category and of the M class (53-63 g) belonged to white shell Leghorn Lohmann LSL-Classic lineage. Utrerana and Leghorn Lohmann LSL-Classic lineage commercial hens were managed in individual cages (50 × 62 × 41 cm) following Council Directive 1999/74/EC of 19 July 1999, setting the minimum standards for the protection of laying hens at the Centro Agropecuario Provincial de Córdoba (Spain). Feed and water were available ad libitum. All birds were reared according to the regulations of the European Union (2010/63/EU) in their transposition to the Spanish law (RD 53/2013). Stocking density was four animals per each $m^2$, nest box density consisted of 29 animals per $m^2$. Circle waterers of 5 cm diameter and 41 cm feeder allotment/space were provided for each animal. Wood shavings were used as a floor substrate covering the floor to a depth of approximately 1 cm. Nest box substrate consisted of plastic turf mats covering the floor at a depth of approximately 1 cm. Further information regarding maintenance system of Leghorn Lohmann LSL-Classic lineage commercial hens and Utrerana native breed can be found in González Ariza, et al. (2019).

Ecologic/organic eggs were obtained from Leghorn Lohmann LSL-Classic lineage hens. The birds were placed in pens comprising a ceiling covered surface of 41.6 $m^2$ and a free-roaming surface of 1000 $m^2$ following the Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. A total of 20 cm of perch was provided per bird. Food and water were available ad libitum. All birds were reared according to the regulations of the European Union (2010/63/EU) in their transposition to the Spanish law (RD 53/2013). Stocking and nest box density, waterers and feeder

space followed the requirements stated at the regulation referenced above. Wood shavings were used as a floor substrate covering the floor at a depth of approximately 1 cm and nest box substrate consisted of plastic turf mats covering the floor at a depth of approximately 1 cm as well.

A description of the recipe and chemical composition of the compound feed used for hen feeding in this study is provided in Table S1. To avoid an effect of storage time on the sensory properties, evaluation of the samples was performed in two locations (Córdoba and Granada) simultaneously. A 10 min cooking time was determined after a preliminary boiling test performed on a random control group of 27 eggs to determine the duration (9, 10 and 11 min) that prevents overcooking. Eggs were first strained in cold water to prevent shelling during cooking. Sixty-four testing stations were set. Eggs were cut following their longitudinal axis. Each panelist tested and scored half an egg from Utrerana, half an egg from a commercial intensive production Leghorn Lohmann LSL-Classic lineage, and half an egg of the same lineage raised under ecological free-range conditions.

2.1.3. Evaluation Sessions

Participants were received in a conference room and placed at individual blind stations under white lighting (700 lx ± 150 lx), as suggested by Guàrdia et al. (2010). First, panelists were briefed on the methodology and the procedure to allow for acquaintance with the vocabulary to describe the three egg types. Each sample was labeled with random three-digit code matched with the panelist number plus an additional random code to identify samples of the same egg type (Commercial eggs-386; Free-range eggs-745; and Utrerana eggs-639), in a randomized complete-block design. A maximum of three samples were presented to each panelist and were assessed in the same tasting session balancing the first-order and the carry-over effects (MacFie et al., 1989), as suggested by Guàrdia, et al. (2010).

Eggs were evaluated at room temperature (20 °C) and presented on white ceramic plates covered with a food grade PVC film (oxygen permeability; 20,000 $cm^3/m^2/24$ h; water-vapor transmission rate 2000 $g/m^2/24$ h; Macopal, S.L., Lliçà de Vall, Spain) to prevent drying. Mineral water and 15 g golden delicious variety apple slices (Bentabol and Afonso, 2011) at room temperature were provided for mouth rinsing and sense saturation reduction in participants between samples.

2.1.4. Sensory Evaluation and Panelist Contextual Records

Each half egg was rated on six egg sensory attributes (yolk color, white color, odor, flavor, texture and overall score). The visual value of the whole egg and a broken egg on plate visual value were also scored for each egg type separately. Collaterally, panelists provided information on five additional sets comprising a total of 31 variables. These questionnaires later allowed characterization of the overall profile of the panel and the panelists' preferences for the products tested. The panel comprised fairly equal amounts of females (44%) and males (56%).

Panelist context is defined by five sets of variables as follows: Panelist diet habits, production context awareness, product consciousness, cuisine applicability and panelist characterization. The definition for each set and its comprising variables and scales is shown in Supplementary Tables S2 and S3. The scales used follow one unit increases to indicate panelists' ratings. Egg sensory attributes were rated on a 1 to 8 hedonic structured or categorized scoring scale extracted from Anzaldua Morales (1994), except for white color, where panelists only provided answers for seven categories. The sensory attributes set was evaluated using an structured 100 mm line scale anchored with the following ordinal categories: (1) I extremely dislike it, (2) I dislike it a lot, (3) I dislike it moderately, (4) I slightly dislike it, (5) I like it, (6) I slightly like it, (7) I like it moderately, (8) I like it a lot and (9) I extremely like it, adapting the criteria in Anzaldua Morales (1994).

*2.2. Free-Choice Profiling Interobserver Correlation Coefficient (ICC)*

The intraclass correlation coefficient (ICC), based on multiple paired Cohen's κ tests, was calculated to determine if there was agreement between the sixty-four panelists. Fleiss and Cohen (1973) established repeatability guidelines for ICC interpretation as less than 0.4 (low), from 0.4 to 0.59 (reasonable), from 0.6 to 0.74 (good), and from 0.75 to 1.0 (excellent). As we used a random sample of consistent raters for all ratees, we used a "Two-Way Random" model. Then, 95% confidence intervals were computed. The ICC and 95% CI were calculated with the reliability analysis routine of the scale procedure of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016) (Table 1).

**Table 1.** Cronbach's Alpha, Cohen's kappa Intra Class Correlation Coefficient and 95% confidence intervals for interobserver reliability testing and scale consistency sorted by egg type.

| Egg type | | | Cronbach's Alpha | | | | |
|---|---|---|---|---|---|---|---|
| Commercial | | | | 0.800 | | | |
| Utrerana | | | | 0.826 | | | |
| Ecologic | | | | 0.829 | | | |
| Egg type | Measure type | Intraclass Correlation | 95% Confidence Interval | F Test | df1 | df2 | Significance |
| Commercial | Single | 0.105 | 0.071-0.158 | 5.003 | 63 | 2079 | 0.00 |
| | Average | 0.800 | 0.723-0.864 | 5.003 | 63 | 2079 | 0.00 |
| Utrerana | Single | 0.122 | 0.084-0.180 | 5.733 | 63 | 2079 | 0.00 |
| | Average | 0.826 | 0.758-0.882 | 5.733 | 63 | 2079 | 0.00 |
| Ecologic | Single | 0.125 | 0.086-0.183 | 5.843 | 63 | 2079 | 0.00 |
| | Average | 0.829 | 0.763-0.884 | 5.843 | 63 | 2079 | 0.00 |

### *2.3. Scale Reliabiltiy*

Scale internal consistency was studied using Cronbach's alpha. As a general criterion, George and Mallery (2003) suggest the following recommendations for evaluating Cronbach's alpha coefficients: >0.9 is excellent, >0.8 is good, >0.7 is acceptable, >0.6 is questionable, >0.5 is poor and <0.5 is unacceptable. Variables with values over 0.5 were retained as they were able to explain the highest percentage of variance.

### *2.4. Quantitative Descriptive Analysis (QDA)*

Variables and scales use agreement was performed at a preliminary open discussion involving 32 professional panelists, following the premises described in Anzaldua Morales (1994). The same author reported the references used to illustrate the criteria for the variables on each set. Several training and refresher training sessions were set up to develop the different sensory attributes and normalize the panelists according to common perceptions (Anzaldua Morales, 1994). Descriptors varied from 8 to 10 for each panelist.

### *2.5. Egg Type Sensory Attributes Difference Analysis*

Descriptive statistics for the variables on each set are reported in Table S1. Variables were not transformed and sorted into six sets considering their common nature, namely, egg sensory attributes, panelist diet habits, production context

awareness, product consciousness, cuisine applicability, and panelist characterization. Shapiro-Francia tests were carried out with the .sfrancia routine of StataCorp Stata version 14.2. (Supplementary Table S4). As normality was not found, a Kruskal-Wallis H test was performed to study differences across variables. Afterwards, interlevel distribution and median differences among Kruskal-Wallis H significant variables were tested using the pairwise comparisons Dunn's test and sorting medians respectively. If we test for multiple comparisons, the likelihood of incorrectly rejecting statistically significant differences between two or more levels (Type I errors) increases. The Bonferroni correction was performed to compensate for that increase. All nonparametric tests were carried out using the independent samples package from the non-parametrical task of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016) and results are provided in Table S4.

*2.6. Statistical Justification*

Although some authors (Dijksterhuis, 1994) have suggested Procrustes analysis to be one of the most common and strict techniques to analyze sensory attributes related to other aspects such as free choice profiling, it is only applicable when all variables measurement dimensions (p) have similar scales. Contrarily, this analysis renders inaccurate (The MathWorks Inc., 2015) if we do not only have different scales but also different measurement units.

The same authors suggest alternatives such as the nonlinear version of canonical correlation analysis, report results with a virtually perfect fit, which may be partly attributed to the freedom to choose non-linear transformations, which enables scoring traits on very different scales.

*2.7. Non-linear Canonical Correlation between Sets*

A nonlinear canonical correlation analysis (OVERALS) was performed to determine interset similarities to maximize the variance in the relationships among two sets of numerical variables in a low dimensional space. Optimal scaling approach in OVERALS expands the standard canonical analysis as first, it allows more than two sets of variables, accommodating varying scaling standards (Van der Burg, et al., 1994). Second, variables can be scaled as nominal, ordinal, or numerical in an intervariable integrative analysis of non-linear relations. Finally, instead of maximizing interset correlations, these are compared to an unknown compromise

set that is defined by the object scores. OVERALS uses the "alternating least squares (ALS) algorithm", to calculate the "fit function" and the "loss function". The loss function states the difference between the number of chosen dimensions to the best calculated adaptation and shows the lack of fit of a solution, being within a p-dimensional case, the minimum equal to 0 and maximum equal to p. Loss represents the proportion of variation in object scores for each dimension and set in Table 2. The mean of sets is the average loss in sets and gives us the difference between the maximum and actual fits. Summation of average loss and fit is equal to the number of dimensions. Therefore, small loss values indicate large multiple correlations between weighted sums of optimally scaled variables and dimensions (Michailidis and De Leeuw, 1998). The eigenvalue can be calculated by dividing loss per dimensions, and carrying out 1 minus loss per dimension. The eigenvalue is a goodness of fit measure, which ranges from 0 to 1, indicating the level of relationship shown by each dimension, and the sum of these values is called total fit (Table 2),

that is, the statistical index widely used in OVERALS to decide analysis solution dimensionality.

**Table 2.** Eigenvalues for the two-dimensional solution of nonlinear canonical correlation analysis for Utrerana native hen egg sensory attributes (yellow), panelist diet habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and panelist characterization (grey) as perceived by cuisine instructed panelists (n = 192).

| | Egg sensory attributes | Assessor diet habits | Production context awareness | Product consciousness | Cuisine applicability | Assessor characterization | Mean | Eigenvalue |
|---|---|---|---|---|---|---|---|---|
| Dimension 1 | 0.446 | 0.069 | 0.46 | 0.055 | 0.081 | 0.849 | 0.327 | 0.673 |
| Dimension 2 | 0.816 | 0.266 | 0.212 | 0.159 | 0.214 | 0.465 | 0.355 | 0.645 |
| FIT | 1.262 | 0.335 | 0.672 | 0.213 | 0.294 | 1.315 | 0.682 | 1.318 |

For visual mapping of the constructed space, we used the nonlinear canonical correlation analysis with the described six sets and their variables. Component loadings are the correlations between object scores and optimal scaled variables and are sorted in dimensions 1 and 2. These loadings act as coordinates of the variable points on the graph given below in Figures 1-6 and help with illustrating the distribution of variables in a bi-dimensional space. To this aim, quantifications of multiple categories or numerical ranges are used. These quantifications present the center for all respondents belonging to one category and account for the

importance of other variables from the set. Variables close to others have more similarities among interviewed persons than variables that are far apart. To interpret the dimensions obtained, attributes with loadings of over 0.5 (Bárcenas et al., 2003) were the most effective variables in relationships among variable sets because they were positioned far from the origin (denoting the mean) (Greenacre and Hastie, 1987) (Supplementary Table S6). The plots of centroids were labeled according to the categories in the scale for each variable and are presented in Figures 1-6, showing how well variables separate groups of objects. Centroids were in the center of gravity of the objects. Matching clusters of categories in centroid plots need to be identified and interpreted to understand intervariable relationships (Meulman and Heiser, 2012).

Contrary to what happens in principal component analysis (PCA), for which a dimensionality criterion of explained variance over 80%, is required. When OVERALS is linked to FCP, if all variables are specified as ordinal, single nominal, or numerical, the maximum number of dimensions is the lesser of the following two values: The number of observations (n = 192) minus 1, or the total number of variables (Meulman and Heiser, 2012). Then, we reduce the dimensions until we reach the maximum number of dimensions that explains the greatest percentage of variance at an acceptable loss level (Table 3). Single variables are only important when containing information independent from information of other variables of the same set (Hsieh, 2000).

In total, 39 variables with either a nominal, numeric or ordinal scaling level are included in the analysis (Supplementary Table S3). Variables can be classified into two or more sets and scaled as multiple nominal, single nominal, ordinal, or numerical and the interpretation of their direction is obtained from the position of projected centroids. Most of the variables considered in the present study are ordinal. This implies that the order of the categories within each variable must be preserved. Then, if actual and projected centroids are not separated, ordinal variables should have been considered as nominal (IBM Knowledge Center, 2019). As suggested by van der Burg and Dijksterhuis (1996), sensory scores were reorganized into fewer new categories to minimize the existence of empty

categories, we decided to adopt this organization system, thus minimizing the occurrence of unique marginal frequencies.

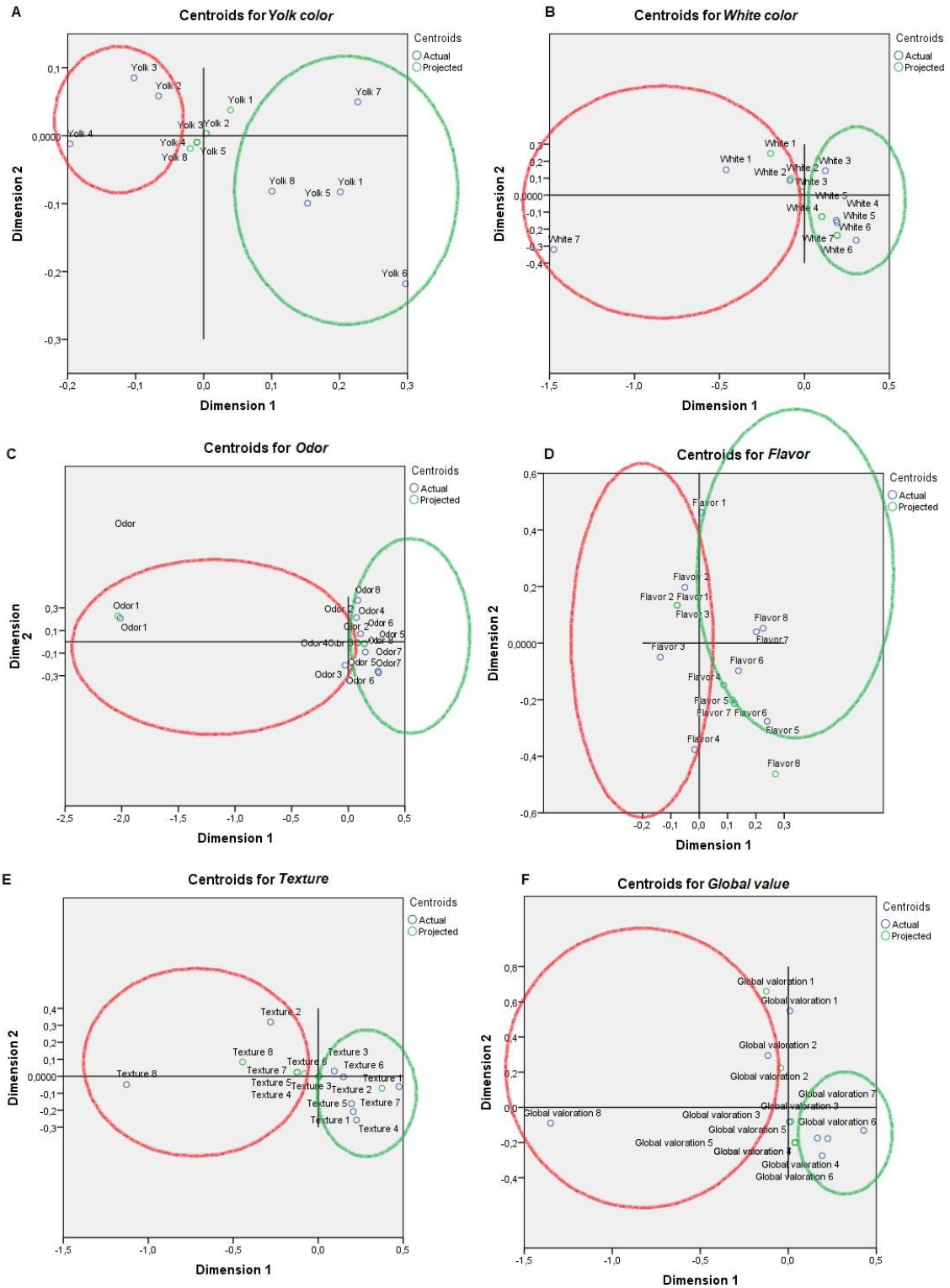



**Figure 1.** *Cont.*

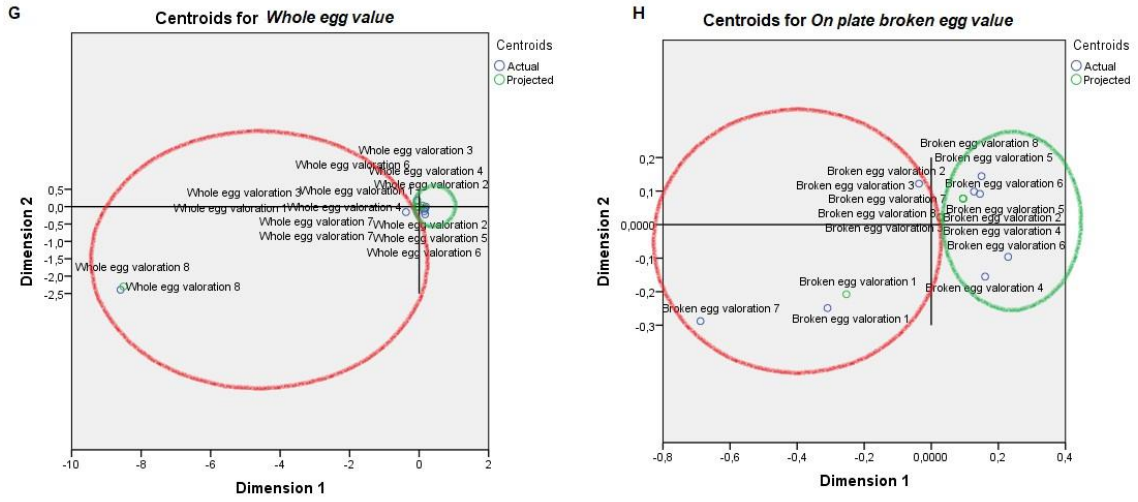



**Figure 1.** Object scores plot visualization of Professional Customer Profiles in regards egg sensory attributes, egg consumption non-affine profile or PPA (red), and affine profile or PPB (green). Egg sensory attributes were as follows: (**A**) Yolk color, (**B**) White color, (**C**) Odor, (**D**) Flavor, (**E**) Texture, (**F**) Global value, (**G**) Whole egg value and (**H**) On plate broken egg value.

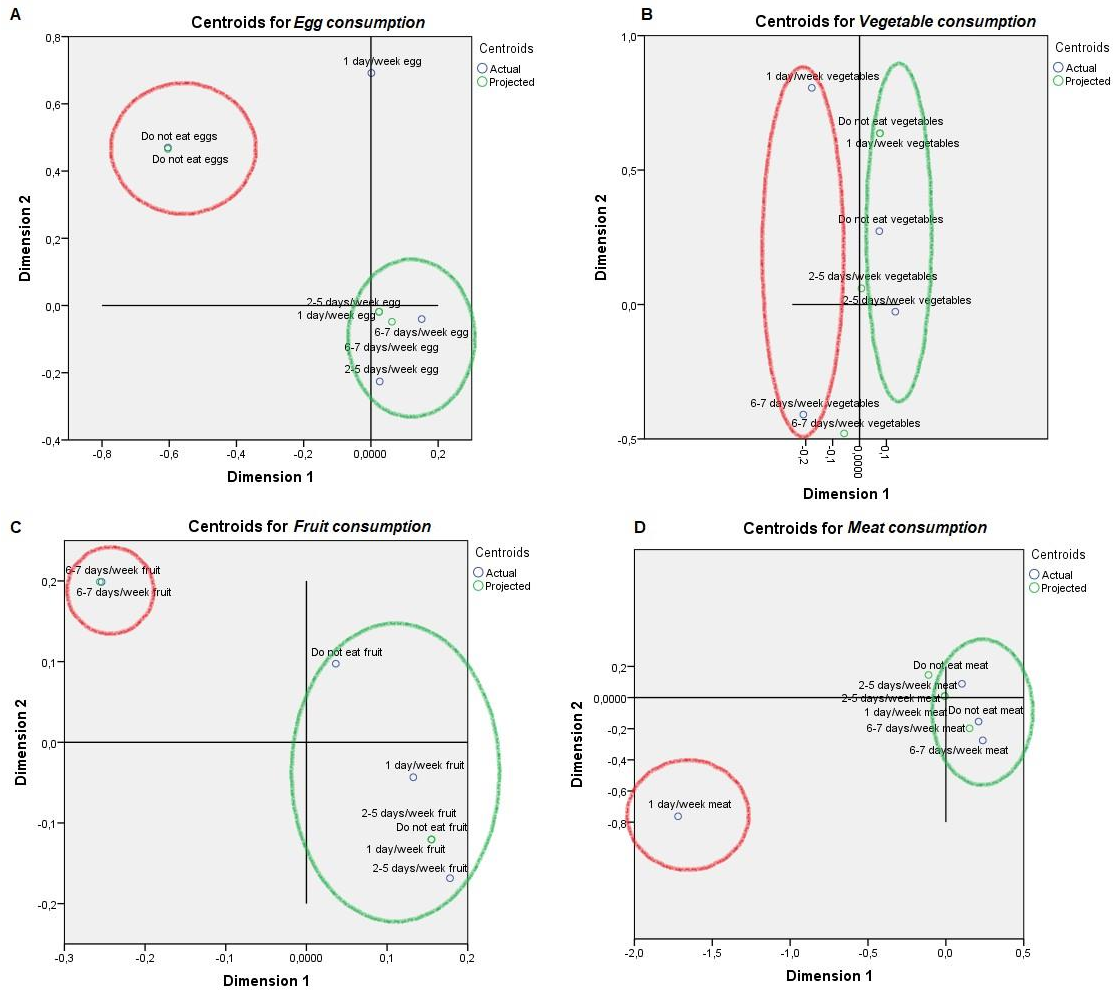



**Figure 2.** *Cont.*

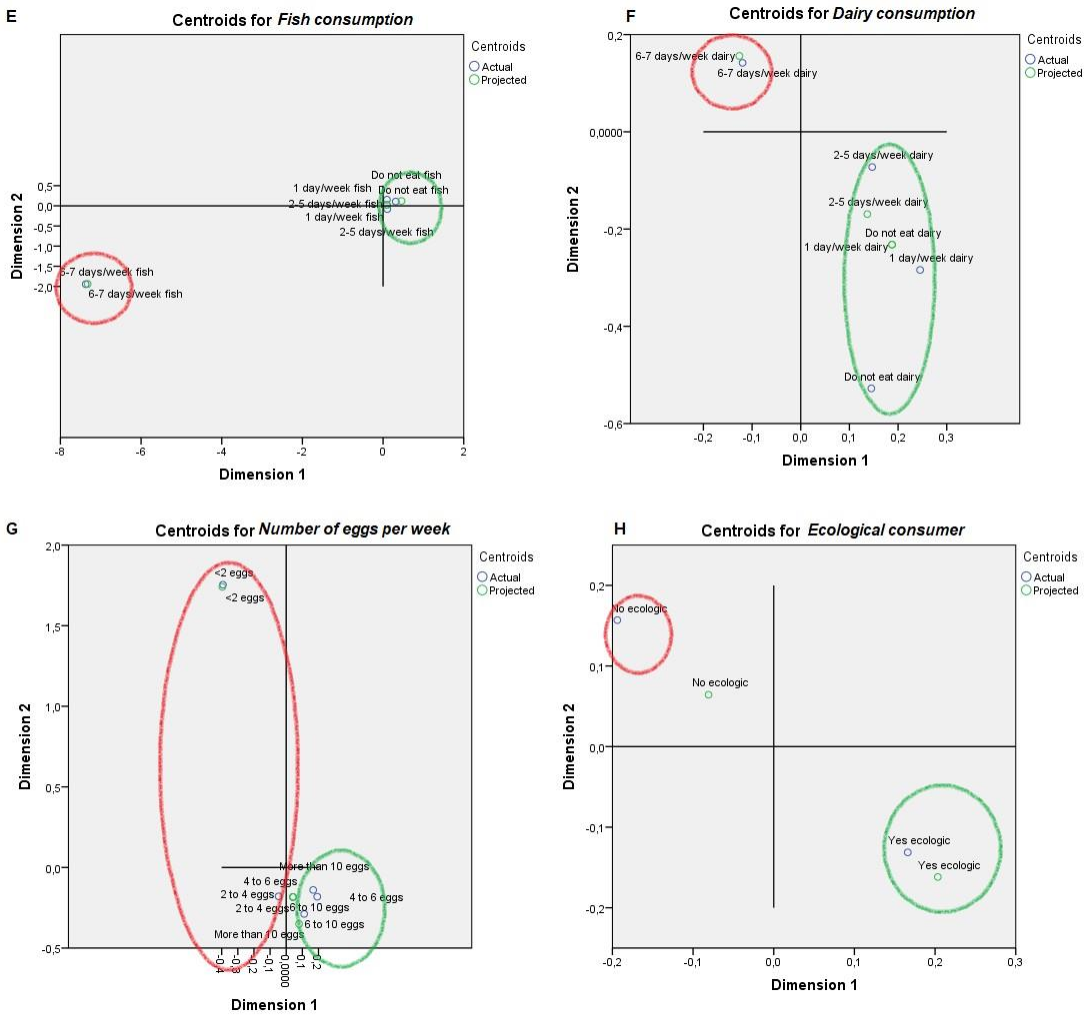



**Figure 2.** Object scores plot visualization of Professional Customer Profiles with regards panelist diet habits, egg consumption non-affine profile or PPA (red), and affine profile or PPB (green). Panelist diet habits were as follows: (**A**) Egg consumption, (**B**) Vegetable consumption, (**C**) Fruit consumption, (**D**) Meat consumption, (**E**) Fish consumption, (**F**) Dairy consumption, (**G**) Number of eggs per week and (**H**) Ecological consumer.

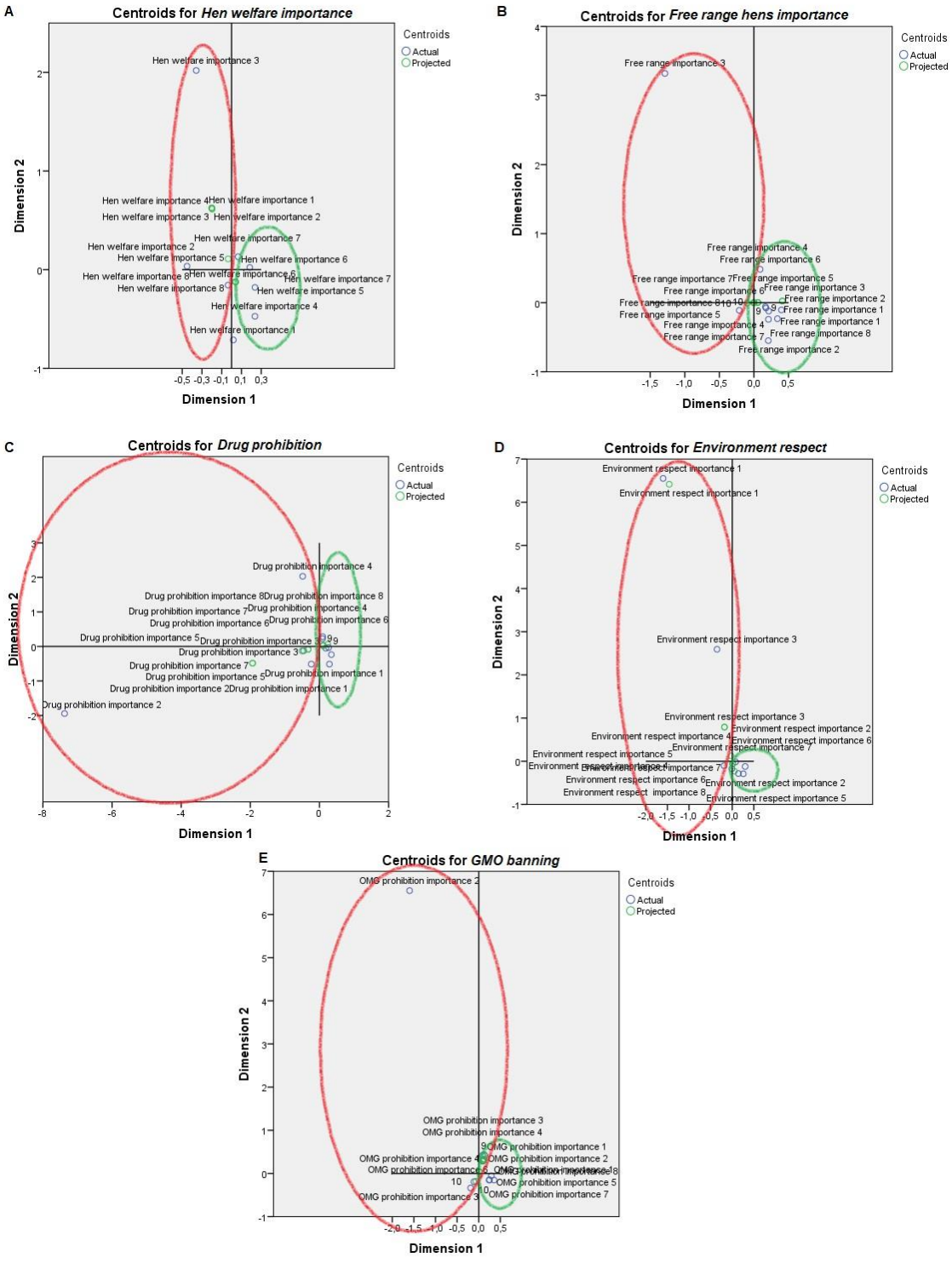



**Figure 3.** Object scores plot visualization of Professional Customer Profiles with regards production context awareness, egg consumption non-affine profile or PPA (red), and affine profile or PPB (green). Production context awareness was scored through: (**A**) Hen welfare importance, (**B**) Free range hens importance, (**C**) Drug prohibition, (**D**) Environment respect and (**E**) GMO banning.

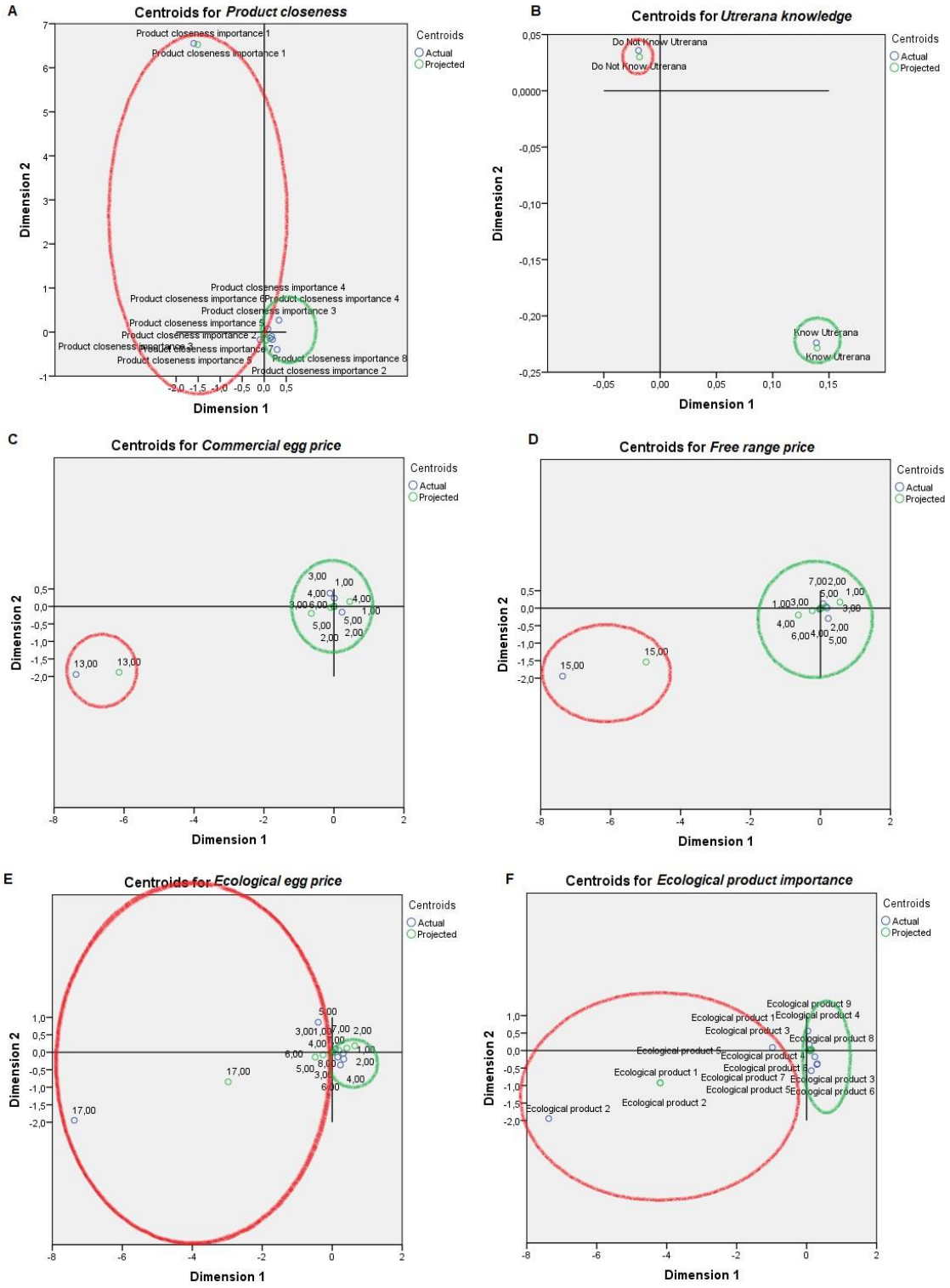



**Figure 4.** *Cont.*

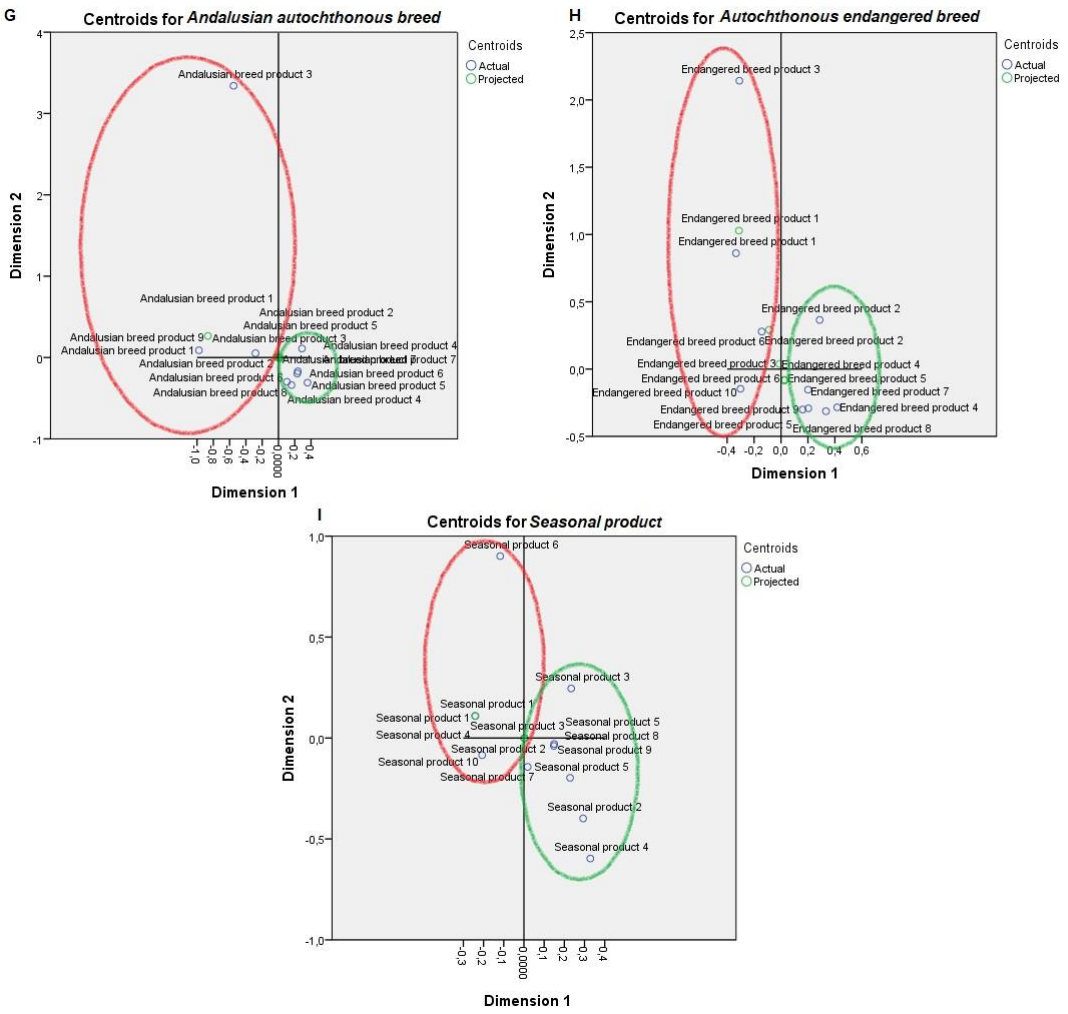



**Figure 4.** Object scores plot visualization of Professional Customer Profiles with regards product consciousness, egg consumption non-affine profile or PPA (red), and affine profile or PPB (green). Product consciousness was scored through: (**A**) Product closeness, (**B**) Utrerana knowledge, (**C**) Commercial egg price, (**D**) Free range price, (**E**) Ecological egg price, (**F**) Ecological product importance, (**G**) Product deriving from an Andalusian autochthonous breed, (**H**) Product deriving from an autochthonous endangered breed and (**I**) Product having a seasonal nature.

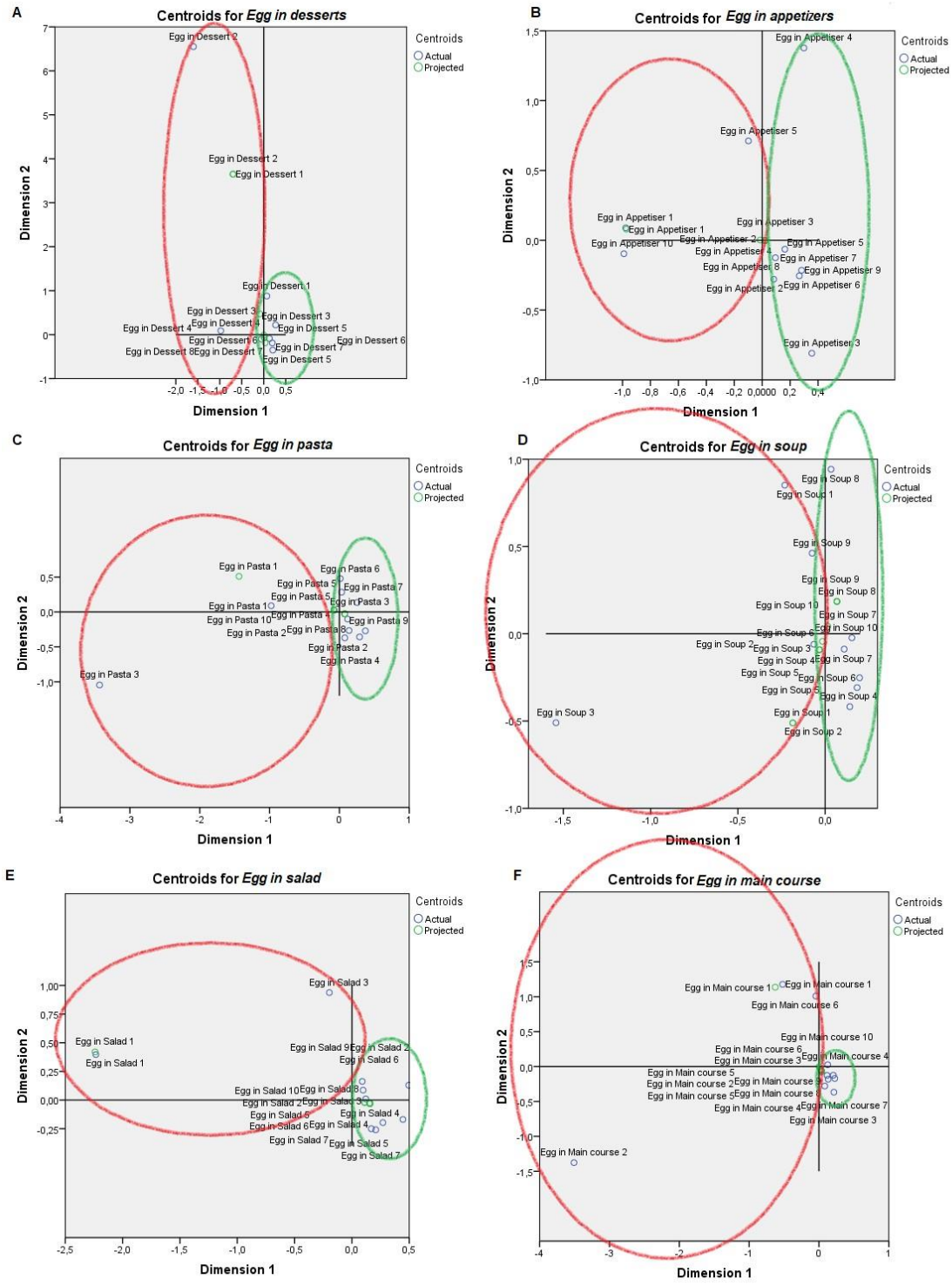



**Figure 5.** Object scores plot visualization of Professional Customer Profiles with regards cuisine applicability, egg consumption non-affine profile or PPA (red), and affine profile or PPB (green). Cuisine applicability was scored through: (**A**) Egg applicability in desserts, (**B**) in appetizers, (**C**) in pasta, (**D**) in soup, (**E**) in salad and (**F**) in main course.

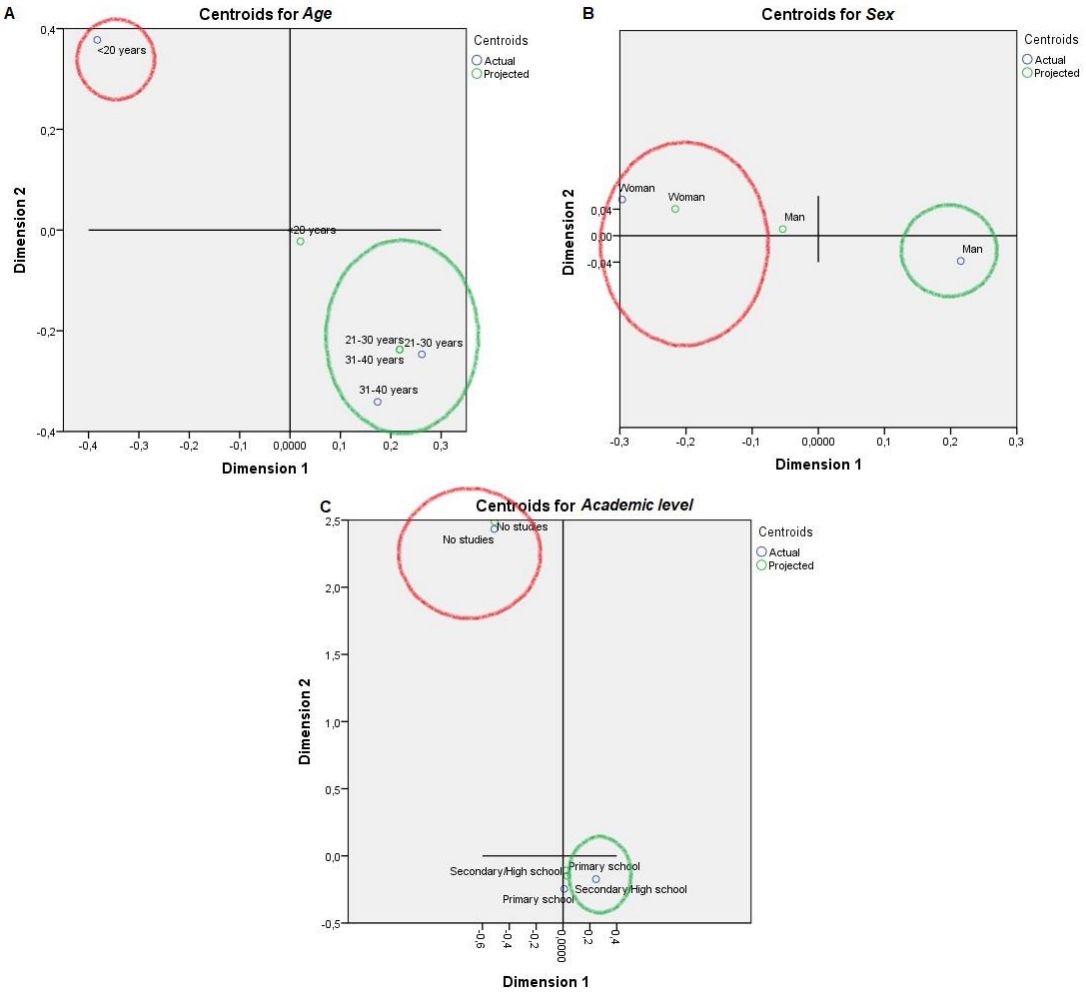



**Figure 6.** Object scores plot visualization of Professional Customer Profiles with regards professional characterization, egg consumption non-affine professional profile or PPA (red), and affine profile or PPB (green). Professional characterization was determined through: (**A**) Age, (**B**) Sex and (**C**) Academic level.

## 3. Results

Kruskal-Wallis H reported significant differences ($p < 0.05$) for all egg sensory attributes across egg type categories except for white color ($p > 0.05$). Dunn's tests reported egg categories were significantly different, with Utrerana egg reporting the highest median (5), followed by ecologic (3), and commercial (2). The Utrerana egg scored one median-unit higher than commercial lineage, which also presented a significantly lower score for flavor, overall and on plate broken egg visual value when compared to ecologic eggs (1 point higher) and Utrerana eggs (2 points higher), with no significant difference between Utrerana and ecologic eggs. Texture was only significantly different between commercial and ecologic eggs ($p < 0.05$),

**Table 3.** Model partitioning fit and loss analysis for Utrerana native hen egg sensory attributes (yellow), panelist diet habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and panelist characterization (grey) as perceived by cuisine instructed panelists (n = 192).

| Set | Variables | Categories | Multiple Fit | | | Single Fit | | | Single Loss | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Dimension 1 | Dimension 2 | Sum | Dimension 1 | Dimension 2 | Sum | Dimension 1 | Dimension 2 | Sum |
| Egg sensory attributes | Yolk colour | 8 | 0.060 | 0.015 | 0.074 | 0.052 | 0.013 | 0.065 | 0.007 | 0.002 | 0.009 |
| | White colour | 7 | 0.078 | 0.026 | 0.105 | 0.077 | 0.019 | 0.096 | 0.002 | 0.007 | 0.008 |
| | Smell | 8 | 0.088 | 0.022 | 0.110 | 0.085 | 0.005 | 0.090 | 0.003 | 0.017 | 0.021 |
| | Flavour | 8 | 0.048 | 0.030 | 0.078 | 0.039 | 0.024 | 0.062 | 0.009 | 0.006 | 0.015 |
| | Texture | 8 | 0.045 | 0.095 | 0.140 | 0.032 | 0.093 | 0.126 | 0.013 | 0.002 | 0.015 |
| | Overall value | 8 | 0.009 | 0.099 | 0.107 | 0.005 | 0.097 | 0.103 | 0.004 | 0.001 | 0.005 |
| | Whole egg visual value | 8 | 0.311 | 0.068 | 0.379 | 0.302 | 0.047 | 0.349 | 0.010 | 0.021 | 0.030 |
| | Broken egg visual value | 8 | 0.017 | 0.042 | 0.059 | 0.012 | 0.041 | 0.053 | 0.005 | 0.002 | 0.006 |
| Assessor diet habits | Egg consumption | 4 | 0.002 | 0.162 | 0.164 | 0.002 | 0.161 | 0.163 | 0.000 | 0.001 | 0.001 |
| | Vegetable consumption | 4 | 0.032 | 0.254 | 0.286 | 0.032 | 0.249 | 0.281 | 0.000 | 0.005 | 0.005 |
| | Fruit consumption | 4 | 0.014 | 0.134 | 0.148 | 0.013 | 0.132 | 0.145 | 0.001 | 0.002 | 0.003 |
| | Meat consumption | 4 | 0.005 | 0.017 | 0.022 | 0.005 | 0.016 | 0.021 | 0.001 | 0.001 | 0.001 |
| | Fish consumption | 4 | 0.906 | 0.067 | 0.974 | 0.906 | 0.055 | 0.961 | 0.001 | 0.012 | 0.013 |
| | Dairy consumption | 4 | 0.006 | 0.116 | 0.122 | 0.004 | 0.101 | 0.105 | 0.002 | 0.015 | 0.017 |
| | Number of eggs per week | 5 | 0.022 | 0.525 | 0.547 | 0.021 | 0.524 | 0.545 | 0.001 | 0.001 | 0.002 |
| | Ecological consumer | 2 | 0.000 | 0.006 | 0.007 | 0.000 | 0.006 | 0.007 | 0.000 | 0.000 | 0.000 |
| Production context awareness | Hen welfare | 8 | 0.040 | 0.025 | 0.065 | 0.003 | 0.008 | 0.011 | 0.038 | 0.017 | 0.054 |
| | Free range hens | 10 | 0.230 | 0.026 | 0.256 | 0.216 | 0.001 | 0.217 | 0.014 | 0.025 | 0.039 |
| | Drug prohibition | 10 | 0.985 | 0.108 | 1.093 | 0.644 | 0.073 | 0.717 | 0.341 | 0.035 | 0.376 |
| | Environment respect | 8 | 0.069 | 0.854 | 0.923 | 0.036 | 0.798 | 0.834 | 0.033 | 0.056 | 0.089 |
| | GMO banning | 10 | 0.048 | 0.024 | 0.072 | 0.045 | 0.012 | 0.058 | 0.002 | 0.012 | 0.014 |
| Product consciousness | Product closeness | 8 | 0.055 | 0.676 | 0.732 | 0.055 | 0.674 | 0.729 | 0.001 | 0.003 | 0.003 |
| | Utrerana knowledge | 2 | 0.000 | 0.002 | 0.002 | 0.000 | 0.002 | 0.002 | 0.000 | 0.000 | 0.000 |
| | Commercial egg price | 13 | 0.021 | 0.051 | 0.072 | 0.020 | 0.050 | 0.071 | 0.001 | 0.001 | 0.001 |
| | Free range egg price | 15 | 0.002 | 0.051 | 0.052 | 0.001 | 0.051 | 0.052 | 0.000 | 0.000 | 0.001 |
| | Ecological egg price | 17 | 0.002 | 0.025 | 0.027 | 0.000 | 0.001 | 0.001 | 0.002 | 0.024 | 0.026 |
| | Ecological product | 9 | 2.097 | 0.080 | 2.177 | 2.094 | 0.056 | 2.150 | 0.002 | 0.024 | 0.027 |
| | Andalusian autochthonous breed product | 9 | 0.803 | 0.009 | 0.812 | 0.803 | 0.001 | 0.804 | 0.000 | 0.008 | 0.009 |
| | Endangered breed product | 10 | 0.011 | 0.140 | 0.150 | 0.008 | 0.127 | 0.135 | 0.003 | 0.013 | 0.016 |
| | Seasonal product | 10 | 0.004 | 0.019 | 0.023 | 0.001 | 0.001 | 0.002 | 0.004 | 0.018 | 0.021 |
| Cuisine applicability | Egg in desserts | 8 | 0.039 | 0.641 | 0.679 | 0.023 | 0.526 | 0.548 | 0.016 | 0.115 | 0.131 |
| | Egg in appetizers | 10 | 0.017 | 0.340 | 0.357 | 0.002 | 0.313 | 0.315 | 0.015 | 0.027 | 0.042 |
| | Egg in pasta | 10 | 0.032 | 0.222 | 0.253 | 0.021 | 0.213 | 0.234 | 0.010 | 0.009 | 0.019 |
| | Egg in soup | 10 | 0.011 | 0.247 | 0.258 | 0.006 | 0.242 | 0.248 | 0.006 | 0.005 | 0.011 |
| | Egg in salad | 10 | 3.594 | 0.255 | 3.849 | 3.585 | 0.215 | 3.800 | 0.009 | 0.040 | 0.049 |
| | Egg in main course | 10 | 2.323 | 0.924 | 3.247 | 2.319 | 0.904 | 3.223 | 0.004 | 0.020 | 0.024 |
| Assessor characterization | Age | 3 | 0.193 | 0.046 | 0.239 | 0.192 | 0.046 | 0.239 | 0.000 | 0.000 | 0.000 |
| | Sex | 2 | 0.183 | 0.012 | 0.195 | 0.183 | 0.012 | 0.195 | 0.000 | 0.000 | 0.000 |
| | Academic level | 3 | 0.015 | 0.475 | 0.490 | 0.008 | 0.475 | 0.483 | 0.007 | 0.000 | 0.007 |

with the latter reporting a one-point-higher median than commercial or Utrerana eggs. Commercial eggs' whole egg visual value was significantly different to that of ecologic and Utrerana eggs.

Single ICC, determining how a single observation taken at random may correlate to another single observation, was 0.105, 0.120 and 0.125, for commercial, Utrerana and ecologic eggs, respectively. This could be expected, given that we were considering panelists' personal appreciation of certain products, and no correlation should be expected beforehand as they may be strongly conditioned by subjective factors. However, average ICC and Cronbach's alpha, that is, how consistent the whole panel of panelists is on average, were 0.800, 0.826 and 0.829, for commercial, Utrerana and ecologic eggs, respectively, reporting an excellent repeatability. This suggested the survey and scales used were sound and the panel was properly instructed and reliable.

Eigenvalues were high (0.673 and 0.645 for dimensions 1 and 2, respectively). Hence, the actual fit value was 1.318. A bi-dimensional solution was chosen, so 1.318/2 = 65.9% of the variation was calculated in the analysis, with 0.673/1.318 = 51.1% of the actual fit calculated by the first dimension and 0.645/1.318 = 48.9% by the second dimension.

Table 2 shows a summary of loss functions for each dimension and set. Average loss was 2 - 1.318 = 0.682 in our study and not necessarily high. The number of dimensions was equal to 2 (0.682 + 1.318). The single and multiple fit of variables is presented in Table 3. Component loadings are presented in Table S6. The visual maps depicted in Figures 1-6 are defined by all variables listed in Tables S1 and S2. Those variables, which in sum showed a multiple fit of more than 0.1 (Table 3), may play a more important role in the explanation of variance. Variable values, which are not displayed, were mainly spread around the axis of coordinates. By neglecting this proportion of data none of the influential values are lost, but the readability of figures is improved. Component loadings (interpanelist agreement) are shown for each variable separately in Table S5. Dimension 1 shows that the panelists agree very much on the attribute whole egg visual, fish consumption, drug prohibition, commercial egg price, free range egg price, ecological egg price, ecological product status relevance, and salad applicability. The second dimension shows panelist

agreement is dominated by environmental aspects, egg applicability in desserts, and academic level.

Apparently, when analyzing each set separately, yolk color, flavor and overall score were the attributes on which the panelists agreed less. These lowest agreement values are also reported for vegetable and meat consumption, hen welfare and genetically modified organism (GMO) banning, Utrerana knowledge, seasonal product conception or egg applicability in soup.

Two very distinct professional profiles are identified regarding attitudes towards eggs (Figures 1-6), Professional Profile A-non-affine profile (PPA, in red in plots), and Professional Profile B-affine profile (PPB, in green in plots). The egg sensory attributes set is assessed in Figure 1. PPA scored yolk color from 2 to 4. The most of the observers from PPB scored yolk color higher than 5 out of 8 levels in the scale. PPA normally scored white color 1 or 2, while PPB scored white yolk from 3 to 6. PPA scored odor from 1 to 4, while PPB scored odor from 4 to 8. PPA scored flavor from 2 to 4, while PPB scored it from 5 to 8. PPA scored texture with the values of 2, from 4 to 5 and from 7 to 8, while PPB provided constantly increasing values from 1 to 7. For the overall score, PPA provided scores of 2 or 8 while PPB scores increasingly ranged from 4 to 7. For whole egg, PPA provided 4 or 8 scores, while PPB provided values from 2 to 6 (excluding 4). The on-plate broken egg value was irregularly scored by PPA and PPB.

PPA does not usually consume eggs, while PPB consumes more than four eggs a week more than two days per week (Figure 2). PPA either does not consume vegetables or consumes them from six to seven days a week, while PPB consumes vegetables from one to five days a week. PPA consumes fruit six to seven days a week while PPB or does not eat fruit or eat it less than five days a week. PPA eats meat one day per week while PPB eats meat more than two days per week. Fish and dairy consumption habits were reported by PPA in six to seven days, while PPB used to consume fish and dairy products less than five days a week or did not consume them at all. PPA did not consume ecological products while PPB did consume ecological products.

PPA provided a low importance to hen welfare, while PPB provided it with a higher importance (Figure 3). Contrastingly, PPA provided the highest importance for hens

being kept in free range conditions in the scale, while PPB scored differently from 1 to 7 and from 8 to 9. PPA scored drug prohibition importance with 1 to 2 values and 4, while PPB scored its importance from 4 to 6 and from 8 to 9. A more irregular trend, supported by the low component loadings (Supplementary Table S6), was described in both profiles for environmental respect and GMO banning.

PPA scored product closeness from 1 to 3, while PPB scored it from 4 to 8. PPB was acquainted with the Utrerana breed while PPA was not (Figure 4). Contrary to PPB, PPA progressively misattributed the highest prices to commercial, free range and ecological eggs, respectively, and provided the lowest importance to ecological products or to the product being linked to an Andalusian autochthonous or endangered breed. The importance conferred to the seasonal attribute of the product described an irregular scoring pattern for both PPA and PPB.

PPA presented a lower trend to use eggs in desserts, as appetizers, in pasta, soup or salad than PPB (Figure 5). PPA mostly comprise women under 20 years old with no studies, while PPB comprised men from 21 to 40 years old and with secondary studies (Figure 6).

## 4. Discussion

The eigenvalues of the two dimensions that result from the nonlinear canonical correlation analysis are quite high, with 0.673 for the first dimension and 0.645 for the second dimension. Our total fitness value of 1.318 can be considered appropriate for this type of treatment (van der Burg and Dijksterhuis, 1996), as it has been reported by several authors and food products. Other two-dimensional solutions reported in the literature have produced total fit indexes of 1.644 in apples, 1.763 in luncheon meat, 1.192 in water and 1.856 in cheese (van der Burg and Dijksterhuis, 1996; Bárcenas, et al., 2003). This makes the conclusions driven from the present study valid and reliable.

European consumers prefer darker yolks, given the psychological healthier egg qualities misattribution. Observers scored Utrerana yolk color and odor significantly higher, which may be based on Utrerana's acknowledged darker yolk color when compared to laying lineages' yolk color (González Ariza, et al., 2019). The higher pigmentation found in some strains may be due to different genetic capabilities to absorb and deposit pigments in yolk (Sirri et al., 2007). Different egg

yolk color preferences have been reported between northern and southern European countries (Hernandez et al., 2001), with a taste towards intensely colored (golden-orange) yolks in southern countries, contrasting with what occurs in the majority of consumers worldwide, where consumers show a greater affinity towards brighter yolks. Similarly, consumers of ecologic/organic eggs generally accept paler yellow yolks, as reported by Grashorn (2016).

Yolk color has been reported to depend directly on the carotenoid level and on the proportion between yellow and red carotenoids in the feed provided to laying hens. The content of yellow carotenoids (lutein, zeaxanthin, cryptoxanthin, violaxanthin, ethyl ester of β-apo-81-carotenoic acid, β-apo-81-carotenal) stabilize the yellow color in the yolk, but do not intensify it. Contrastingly, for a rather intense, golden-orange color, red carotenoids, such as capsanthin/capsorubin, canthaxanthin, and citranaxanthin have to be added to the feed. Red carotenoids cover yellow carotenoids, and if their content is further increased then the yolk color presents a pinkish or red tone (Grashorn, 2016).

A stronger egg odor has been attributed to dual-purpose hens when compared to laying hens (Rizzi and Marangon, 2012). Additionally, native breeds' eggs have reported similar or superior values for aroma than improved breeds eggs (Haunshi et al., 2010). These results contrast with those of Olugbemi et al. (2013), who did not find significant differences between commercial laying hens and local breeds. Highly significant differences have been reported for egg composition across hen breeds and avian species, particularly regarding egg volatiles, fatty acids content, and albumen proteome composition (Jin et al., 2019). Supportive findings by Rizzi and Marangon (2012) indicated that dual-purpose hens present a stronger flavor. Haunshi, et al. (2010) also found flavor acceptability was significantly higher in local breeds than in improved breeds. No significant differences were found in either the texture across egg types, or in the literature for the texture of processed scrambled eggs belonging to hens fed on alternative products to dietary molt (Landers et al., 2005).

The Utrerana eggs' overall score was significantly higher than commercial eggs' score, agreeing with earlier reports (Haunshi, et al., 2010). The on-plate broken egg visual values were significantly lower in commercial eggs than ecologic and

Utrerana eggs. This suggests that the Utrerana egg's higher proportion of yolk and a darker yolk (González Ariza, et al., 2019) would have a greater market acceptability. Panelists agreed very much on the whole egg visual value, as previously reported (González Ariza, et al., 2019). Furthermore, external appearance and eggshell color has been suggested to hold some positive correlations with egg quality parameters (Samiullah et al., 2015).

Panelists' agreement was higher for fish consumption; drug prohibition; commercial, free range and ecological egg price; ecological product status relevance and egg in salad. Contextually, total drug banning led to the promotion of the consciousness of drug use, which develops a popular sense towards the effects of antibiotics and growth promoters on food safety and health in farms in Europe, which may be the basis for the panelists' high agreement on the subject (Donoghue, 2003). Furthermore, it can be inferred that when prices are higher, environmentally friendly food production and ecological products are regarded as secondary priorities (Uusitalo, 1990). Still, a tendency for consumers to pay higher prices for these environmentally-friendly products has been described in the literature (Beckmann and Kristensen, 1994).

Panelists agreed with respect to the environment, use of eggs in dessert and academic level (second dimension). Food consumption and production trends and patterns are one of the main causes of environmental pressure. Consumers are aware of this; hence, their consumption choices guide the search for more sustainable productive systems (Roy et al., 2009). Not only did the PPB group consume more eggs, but also scored sensory attributes higher than the PPA group (non-usual eggs consumers, <4 eggs/week) and were more conscious about ecological production. Contrastingly, PPA reported a higher fruit, fish and dairy consumption, contrasting with the more frequent PPB meat and ecological products consumption habits. A lower frequency of consumption of eggs or meat in habitual fish consumers has been observed (Barberger-Gateau et al., 2005). This could be explained by the division of panelists depending on their consumption livestock derived products. European diets are characterized by a high intake of livestock products (meat, dairy and eggs) (Pan et al., 2012).

The PPA group scored product closeness lower, was not acquainted with the Utrerana breed, misattributed a higher price to Utrerana eggs than to other egg types, scored ecological product status relevance lower and provided a relatively low importance to the product being linked to an Andalusian autochthonous and endangered breed. Contrastingly, the PPB group was acquainted with the Utrerana breed and scored the product and local endangered breeds-based production systems higher. Links with local breeds and their products starting from childhood improve their marketing strategies (Cerjak et al., 2014). Hence, alternative markets may help conserving endangered native breeds and valuable animal genetic resources, as consumer demand for specialty livestock products and the willingness of consumers to pay for them largely depends on their lack of availability and their knowledge on the breed involved (Tienhaara et al., 2013).

The functions of eggs, like coagulating, foaming, emulsifying and contributing nutrients, make them a useful ingredient in a lot of gastronomic preparations (Stadelman et al., 1995). PPB provided a higher cuisine applicability to Utrerana eggs (desserts, appetisers, pasta, soup, salad and main courses) than PPA. Professionals acquainted with the product and its qualities, find a greater applicability than those who are not familiar. Polesel et al. (2013) suggested egg consumption as an indicator of a diet rich in foods such as desserts and meat.

Women under 20 years old with no studies fit the PPA, while PPB would be characterized by men from 21 to 40 years old with secondary studies. These results agree with those of a previous study reporting that men usually consume more eggs than women, and individuals of 20-30 years old had the lowest odds of consuming eggs (Conrad et al., 2017). Stefanikova et al. (2006)suggested men eat more meat, eggs and milk, while women eat more fruit and vegetables. Another study conducted in college students and nutrition educators suggested men consumed more meat, poultry, fish and eggs, while women consumed more vegetables and fruit (Hertzler and Bruce, 2002). Bejaei et al. (2011) showed that free-run, free range, and organic eggs consumers have higher education levels compared with consumers of other egg types; this is supported by the fact that PPB-which valued more highly ecological and local hens' eggs, than commercial ones-also presented higher education levels.

The differentiated quality of a product can be protected in markets offering a wider scope of valuable products, adapted to the consumer's special needs (Toften and Hammervoll, 2013). Breed choice is mainly prescribed by the regulations of the producers, while the high quality of the products is appreciated by a small group of consumers, which indirectly promotes a local breed's preservation (Ligda and Casabianca, 2013). Customer profile analysis adds value to the formulation of investment projects, providing information on consumers' reactions to alternative products, generated through innovation or trend. In this context, the preferences of the target market among similar products, their purchase incentives, needs, among others, must be established. In this way, market research and the analysis of data collected enables the issuing of a diagnosis on the viability of the product in question, in turn translating into the sustainability of the breed that it originated from.

## 5. Conclusions

Involving autochthonous breeds, such as Utrerana, in common production systems and commercial chains seeking the characterization of differentiated products could be the key to improving profitability in future sustainable poultry productions. Defining different attitudes of costumers towards eggs may help outlining potential strategies for the design and implementation of marketing campaigns, indirectly identifying those sectors to which a greater effort should be made in an attempt to revalue a native breed's egg products. These profiles may also suggest strategies on how to successfully achieve the aim of covering the currently increasing demand for non-conventional quality products linked to particular breeds and production systems from markets that are different from those normally established for classical highly productive systems.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2076-2615/9/11/920/s1: Table S1. Recipe and chemical composition of the compound feed used for feeding the hen sets in the study; Table S2. Descriptive statistics for Utrerana native hen egg sensory attributes (yellow), Panelist diet habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and consumer characterization (grey) as perceived by cuisine instructed panelists (n = 192); Table S3. Scales for Utrerana native hen variables included in the sets of egg sensory attributes, Panelist diet habits, production context awareness, product consciousness, cuisine applicability and panelist characterization as perceived by cuisine instructed panelists (clustering set in bold); Table S4. Testing for normality using Shapiro-Francia W' in Utrerana native hen egg sensory attributes (yellow), Panelist diet habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and Panelist characterization (grey) as perceived by cuisine instructed panelists (n = 192); Table S5. Kruskal

Wallis H Ranks, Dunn's test and Bonferroni's significance correction and Median sorted by egg type for Utrerana native hen egg sensory attributes; Table S6. Components loadings for nonlinear canonical correlation analysis for Utrerana native hen egg sensory attributes (yellow), Panelist diet habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and Panelist characterization (grey) as perceived by cuisine instructed panelists (n = 192).

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**Supplementary Table S1.** Recipe and chemical composition of the compound feed used for feeding the hen sets in the study.

| **Utrerana breed and Leghorn Lohmann LSL-Classic lineage fed on commercial conditions** | | **Leghorn Lohmann LSL-Classic lineage fed on ecological conditions[a]** | |
|---|---|---|---|
| *RECIPE* | | *RECIPE* | |
| Corn | | Corn | |
| Wheat | | Wheat | |
| Shelled toasted soybeans flour | | Shelled toasted soybeans flour | |
| Calcium carbonate | | Calcium carbonate | |
| Barley | | Barley | |
| Monocalcium phosphate | | Monocalcium phosphate | |
| Soybean oil | | Soybean oil | |
| Sodium chloride | | Sodium chloride | |
| Sodium bicarbonate | | Sodium bicarbonate | |
| *CHEMICAL COMPOSITION (%)* | | *CHEMICAL COMPOSITION (%)* | |
| Crude protein | 15.7 | Crude protein | 15.8 |
| Crude fat and oils | 3.6 | Crude fat and oils | 4.2 |
| Crude fiber | 2.4 | Crude fiber | 3.8 |
| Crude ashes | 14 | Crude ashes | 12.4 |
| Calcium | 4.1 | Calcium | 4.10 |
| Phosphorus | 0.66 | Phosphorus | 0.65 |
| Sodium | 0.15 | Sodium | 0.10 |
| Methionine | 0.38 | Methionine | 0.30 |
| Lysine | 0.79 | Lysine | 0.80 |

[a]The selection of the ecologic compound feed to be fed to the hens was chosen following the premises described in the Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control.

**Supplementary Table S2.** Descriptive statistics for Utrerana native hen egg sensory attributes (yellow), Panelist diet habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and consumer characterization (grey) as perceived by cuisine instructed panelists (n=192).

| | Variables | Categories/Maximum[a]/Hedonic points | Optimal Scaling Level | Mean | Std. Error of Mean | Median | Variance | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|---|
| Egg sensory attributes | Yolk colour | 1-8 | Ordinal | 3.60 | 0.128 | 3 | 3.132 | 1 | 8 |
| | White colour | 1-7 | Ordinal | 3.23 | 0.102 | 3 | 2.010 | 1 | 7 |
| | Smell | 1-8 | Ordinal | 3.79 | 0.118 | 4 | 2.668 | 1 | 8 |
| | Flavour | 1-8 | Ordinal | 3.36 | 0.125 | 3 | 3.017 | 1 | 8 |
| | Texture | 1-8 | Ordinal | 3.39 | 0.119 | 3 | 2.741 | 1 | 8 |
| | Overall value | 1-8 | Ordinal | 3.35 | 0.11 | 3 | 2.344 | 1 | 8 |
| | Whole egg visual value | 1-8 | Ordinal | 3.07 | 0.092 | 3 | 1.634 | 1 | 8 |
| | Broken egg visual value | 1-8 | Ordinal | 3.32 | 0.122 | 3 | 2.838 | 1 | 8 |
| Panelist diet habits | Egg consumption | 1-4 | Ordinal | 2.73 | 0.039 | 3 | 0.290 | 1 | 4 |
| | Vegetable consumption | 1-4 | Ordinal | 3.08 | 0.048 | 3 | 0.449 | 1 | 4 |
| | Fruit consumption | 1-4 | Ordinal | 3.14 | 0.060 | 3 | 0.687 | 1 | 4 |
| | Meat consumption | 1-4 | Ordinal | 2.95 | 0.037 | 3 | 0.265 | 1 | 4 |
| | Fish consumption | 1-4 | Ordinal | 2.44 | 0.049 | 3 | 0.467 | 1 | 4 |
| | Dairy consumption | 1-4 | Ordinal | 3.34 | 0.065 | 4 | 0.824 | 1 | 4 |
| | Number of eggs per week | 1-5 | Ordinal | 2.36 | 0.059 | 2 | 0.671 | 1 | 5 |
| | Ecological consumer | 1-2 | Single Nominal | 1.45 | 0.036 | 1 | 0.249 | 1 | 2 |
| Production context awareness | Hen welfare | 1-8 | Ordinal | 6.81 | 0.128 | 8 | 3.137 | 1 | 8 |
| | Free range hens | 1-10 | Ordinal | 7.69 | 0.192 | 9 | 7.096 | 1 | 10 |
| | Drug prohibition | 1-10 | Ordinal | 8.28 | 0.192 | 10 | 7.051 | 1 | 10 |
| | Environment respect | 1-8 | Ordinal | 7.00 | 0.123 | 8 | 2.89 | 1 | 8 |
| | GMO banning | 1-10 | Ordinal | 8.13 | 0.193 | 10 | 7.115 | 1 | 10 |
| Product consciousness | Product closeness | 1-8 | Ordinal | 6.66 | 0.129 | 8 | 3.211 | 1 | 8 |
| | Utrerana knowledge | 1-2 | Single Nominal | 1.86 | 0.025 | 2 | 0.121 | 1 | 2 |
| | Commercial egg price | 1-13[a] | Numerical | 2.15 | 0.114 | 2.3 | 2.498 | 1 | 13 |
| | Free range egg price | 1-15[a] | Numerical | 2.45 | 0.143 | 2.8 | 3.951 | 1 | 15 |
| | Ecological egg price | 1-17[a] | Numerical | 2.51 | 0.172 | 1 | 5.652 | 1 | 17 |
| | Ecological product | 1-9 | Ordinal | 6.94 | 0.147 | 7 | 4.143 | 1 | 9 |
| | Andalusian autochtonous breed product | 1-9 | Ordinal | 6.94 | 0.155 | 7 | 4.614 | 1 | 9 |
| | Endangered breed product | 1-10 | Ordinal | 6.91 | 0.209 | 7 | 8.347 | 1 | 10 |
| | Seasonal product | 1-10 | Ordinal | 7.42 | 0.175 | 8 | 5.900 | 1 | 10 |
| Cuisine applicability | Egg in desserts | 1-8 | Ordinal | 6.97 | 0.109 | 8 | 2.292 | 1 | 8 |
| | Egg in appetizers | 1-10 | Ordinal | 6.94 | 0.135 | 7 | 3.483 | 1 | 10 |
| | Egg in pasta | 1-10 | Ordinal | 7.31 | 0.160 | 7.5 | 4.928 | 1 | 10 |
| | Egg in soup | 1-10 | Ordinal | 6.28 | 0.169 | 6 | 5.481 | 1 | 10 |
| | Egg in salad | 1-10 | Ordinal | 6.27 | 0.178 | 7 | 6.102 | 1 | 10 |
| | Egg in main course | 1-10 | Ordinal | 6.75 | 0.183 | 7 | 6.408 | 1 | 10 |
| Panelist characterizatio | Age | 1-3 | Ordinal | 2.06 | 0.081 | 2 | 1.253 | 1 | 5 |
| | Sex | 1-2 | Single Nominal | 1.44 | 0.036 | 1 | 0.247 | 1 | 2 |
| | Academic level | 1-3 | Single Nominal | 2.02 | 0.030 | 2 | 0.173 | 1 | 3 |

[a]For numerical variables maximun value is reported instead of number of categories.

**Supplementary Table S3.** Scales for Utrerana native hen variables included in the sets of egg sensory attributes, Panelist diet habits, production context awareness, product consciousness, cuisine applicability and panelist characterization as perceived by cuisine instructed panelists (clustering set in bold).

| | |
|---|---|
| **Sensory attributes** (Yolk colour, white colour, smell, flavour, texture, overall value, whole egg visual value and broken egg visual value) | (1) I extremely dislike it |
| | (2) I dislike it a lot |
| | (3) I dislike it moderately |
| | (4) I slightly dislike it |
| | (5) I like it |
| | (6) I slightly like it |
| | (7) I like it moderately |
| | (8) I like it a lot |
| | (9) I extremely like it |
| **Sensory attributes** (White colour) | (1) I extremely dislike it to (8) I extremely like it |
| **Panelist diet habits** (Vegetable consumption, fruit consumption, meat consumption fish consumption and dairy consumption) | (1) No consumption |
| | (2) One day/week |
| | (3) 2 to 5 days/week |
| | (4) 6 to 7 days/week |
| **Panelist diet habits** (Egg consumption) | (1) ≤2 eggs per week |
| | (2) 2 to 4 eggs per week |
| | (3) 4 to 6 eggs per week |
| | (4) 6 to 10 eggs per week |
| | (5) >10 eggs per week |
| **Panelist diet habits** (Ecological consumer) | (1) Yes |
| | (2) No |
| **Production context awareness** (Free range hens, Drug prohibition, GMO banning), **product consciousness** (Product closeness, Endangered breed product, Seasonal product) and **cuisine applicability** (Egg in appetizers, Egg in pasta, Egg in soup, Egg in salad, Egg in main course) | (1) No importance to (10) Extremely important) |
| **Production context awareness** (Hen welfare and environment respect) and **cuisine applicability** (Egg in desserts) | (1) No importance to (8) Extremely important) |
| **Product consciousness** (Ecological product, Andalusian autochthonous breed product and product closeness) | (1) No importance to (9) Extremely important) |
| **Product consciousness** (Utrerana knowledge) | (1) Customer acquainted to the breed |
| | (2) Customers not acquainted to the breed |
| **Product consciousness** (Prices for commercial, free range or ecological eggs) | from 1 to 13, 1 to 15 and 1 to 17, respectively. |
| **Panelist characterization** (Age) | (1) ≤20 years old |
| | (2) from 21 to 30 years old |
| | (3) from 31 to 40 years old |
| | (4) from 41 to 50 years old |
| | (5) ≥51 years old |
| **Panelist characterization** (Sex) | (1) Man |
| | (2) Woman |
| **Panelist characterization** (Academic level) | (1) No studies |
| | (2) Primary school |
| | (3) High school |
| | (4) College or University studies |

**Supplementary Table S4.** Testing for normality using Shapiro-Francia W' in Utrerana native hen egg sensory attributes (yellow), Panelist diet habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and Panelist characterization (grey) as perceived by cuisine instructed panelists (n=192).

| | Variables | W' | V' | z | Prob>z | Skewness | Kurtosis |
|---|---|---|---|---|---|---|---|
| Egg sensory attributes | Yolk colour | 0.97 | 4.33 | 3.02 | 0.00 | 0.62 | -0.41 |
| | White colour | 0.99 | 1.53 | 0.88 | 0.19 | 0.49 | -0.10 |
| | Smell | 0.99 | 2.19 | 1.62 | 0.05 | 0.49 | -0.21 |
| | Flavour | 0.96 | 6.08 | 3.72 | 0.00 | 0.82 | 0.00 |
| | Texture | 0.97 | 5.06 | 3.34 | 0.00 | 0.80 | 0.45 |
| | Overall value | 0.96 | 6.46 | 3.85 | 0.00 | 0.94 | 1.13 |
| | Whole egg visual value | 0.98 | 2.59 | 1.96 | 0.03 | 0.69 | 0.96 |
| | Broken egg visual value | 0.98 | 3.33 | 2.48 | 0.01 | 0.66 | -0.17 |
| Panelist diet habits | Egg consumption | 0.98 | 3.04 | 2.30 | 0.01 | -1.33 | 1.75 |
| | Vegetable consumption | 1.00 | 0.18 | -3.58 | 1.00 | -0.41 | 0.35 |
| | Fruit consumption | 0.99 | 1.10 | 0.19 | 0.42 | -0.77 | 0.09 |
| | Meat consumption | 0.96 | 6.43 | 3.84 | 0.00 | -1.47 | 5.83 |
| | Fish consumption | 0.99 | 1.28 | 0.51 | 0.30 | -0.52 | -0.40 |
| | Dairy consumption | 0.96 | 5.86 | 3.65 | 0.00 | -1.37 | 1.04 |
| | Number of eggs per week | 0.97 | 4.99 | 3.32 | 0.00 | 0.98 | 1.85 |
| | Ecological consumer | 1.00 | 0.00 | -59.37 | 1.00 | 0.19 | -1.99 |
| Production context awareness | Hen welfare | 0.94 | 10.20 | 4.79 | 0.00 | -1.63 | 1.92 |
| | Free range hens | 0.94 | 8.66 | 4.45 | 0.00 | -1.02 | -0.14 |
| | Drug prohibition | 0.90 | 15.87 | 5.70 | 0.00 | -1.56 | 1.23 |
| | Environment respect | 0.90 | 15.80 | 5.69 | 0.00 | -1.94 | 3.04 |
| | GMO banning | 0.92 | 12.10 | 5.14 | 0.00 | -1.44 | 0.93 |
| Product consciousness | Product closeness | 0.97 | 3.95 | 2.83 | 0.00 | -1.24 | 0.70 |
| | Utrerana knowledge | 1.00 | 0.00 | -56.82 | 1.00 | -2.08 | 2.37 |
| | Commercial egg price | 0.55 | 70.58 | 8.78 | 0.00 | 5.04 | 33.13 |
| | Free range egg price | 0.68 | 49.90 | 8.06 | 0.00 | 3.91 | 22.94 |
| | Ecological egg price | 0.75 | 38.81 | 7.55 | 0.00 | 3.64 | 19.62 |
| | Ecological product | 0.97 | 4.30 | 3.01 | 0.00 | -0.86 | -0.06 |
| | Andalusian autochthonous breed product | 0.97 | 5.12 | 3.37 | 0.00 | -0.99 | 0.20 |
| | Endangered breed product | 0.98 | 3.62 | 2.66 | 0.00 | -0.62 | -0.73 |
| | Seasonal product | 0.99 | 2.06 | 1.49 | 0.07 | -0.76 | -0.06 |
| Cuisine applicability | Egg in desserts | 0.91 | 13.65 | 5.39 | 0.00 | -1.95 | 4.08 |
| | Egg in appetizers | 0.98 | 3.51 | 2.59 | 0.00 | -0.63 | 0.99 |
| | Egg in pasta | 0.99 | 1.14 | 0.26 | 0.40 | -0.56 | -0.15 |
| | Egg in soup | 1.00 | 0.45 | -1.65 | 0.95 | -0.19 | -0.62 |
| | Egg in salad | 0.99 | 1.43 | 0.73 | 0.23 | -0.39 | -0.50 |
| | Egg in main course | 0.98 | 3.00 | 2.26 | 0.01 | -0.60 | -0.31 |
| Panelist characterization | Age | 0.96 | 6.06 | 3.72 | 0.00 | 1.10 | 0.48 |
| | Sex | 1.00 | 0.00 | -59.89 | 1.00 | 0.25 | -1.96 |
| | Academic level | 1.00 | 0.02 | -7.78 | 1.00 | 0.11 | 2.92 |

**Supplementary Table S5.** Kruskal Wallis H Ranks, Dunn's test and Bonferroni's significance correction and Median sorted by egg type for Utrerana native hen egg sensory attributes.

| | Yolk colour | White colour | Smell | Flavour | Texture | Global value | Whole egg visual value | Broken egg visual value |
|---|---|---|---|---|---|---|---|---|
| Chi-Square | 61.28 | 3.669 | 15.718 | 18.435 | 7.083 | 23.082 | 10.354 | 41.937 |
| df | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Asymp. Significance | 0.000 | 0.160 | 0.000 | 0.000 | 0.029 | 0.000 | 0.006 | 0.000 |

| | Egg Type pairwise comparison | Test Statistic | Sig. | Bonferroni Adj. Sig. |
|---|---|---|---|---|
| Yolk colour | Commercial-Ecologic | 12.522 | 0.000 | 0.001 |
| Yolk colour | Commercial-Utrerana | 50.443 | 0.000 | 0.000 |
| Yolk colour | Ecologic-Utrerana | 20.666 | 0.000 | 0.000 |
| Smell | Commercial-Utrerana | 16.020 | 0.000 | 0.000 |
| Smell | Commercial-Ecologic | 2.002 | 0.157 | 0.471 |
| Smell | Utrerana-Ecologic | 2.776 | 0.096 | 0.287 |
| Flavour | Commercial-Ecologic | 7.880 | 0.005 | 0.015 |
| Flavour | Commercial-Utrerana | 13.333 | 0.000 | 0.001 |
| Flavour | Ecologic-Utrerana | 0.781 | 0.377 | 1.000 |
| Texture | Commercial-Utrerana | 1.222 | 0.269 | 0.807 |
| Texture | Commercial-Ecologic | 9.141 | 0.002 | 0.007 |
| Texture | Utrerana-Ecologic | 3.782 | 0.052 | 0.155 |
| Overall value | Commercial-Ecologic | -36.867 | 0.000 | 0.000 |
| Overall value | Commercial-Utrerana | -42.281 | 0.000 | 0.000 |
| Overall value | Ecologic-Utrerana | 5.414 | 0.572 | 1.000 |
| Whole egg visual value | Commercial-Ecologic | 11.782 | 0.001 | 0.002 |
| Whole egg visual value | Commercial-Utrerana | 21.014 | 0.000 | 0.000 |
| Whole egg visual value | Ecologic-Utrerana | 0.667 | 0.414 | 1.000 |
| Broken egg visual value | Commercial-Ecologic | 9.581 | 0.002 | 0.006 |
| Broken egg visual value | Commercial-Utrerana | 22.533 | 0.000 | 0.000 |
| Broken egg visual value | Ecologic-Utrerana | 3.125 | 0.077 | 0.231 |

| Egg type | Parameter | Yolk colour | White colour | Smell | Flavour | Texture | Overall value | Whole egg visual value | Broken egg visual value |
|---|---|---|---|---|---|---|---|---|---|
| Commercial | Median | 2 | 3 | 3 | 2 | 3 | 2 | 3 | 2 |
| Utrerana | Median | 5 | 3 | 4 | 4 | 3 | 4 | 3 | 4 |
| Ecologic | Median | 3 | 3 | 4 | 3 | 4 | 3 | 3 | 3 |

**Supplementary Table S6.** Components loadings for nonlinear canonical correlation analysis for Utrerana native hen egg sensory attributes (yellow), Panelist diet habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and Panelist characterization (grey) as perceived by cuisine instructed panelists (n=192).

| Set | Variables | Dimension | |
|---|---|---|---|
| | | 1 | 2 |
| Egg sensory attributes | Yolk colour | -0.013 | -0.013 |
| | White colour | 0.107 | -0.132 |
| | Smell | 0.450 | -0.050 |
| | Flavour | 0.097 | -0.167 |
| | Texture | -0.146 | 0.028 |
| | Overall value | 0.047 | -0.248 |
| | Whole egg visual value | -0.617 | -0.167 |
| | Broken egg visual value | 0.103 | 0.084 |
| Panelist diet habits | Egg consumption | 0.110 | -0.085 |
| | Vegetable consumption | -0.042 | -0.351 |
| | Fruit consumption | -0.199 | 0.155 |
| | Meat consumption | 0.047 | -0.062 |
| | Fish consumption | -0.930 | -0.246 |
| | Dairy consumption | -0.139 | 0.173 |
| | Number of eggs per week | 0.128 | -0.563 |
| | Ecological consumer | -0.160 | 0.127 |
| Production context awareness | Hen welfare | 0.076 | -0.237 |
| | Free range hens | -0.121 | -0.007 |
| | Drug prohibition | 0.534 | 0.134 |
| | Environment respect | 0.191 | -0.841 |
| | GMO banning | -0.078 | -0.217 |
| Product consciousness | Product closeness | 0.192 | -0.829 |
| | Utrerana knowledge | -0.055 | 0.090 |
| | Commercial egg price | -0.839 | -0.257 |
| | Free range egg price | -0.763 | -0.235 |
| | Ecological egg price | -0.621 | -0.176 |
| | Ecological product | 0.750 | 0.166 |
| | Andalusian autochthonous breed product | 0.110 | -0.034 |
| | Endangered breed product | 0.092 | -0.302 |
| | Seasonal product | 0.043 | -0.019 |
| Cuisine applicability | Egg in desserts | 0.127 | -0.663 |
| | Egg in appetizers | 0.123 | -0.010 |
| | Egg in pasta | 0.195 | -0.069 |
| | Egg in soup | 0.067 | 0.185 |
| | Egg in salad | 0.579 | -0.109 |
| | Egg in main course | 0.139 | -0.252 |
| Panelist characterization | Age | 0.154 | -0.168 |
| | Sex | -0.140 | 0.026 |
| | Academic level | 0.145 | -0.707 |

# Discriminant Canonical Analysis as a Validation Tool for Multivariety Native Breed Egg Commercial Quality Classification

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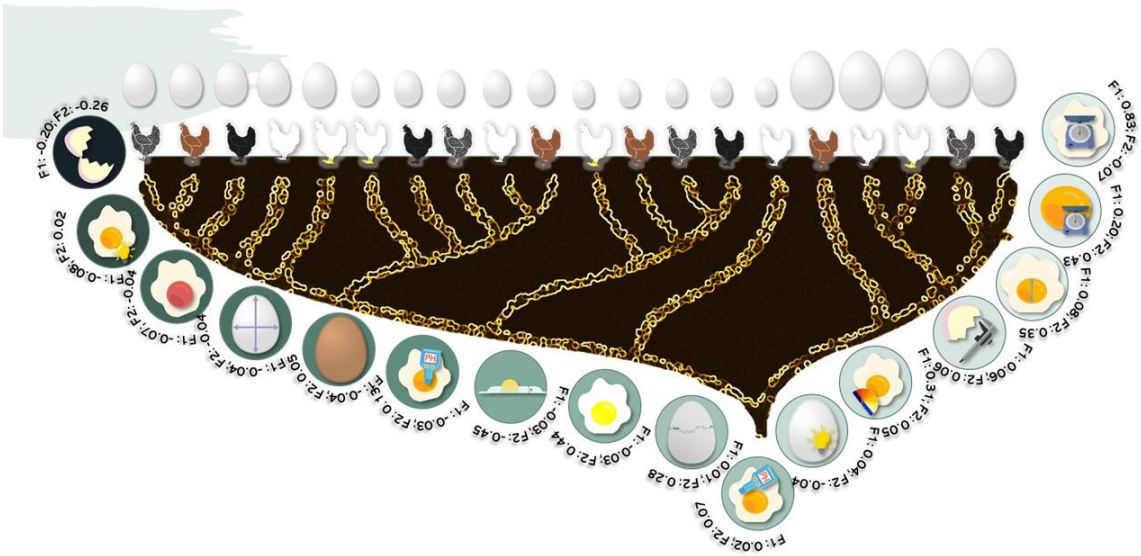
**Abstract:** This study aimed to develop a tool to validate multivariety breed egg quality classification depending on quality-related internal and external traits using a discriminant canonical analysis approach. A flock of 60 Utrerana hens (Franciscan, White, Black, and Partridge) and a control group of 10 Leghorn hens were placed in individual cages to follow the traceability of the eggs and perform an individual internal and external quality assessment. Egg groups were determined depending on their commercial size (S, M, L, and XL), laying hen breed, and variety. Egg weight, major diameter, minor diameter, shell b*, albumen height, and the presence or absence of visual defects in yolk and/or albumen showed multicollinearity problems (variance inflation factor (VIF) > 5) and were discarded. Albumen weight, eggshell weight, and yolk weight were the most responsible traits for the differences among egg quality categories (Wilks' lambda: 0.335, 0.539, and 0.566 for albumen weight, eggshell weight, and yolk weight, respectively). The combination of traits in the first two dimensions explained 55.02% and 20.62% variability among groups, respectively. Shared properties between Partridge and Franciscan varieties may stem from their eggs presenting heavier yolks and slightly lower weights, while White Utrerana and Leghorn hens' similarities may be ascribed to hybridization reminiscences.

**Graphical abstract:**



**Keywords**: Egg quality; external quality traits; internal quality traits; DSM color; fan color coordinate decomposition; mechanical eggshell strength; pH-related traits

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## 1. Introduction

In 2019, the world's hen egg production exceeded 1.6 billion eggs, 28.7% higher production than a decade before (FAOSTAT, 2021). Such a remarkable increase brought about a parallel increase in the concerns for animal welfare and environment in the European Union. Contextually, more than 50% of hens were reared in cage-free systems, while 18% of hens were reared in alternative production systems (free-range and organic) in Europe in 2019 (Agri, 2021).

This increasing interest in products obtained under non-industrial production systems allows the development of sustainable farming practices (Dróżdż et al., 2020). These sustainable farming practices may involve the use of native breeds adapted to the local environment, with great rusticity and resistance to meteorological situations and diseases, as well as great ability to search for food in the wild (Lordelo et al., 2017; Laouadi et al., 2020). Consequently, it is through the conservation of animal genetic resources, that economic sustainability in the rural areas is promoted (Alderson, 2018; Toalombo et al., 2019).

The Utrerana avian breed is one example of rustic Spanish hen, located in Andalusia (Southern Spain), which is officially considered to be endangered as stated in the Royal Decree-Law 45/2019 from the 8 February 2019. Its four varieties, namely, White, Franciscan, Black, and Partridge, are classified depending on the color of its feathers and tarsi (Orozco, 1989).

It was initially oriented toward egg production, however, the introduction of rather productive commercial hybrid genotypes in Europe caused the displacement of the Utrerana hen breed to a secondary position (Campo, 2007). As a result, the breed census reached a critical situation, which was parallel to a decrease in its productive indices derived from the patent lack of productive selection (Fernández et al., 2009). Although the number of individuals has multiplied in the last years, only 1548 animals were registered in the studbook of the breed during 2019 (MAPA, 2021).

The enhancement of local products can be a strategy for the conservation of autochthonous genotypes, by avoiding the loss of connection between products, the local breeds from which they derive, and the area in which these were produced, as has been described for industrial products (Mordenti et al., 2017). In line with this situation, the definition of the breed's productive role became compulsory to maximize the breed's potential to satisfy current commercial demands. The characterization of Utrerana's egg as the main product of the breed was configured in the context of a set of strategies that sought the obtention of competitive sustainable products in the framework of the recent emerging diseases and climate change (Lordelo et al., 2020).

The acceptability of the eggs by consumers is mainly affected by the characteristics that describe their quality. Egg quality depends on several parameters, which are related to the eggshell, the albumen, and the yolk. Quality traits can be classified into external and internal quality, depending on whether the egg has to be broken to be scored (internal quality) or not (external quality) (Begli et al., 2010; González Ariza et al., 2019b). Egg weight, eggshell strength, albumen quality, and yolk color intensity are among the most important egg traits of commercial interest (Hanusova et al., 2015; Sirri et al., 2018; Sokołowicz et al., 2018; González Ariza, et al., 2019b). Eggs are commercially classified into four classes depending on their total egg weight: S (<53 g), M (53-63 g), L (63-73 g), and XL (>73 g).

Egg quality traits have been reported to be multifactorially dependent mainly on the laying hen's age and nutritional factors (Krawczyk, 2009; Samiullah et al., 2015; Samiullah et al., 2016; Sokołowicz, et al., 2018). However, there are some relevant pieces of evidence for the influence of the genotype on some of these egg quality traits on the relative proportion of yolk and albumen, albumen quality, or chemical composition (Washburn, 1979; González Ariza, et al., 2019b; Di Rosa et al., 2020; Krawczyk et al., 2020; González Ariza et al., 2021; Ianni et al., 2021).

Utrerana's egg not only constitutes a differentiated product in terms of internal and external quality-related traits (González Ariza, et al., 2019b) and chemical composition (González Ariza, et al., 2021). Additionally, its sensory characteristics have been reported to differ from the eggs of commercial lines (González Ariza et al., 2019a).

Consequently, the present study aimed to determine the contributions of external and internal quality parameters to the eggs produced by each of the four varieties of Utrerana hens and a control flock of a commercial laying lineage. Canonical discriminant analysis was used to design a statistical tool that permits determining whether specific eggs may correctly fit the features of the different commercial size categories (S, M, L, and XL), which may support the standardization of the Utrerana varieties' eggs as products and may address and support their suitability to cover particular sections of the market for egg consumption.

## 2. Materials and Methods

### *2.1. Institutional Animal Care and Use Committee Statement*

Avian-specific codes for good practices and the national guidelines for the care and use of laboratory and farm animals were followed in agreement with the standards found under the scope of the European Union legislation (2010/63/EU, from the 22 September 2010) and its transposed Spanish law document (Royal Decree Law 53/2013). As recommended by Royal Decree Law 53/2013 and its credited entity, the Ethics Committee of Animal Experimentation from the University of Córdoba, no additional permission was required as stated in the 5th section of the 2nd article of the aforementioned document given the zootechnical credited utilization of the animals participating in the present study.

### *2.2. Layer Flock and Environmental Conditions*

The public farm in which the study took place is located at the Agropecuary Provincial Center of Diputación of Córdoba, south Spain (Plus code: W77Q + MF El Levigar/37°54'50.9'' N 4°42'40.4'' W). The eggs used in the experiment were obtained from a layer flock comprising 60 Utrerana hens and 10 Leghorn Lohmann LSL-Classic lineage hens (hereinafter referred to as "Leghorn hens"), distributed depending on their breed or variety as described in Table 1. The laying flock was housed in individual cages (50 × 62 × 41 cm), to ensure that the traceability of each egg daily was feasible and following the Council Directive 1999/74/EC of 19 July 1999, which states minimum standards for the protection of laying hens. This whole year study ran from February 2019 to February 2020. All the birds were fed on the same commercial feed (15.20% crude protein, 4.60% crude fat and oils, 3.20% crude fiber, 14.00% crude ashes, 4.10% calcium, 0.66% phosphorus, 0.19% sodium, 0.31% methionine, 0.72% lysine). Feed and water were available *ad libitum*.

**Table 1.** Flock management information. All cages were chosen according to Council Directive 1999/74/EC of 19 July 1999, laying down minimum standards for the protection of laying hens.

| Flock Management Parameter | Utrerana varieties | | | | Leghorn (Control) |
|---|---|---|---|---|---|
| | White | Franciscan | Black | Partridge | |
| Laying hens | 15 | 15 | 15 | 15 | 10 |
| Hens (70 weeks old) | 8 | 8 | 8 | 8 | 0 |
| Pullets (28 weeks old) | 7 | 7 | 7 | 7 | 10 |
| Stocking density [1] | 4 animals per each $m^2$ | | | | |
| Nest box density [1] | 29 animals per each $m^2$ | | | | |
| Waterer allotment/space | Circle waterers of 5 cm of diameter per animal | | | | |
| Feeder allotment/space | 41 cm per animal | | | | |
| Floor substrate | Wood shavings covering the floor at a depth of approximately 1 cm | | | | |
| Nest box substrate | Plastic sturf mats covering the floor at a depth of approximately 1 cm | | | | |

[1] Stocking density and nest box density were determined after computing the whole cage's surface considering dimensions were 50 x 62 x 41 cm and its surface area was 0.25 $m^2$.

### *2.3. Work Sample*

All statistical tests were performed on an egg sample comprising 541 eggs, laid during a complete laying cycle. The eggs were classified depending on their breed and variety and commercial size as shown in Table 2. The protocols are described below in Sections 2.4 and 2.5 were performed on each egg individually.

**Table 2.** Number of observations (eggs) classified per breed/variety and commercial size.

| | S (<53 g) | M (53-63 g) | L (63-73 g) | XL (>73 g) | Total |
|---|---|---|---|---|---|
| White | 2 | 46 | 45 | 3 | 96 |
| Franciscan | 7 | 71 | 25 | 2 | 105 |
| Black | 8 | 43 | 34 | 10 | 95 |
| Partridge | 8 | 32 | 32 | 3 | 75 |
| Leghorn | 12 | 83 | 60 | 15 | 170 |
| Total | 37 | 275 | 196 | 33 | 541 |

*2.4. External and Internal Quality-Related Traits Description*

External and internal quality-related traits were measured separately. For the external quality of the egg, noninvasive methods were used, and measurements were taken without breaking the eggshell. The external quality traits that were measured were as follows: egg weight; major and minor diameters of the egg; eggshell color lightness, redness, and yellowness coordinates (shell L*, shell a*, and shell b*). Shape index (SI) was computed through the following formula (Anderson et al., 2004):

$$SI = (\emptyset M/(\emptyset m) * 100,$$

where ØM is the major diameter and Øm is the minor diameter.

Eggs were classified depending on their shape index as follows: sharp egg (SI < 72), standard egg (SI = 72-76), or round egg (SI > 76) (Duman et al., 2016).

Internal egg quality-related traits were evaluated after breaking the egg. Internal egg quality traits measures were as follows: eggshell resistance (eggshell strength and area under the force-displacement curve); albumen height; yolk color; yolk lightness, redness, and yellowness variables (yolk L*, yolk a*, and yolk b*); yolk diameter; eggshell weight; yolk weight, albumen weight; yolk pH; albumen pH; eggshell thickness; and the presence or absence of visual defects in yolk and/or albumen. Haugh units (HU) were calculated as a measure of the albumen quality, from the variables albumen height and egg weight via the following formula (Eisen et al., 1962):

$$HU = 100 * \log(h - 1.7e^{0.37} + 7.6),$$

where h is albumen height (mm) and w is egg weight (g).

*2.5. Measurements on Eggs*

The egg quality measurements were registered fortnightly for the whole duration of the study. Egg quality was assessed at 22 ± 1 °C. The traceability of the egg and the external and internal characterization of each individual egg were performed and registered within 24 h after oviposition. Eggs were weighed individually using an electronic scale (Cobos, CSB-600C, Barcelona, Spain). Eggshell color was assessed using a portable spectrophotometer (CM 700d, Konica Minolta Holdings Inc., Tokyo, Japan). Eggshell color results were expressed using the International Commission on Illumination (CIE) L*a*b* system color profile. Major and minor diameters were measured using a Vernier scale (Electro DH M 60.205, Barcelona, Spain).

Mechanical eggshell strength measurement was performed using a texturometer TA.XT2 Texture Analyzer (TA.XT2; Texture Technologies Corp., Scarsdale, NY, USA). Eggshells were punctured at the bottom (large end) of the eggshell with a polyoxymethylene (POM) probe with a 5 mm diameter. Eggshell strength and the area under the curve were determined from the graphical curve obtained by the texturometer. The approach followed started when each individual eggshell was broken, and the yolk and albumen were deposited on a glass surface to take measurements of the internal quality-related variables described above. Albumen height was computed as the arithmetic mean of three measurements performed using a Haugh digital micrometer (Baxlo, Barcelona, Spain). The intensity of the yellow-orange color of the yolk was measured both with the portable spectrophotometer (L*, a*, b*) and using a Roche color fan (yolk color) (DSM, DSM® YolkFanTM, Heerlen, The Netherlands). The yolk diameter was measured on a Vernier scale. The eggshell, albumen, and yolk were separated and weighed using a precision balance. The pH was measured using a pH meter (Crison®, PH-25, Barcelona, Spain). Eggshell thickness was measured averaging three measurements around the blowhole near the equator of the egg upon a Vernier scale. All the eggs were visually evaluated to detect blood or meat spots in the albumen, yolk, or both.

*2.6. Canonical Discriminant Analysis*

A canonical discriminant analysis was performed using egg weight, major diameter, minor diameter, shell L*, shell a*, shell b*, shape index, eggshell strength,

area under the force-displacement curve, albumen height, Haugh units, yolk color, yolk L*, yolk a*, yolk b*, yolk diameter, eggshell weight, yolk weight, albumen weight, yolk pH, albumen pH, eggshell thickness, presence or absence of visual defects in yolk and/or albumen, and Haugh units per egg as explanatory variables. The commercial classification and the hen breed/variety were used as the labeling classification criteria to measure the variability in quality-related traits between and within classification groups, to establish, identify, and outline clusters (Cuadras and Augé, 1981; Marín Navas et al., 2021).

The present discriminant tool permits to sort eggs across hen genotype and quality categories and to determine the clustering patterns described by the egg sample through a linear combination of quality-related traits. Canonical discriminant analysis was also used to plot pairs of canonical variables building a territorial map to graphically interpret group differences. Variable selection was performed using regularized forward stepwise multinomial logistic regression algorithms as suggested by Marín Navas, et al. (2021). Priors were regularized based on group sizes computed from the prior probability option in SPSS version 26.0 software rather than considering them to be equal, to prevent group with different sample sizes from affecting the quality of classification (Tai and Pan, 2007). As the previous authors suggested, the statistical analysis used in the present research has been reported to be robust when sample sizes between groups are highly unequal. To palliate potential distortion effects, the smallest sample size should be at least 20 for every 4 or 5 predictors, and the maximum number of independent variables should be n-2, where n is the sample size (Poulsen and French, 2008). However, the fact of having 4 or 5 times more observations and dependent variables than previously described makes the discriminant approaches efficient (Marín Navas, et al., 2021). This requirement is far surpassed in the present study, so the distorting effects mentioned are avoided.

Multicollinearity is a statistical phenomenon in which two or more variables are reciprocally dependent upon other variables in a way such that one can be linearly predicted from the rest with a high degree of accuracy. Multicollinearity analysis was performed before discriminant analysis to ensure that the regressors used were independent, so the variables chosen by the forward or backward stepwise selection

methods were the same. Then, the forward stepwise selection method was chosen, as it is less time-demanding than the backward selection method.

Canonical discriminant analysis was performed by the use of the Discriminant routine of the Classify package of the SPSS version 26.0 software and the Discriminant Analysis routine of the Analyzing Data package of XLSTAT Pearson Edition.

2.6.1. Multicollinearity Preliminary Testing

The multicollinearity assumption was tested to discard redundancies in the variables considered so that this phenomenon does not condition the structure of the matrices or overinflate the explanatory potential of variance, before performing a discriminant canonical analysis (Marín Navas, et al., 2021). The variance inflation factor (VIF) was computed and used as an indicator of multicollinearity, following the formula:

$$VIF = 1/(1 - R^2)$$

where $R^2$ is the coefficient of determination of the regression equation. A VIF value of 5 was accepted in the present research, as reported by other authors (Rogerson, 2001). The amount of variability in a certain independent variable that is not explained by the rest is called the tolerance and is calculated as 1 - $R^2$ (Daoud, 2017). If tolerance has values lower than 0 and, simultaneously, the value of VIF is ≥10, multicollinearity can be considered a problem. For this, the Linear routine of the Regression package of the SPSS, version 26.0 software was used.

2.6.2. Canonical Correlation Dimension Determination

The maximum number of canonical correlations (interpreted as Pearson's ρ) between two sets of variables is the number of variables in the smaller set. Although the first canonical correlation may often explain most of the relationship between sets, all canonical correlations must be considered (Tabachnick and Fidell, 1989). Canonical correlation values of ≥0.30 may be indicative of a statistically significant dimension.

2.6.3. Canonical Discriminant Analysis Efficiency

Variables that may significantly contribute to the discriminant function are evaluated by Wilks' lambda test. As Wilks' lambda approximates to 0, the contribution of the variable to the discriminant function increases. Functions can be used to explain group ascription if the significance (tested using $\chi^2$) is below 0.05 (Anuthama et al., 2011).

2.6.4. Canonical Discriminant Analysis Model Reliability

The assumption of equal covariance matrices was evaluated through Pillai's trace criterion, which is the only acceptable test to be used in cases of unequal sample sizes (Zhang et al., 2020; Marín Navas, et al., 2021). Pillai's trace criterion was computed using the Multivariate routine of General Linear Model package of the software SPSS, version 26.0 software. Statistical differences in the dependent variables across the levels of independent variables are considered when significance is below 0.05.

2.6.5. Variable Dimensionality Reduction

The overall variables were minimized to a few significant variables that contributed most to the different variations in the different types of eggs using a preliminary principal component analysis (PCA).

2.6.6. Canonical Coefficients and Loading Interpretation and Spatial Representation

The percentage of allocation of an egg within its group (defined by its commercial size and the genotype of the hen that laid it) was determined using a discriminant function analysis. The variables that presented a discriminant loading of ≥|0.40|, were considered to be substantially discriminant. Non-significant variables were excluded from the function using stepwise procedures. The larger the absolute coefficients for each particular variable within a set, the better the discriminating ability (Marín Navas, et al., 2021). Data were standardized following the premises described by Manly and Alberto (2016). Afterward, squared Mahalanobis distances were calculated. Squared Mahalanobis distances between groups were obtained using the following formula:

$$D_{ij}^2 = (\bar{Y}_i - \bar{Y}_j)\, COV^{-1}(\bar{Y}_i - \bar{Y}_j)$$

where $D_{ij}^2$ is the distance between population i and j; $\bar{Y}_i$ and $\bar{Y}_j$ are the means of variable x in the ith and jth populations, respectively; $COV^{-1}$ is the inverse of the covariance matrix of measured variable x.

The squared Mahalanobis distance was used to graphically depict the clustering patterns defined by the differences in the values for quality-related traits across the potential egg classifications considered in the present research. To this aim, a dendrogram representing the possible categories within egg quality classification was constructed using the underweighted pair-group method arithmetic averages (UPGMA) from the Universitat Rovira i Virgili (URV), Tarragona, Spain, and the Phylogeny procedure of MEGA X 10.0.5 (Institute of Molecular Evolutionary Genetics, The Pennsylvania State University, State College, PA, USA).

2.6.7. Discriminant Function Cross-Validation

The hit ratio can be defined as the percentage of correctly classified observations (Ramayah et al., 2010). The leave-one-out cross-validation option was used to validate the discriminant functions used. The classification rate must be at least 25% higher than obtained by chance to be considered accurately enough (Marín Navas, et al., 2021).

Press' Q significance test was used to compare the discriminating power of the cross-validated function by using the following formula:

$$Press'Q = [N - (nk)]^2/N(K - 1)$$

where N is the number of observations in the sample; n is the number of observations correctly classified; and K is the number of groups. Subsequently, the value of Press' Q statistic was compared with the critical value of 6.63 for $\chi^2$ with one degree of freedom in a significance of 0.01. If Press' Q exceeds the critical value of $\chi^2 = 6.63$, the cross-validated classification can be considered significantly better than chance.

## 3. Results

### *3.1. Canonical Discriminant Analysis Model Reliability*

Egg weight, major diameter, minor diameter, shell b*, albumen height, and presence or absence of visual defects in yolk and/or albumen showed VIF values

over 5 and were discarded from further analyses. A summary of the value of tolerance and VIF for each variable is shown in Table 3.

**Table 3.** Multicollinearity analysis of quality-related traits of eggs.

| Statistics/Parameters | Tolerance (1 - $R^2$) | VIF |
|---|---|---|
| Shell L* | 0.2042 | 4.8980 |
| Shell a* | 0.2657 | 3.7642 |
| Yolk weight | 0.4428 | 2.2585 |
| Yolk diameter | 0.4504 | 2.2204 |
| Egshell strenght | 0.5250 | 1.9047 |
| Eggshell weight | 0.5526 | 1.8096 |
| Area under the force-displacement curve | 0.5733 | 1.7441 |
| Yolk color | 0.5877 | 1.7016 |
| Yolk a* | 0.6125 | 1.6326 |
| Yolk b* | 0.6184 | 1.6171 |
| Albumen weight | 0.6744 | 1.4829 |
| Eggshell thickness | 0.7037 | 1.4212 |
| Yolk L* | 0.7044 | 1.4196 |
| Haugh units | 0.7541 | 1.3261 |
| Albumen pH | 0.8314 | 1.2027 |
| Yolk pH | 0.8445 | 1.1841 |
| Shape index | 0.8735 | 1.1448 |

Interpretation thumb rule: VIF = 1 (Not correlated); 1 < VIF < 5 (Moderately correlated); VIF ≥ 5 (Highly correlated).

Pillai's trace criterion reported a significant difference across the different egg quality classification groups considered in the study ($p < 0.05$; Table 4).

**Table 4.** Summary of the results of Pillai's Trace of Equality of Covariance Matrices of Canonical Discriminant Functions.

| Parameter | Value |
|---|---|
| Pillai's Trace Criterion | 2.5016 |
| F (Observed value) | 4.7313 |
| F (Critical value) | 1.1357 |
| df1 | 323 |
| df2 | 8857 |
| *p*-value | < 0.0001 |
| alpha | 0.05 |

F, Snedecor's F; df1, numerator degrees of freedom for the F-approximation; df2, denominator degrees of freedom for the F-approximation.

### *3.2. Canonical Coefficients, Loading Interpretation, and Spatial Representation*

Six discriminating canonical functions were identified in the discriminating canonical analysis (Table 5). Table 6 reports the outcomes of discriminating ability testing. Higher eigenvalues were indicative of higher discriminatory power. Functions F1 and F2 with eigenvalues greater than 1 explain 75.63% of the total variance, while the rest contribute to the explanation of the variance with a low percentage of the information to the analysis.

**Table 5.** Canonical variable pairs found in canonical discriminant analysis.

| Function | Eigenvalue | Variance, % | Canonical Correlation | Cumulative variance, % |
|---|---|---|---|---|
| F1 | 3.2788 | 55.0163 | 0.8754 | 55.0163 |
| F2 | 1.2287 | 20.6172 | 0.7425 | 75.6335 |
| F3 | 0.4021 | 6.7477 | 0.5355 | 82.3812 |
| F4 | 0.3055 | 5.1253 | 0.4837 | 87.5064 |
| F5 | 0.2160 | 3.6241 | 0.4215 | 91.1306 |
| F6 | 0.1430 | 2.4002 | 0.3538 | 93.5307 |

**Table 6.** Canonical Discriminant analysis efficienty parameters.

| Test of Functions | Wilks' Lambda | Chi-square | df | Sig. |
|---|---|---|---|---|
| 1 through 17 | 0.011 | 1320.792 | 323 | <0.001 |
| 2 through 17 | 0.063 | 802.254 | 288 | <0.001 |
| 3 through 17 | 0.155 | 541.386 | 255 | <0.001 |
| 4 through 17 | 0.254 | 398.607 | 224 | <0.001 |
| 5 through 17 | 0.358 | 298.246 | 195 | <0.001 |
| 6 through 17 | 0.474 | 217.171 | 168 | 0.010 |

After discarding redundant variables, the test of equality of group means across egg quality classification groups was used to rank variables depending on their discriminating properties (Table 7).

The greater the value of F and the lower the value of Wilks' lambda for a certain variable, the better its discriminating power was, and hence, the higher its position in the rank was as well.

As shown in Table 8, standardized discriminant coefficients were evaluated. This allowed us to determine the possibility of a reduction in the discriminant power of individual variables as a result of multicollinearity between pairs.

**Table 7.** Results for the tests of equality of group means to test for difference across breeds once redundant variables have been removed.

| Variables | Rank | Wilks' Lambda | F | df1 | df2 | Significance |
|---|---|---|---|---|---|---|
| Albumen weight | 1 | 0.335 | 54.380 | 19 | 521 | < 0.0001 |
| Eggshell weight | 2 | 0.539 | 23.440 | 19 | 521 | < 0.0001 |
| Yolk weight | 3 | 0.566 | 21.010 | 19 | 521 | < 0.0001 |
| Yolk diameter | 4 | 0.651 | 14.680 | 19 | 521 | < 0.0001 |
| Haugh units | 5 | 0.700 | 11.760 | 19 | 521 | < 0.0001 |
| Yolk b* | 6 | 0.764 | 8.470 | 19 | 521 | < 0.0001 |
| Shape index | 7 | 0.800 | 6.860 | 19 | 521 | < 0.0001 |
| Yolk color | 8 | 0.812 | 6.360 | 19 | 521 | < 0.0001 |
| Area under the Force-displacement curve | 9 | 0.832 | 5.550 | 19 | 521 | < 0.0001 |
| Eggshell strength | 10 | 0.837 | 5.350 | 19 | 521 | < 0.0001 |
| Shell L* | 11 | 0.844 | 5.080 | 19 | 521 | < 0.0001 |
| Yolk a* | 12 | 0.845 | 5.050 | 19 | 521 | < 0.0001 |
| Shell a* | 13 | 0.870 | 4.090 | 19 | 521 | < 0.0001 |
| Eggshell thickness | 14 | 0.892 | 3.320 | 19 | 521 | < 0.0001 |
| Yolk L* | 15 | 0.908 | 2.780 | 19 | 521 | < 0.0001 |
| Yolk pH | 16 | 0.941 | 1.710 | 19 | 521 | 0.0300 |
| Albumen pH | 17 | 0.957 | 1.250 | 19 | 521 | 0.2200 |

F, Snedecor's F; df1, numerator degrees of freedom for the F-approximation (groups minus 1); df2, denominator degrees of freedom for the F-approximation (observations minus 1).

**Table 8.** Discriminant loadings for external and internal quality-related traits determining the relative weight of each trait on each canonical discriminant function.

| | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| Eggshell strength | −0.20 | −0.26 | −0.15 | 0.51 | 0.33 | −0.06 |
| Yolk L* | −0.08 | 0.02 | 0.07 | −0.10 | 0.51 | 0.18 |
| Yolk a* | −0.07 | −0.35 | −0.13 | −0.53 | −0.11 | −0.03 |
| Shape index | −0.04 | −0.04 | 0.69 | −0.13 | −0.05 | 0.27 |
| Shell a* | −0.04 | 0.05 | 0.09 | 0.64 | −0.22 | −0.62 |
| Yolk pH | −0.03 | 0.13 | −0.12 | −0.07 | 0.11 | 0.32 |
| Haugh units | −0.03 | −0.45 | 0.00 | 0.21 | 0.37 | −0.27 |
| Yolk b* | −0.02 | 0.44 | −0.01 | 0.15 | −0.47 | −0.48 |
| Area under the force-displacement curve | 0.01 | 0.28 | 0.43 | 0.02 | −0.06 | 0.04 |
| Albumen pH | 0.02 | 0.07 | 0.07 | 0.03 | −0.10 | −0.04 |
| Shell L* | 0.04 | −0.04 | 0.14 | 0.60 | 0.02 | −0.55 |
| Yolk color | 0.05 | 0.31 | 0.37 | −0.08 | −0.01 | 0.28 |
| Eggshell thickness | 0.06 | 0.06 | 0.23 | 0.29 | 0.09 | 0.05 |
| Yolk diameter | 0.08 | 0.35 | −0.12 | −0.08 | 0.29 | −0.36 |
| Yolk weight | 0.20 | 0.43 | 0.12 | 0.12 | 0.43 | 0.08 |
| Eggshell weight | 0.56 | −0.20 | 0.40 | −0.33 | −0.07 | −0.47 |
| Albumen weight | 0.83 | −0.07 | −0.21 | 0.30 | −0.27 | 0.27 |

The substitution of the values for quality-related traits in the first two discriminating functions was performed to obtain x and y-axis coordinates, for the first and second dimensions, respectively. Once coordinates were obtained, each egg observation was sorted and classified across the different egg quality classification categories and laying hen genotype. Coordinates were used to depict eggs on a territorial map (Figure 1). Centroids represent the means of the discriminant function scores by egg quality classification group for each function calculated.

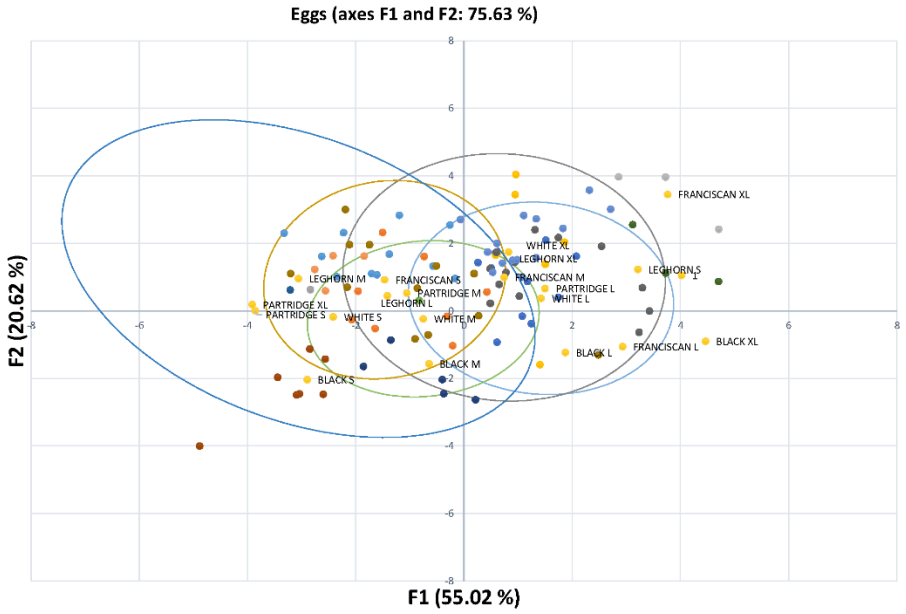


**Figure 1.** Territorial map depicting the eggs considered in the canonical discriminant analysis sorted across commercial quality categories (S, M, L, and XL) and laying hens genotypes (Leghorn and White, Black Franciscan, and Partridge Utrerana varieties). Centroids or canonical group means are the means for each group's canonical observation scores. The larger the difference between centroids, the better the predictive power of the canonical discriminant function in classifying observations.

In this regard, Mahalanobis distances were used as they represent the probability that a case of an unknown background belongs to a particular egg quality classification group. It can be calculated through the relative distance of the problem egg to the centroid of its closest group. The probability of classification of observation into a group was calculated, following the premises in Hair et al. (2010).

Consequently, the hit ratio, or successfully classified cases, was determined (Supplementary Table S1). Mahalanobis distances obtained after the evaluation of the discriminant analysis matrix were transformed into squared Euclidean distance and represented in Figure 2.

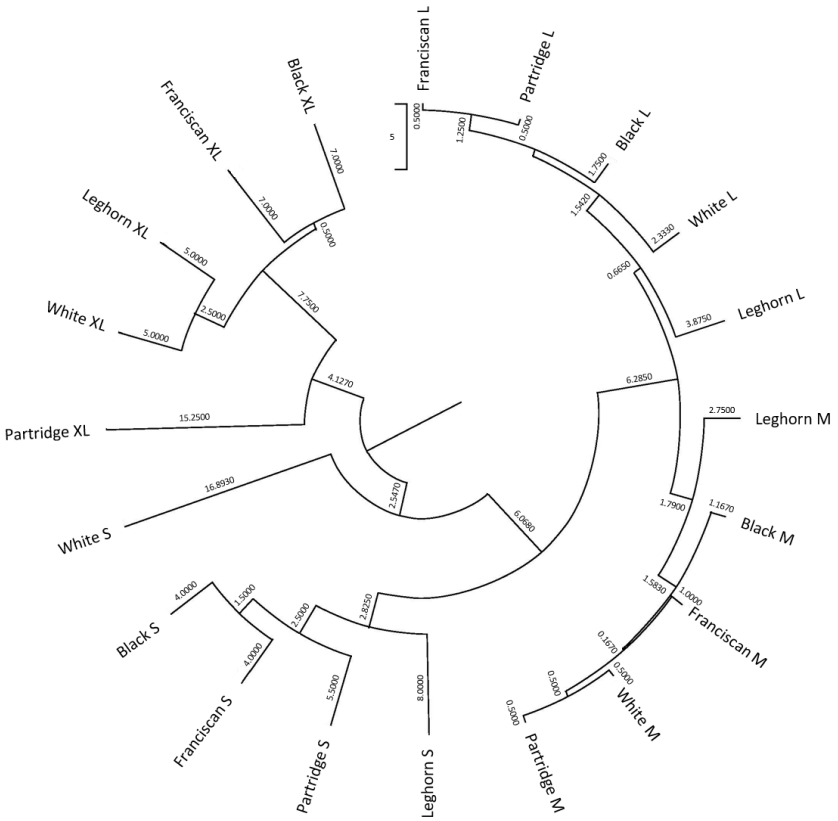


**Figure 2.** Cladogram constructed from Mahalanobis's distances between commercial quality classification categories and laying hen genotypes.

*3.3. Discriminant Function Cross-Validation*

Classification and leave-one-out cross-validation matrices were evaluated (Supplementary Tables S1 and S2). In all, 73.2% of original grouped cases were correctly classified in the different egg quality classification group, from which 57.1% of clustered observations were cross-validated. A Press' Q value of 5297.17 (N: 541; n: 396; K: 20) was obtained; hence, predictions were considered to be significantly better than those that would be obtained by chance at 95% (Chan, 2005).

## 4. Discussion

Involving autochthonous breeds in animal production systems may promote the evolution of sustainable ways of producing. Native breeds can be used in search of productive improvement by taking advantage of genotypes adapted to the climatology and orography, as well as to the technical, productive, and cultural conditions of the area. On the other hand, commercial chains are increasingly requesting more products derived from non-industrial processes. This context

makes it necessary to characterize the eggs of the Utrerana avian breed according to their commercial size while defining how the different quality-related parameters affect the differentiation between eggs across the different varieties and breeds studied. The results obtained in the present study may suggest how to approach the different strategies to make an endangered breed profitable, thus ensuring its conservation by establishing production models to which it is adapted.

The selection of the individuals in the sample was performed considering that the hybrid commercial cycle and both genotypes reach 50% of laying (egg production during a laying cycle). Contextually, the typical production cycle in commercial layers (Leghorn hens among others) lasts about 72 weeks (Seidler, 2003). However, this cycle may extend until 156 weeks in around a third of the Utrerana population (Orozco, 1987). Additionally, according to Kuo et al. (1991), the age at sexual maturity is estimated by age in weeks when 50% egg production is reached. In this regard, the same authors suggested the age when 50% egg production in White Leghorn is reached to be around 21 weeks. By contrast, the information reported by Orozco (1987) suggested the average age of Utrerana hens at the moment of the first laying was 25 weeks. Furthermore, the breeding criterion of both breeds may differ, as while White Leghorn hens breeders have traditionally selected animals for precocity (Keshavarz, 1987), Utrerana breeders have not sought this trait as a priority rather benefiting from the natural lay cycle of the breed (Orozco, 1987). Zita et al. (2009) suggested that egg quality characteristics are affected by the interaction of genotype (breed and strain) and hen's age, rather than exerting their effects independently.

Multicollinearity analyses revealed high correlations between major diameter and minor diameter and egg shape index, since both measurements comprise the formula for its calculation. The same happens with the formula of Haugh units, which includes the variables of egg weight and albumen height, which consequently were eliminated due to multicollinearity problems. Moreover, egg weight can be calculated by separately summing albumen weight, yolk weight, and eggshell weight variables, which may be the logical source for the redundancies detected.

Degree of lightness (L*) and chromaticity coordinates (a* and b*) comprise the L*a*b* color space (Leleu et al., 2011). In this context, coordinates of shell a* and

shell b* are difficult to interpret and can be correlated in white-shelled eggs, such as those of the Utrerana avian breed (Samiullah, et al., 2015). As suggested by other authors (Odabaşi et al., 2007), a* and b* parameters measure chromaticity. More specifically, redness-greenness and yellowness-blueness, respectively. Positive values of a* are linked to increased amounts of redness in eggshell color, whereas negative values of a* relate to increased amounts of greenness in the eggshell color.

Similarly, the representativity of yellow and blue components in eggs of any color are represented by positive and negative values of b*, respectively. In this context, Odabaşi, et al. (2007), suggested that the lighter the shell color (higher L*), the lesser the redness of the color of the eggshell is as well. This was in line with the results reported by Aygun (2014), who reported shell L* could be considered as a discriminative color criterion as the lesser the amount of shell L*, the darker the eggshell color turns to be.

The visual defects in yolk and albumen are produced by meat and blood spots. The presence of these visual defects is regarded as an undesirable feature in eggs that causes rejection by consumers (Brant et al., 1953).

These undesirable findings may stem from the synthesis of the different parts of the egg during ovulation due to the rupture of an ovarian follicle at a different position from the stigma (Rizzi, 2020). In these situations, variations in the chromaticity coordinates of the yolk color could appear, thus, may be one of the sources of multicollinearity problems between the presence of visual defects and yolk a* and yolk b*.

Albumen weight, eggshell weight, and yolk weight variables reported the best discriminating properties (Table 7). These three quality-related traits compose the egg weight, which is the main criterion on which the commercial classification of eggs relies. At the same time, albumen represents about 55-65% of the egg weight (Baykalir and Simsek, 2018; Rizzi, 2021). This explains the fact that albumen weight was ranked first at the test of equality of group means.

Hen strain has been reported to significantly affect albumen ratio (Wan et al., 2019; Rizzi, 2020). Albumen is critical for the survival of the chicken embryo and the variations in the content of albumen in hen eggs can generate differences in skeletal muscle or liver metabolism during embryonic development (Peña-Villalobos et al.,

2017). In laying hens, albumen has great commercial importance, provided its unique functional properties and its use as an ingredient in a large number of culinary international preparations (Secci et al., 2020). In previous studies, the Leghorn has been demonstrated to have a higher albumen weight than the Utrerana avian breed, due to the Leghorn's higher concentration of energy reserves (González Ariza, et al., 2019b). Contextually, Peña-Villalobos, et al. (2017) suggested a significant reduction in metabolic rate occurs in the last fifth of embryonic life in albumen-removed eggs, which in turn derives into reduced catabolic activities in the skeletal muscle of chicks that eventually hatch.

Utrerana has been reported to present a lower eggshell weight and a higher yolk weight than Leghorns (González Ariza, et al., 2019b). These results agree with the present research since these parameters have a high discriminating power when clusters differentiate. Modern commercial breeds showed clear differences in terms of eggshell weight when compared to native poultry, due to the high selection of all egg traits of eggs for its transport and commercial purposes (Tuiskula-Haavisto et al., 2018; Knaga et al., 2019).

Differences in the proportion of egg yolk have been reported between breeds and within highly productive laying hens strains such as the White Leghorn, which may be indicative of the presence of sufficient additive genetic variation (Hartmann et al., 2000). Furthermore, selection based on additive genetic variation in yolk weight has been suggested as an option to promote seeking sustainability of local eggs (Hartmann et al., 2003), as native breeds could satisfy the growing demand for more energetically efficient eggs in the market (González Ariza, et al., 2019b; Di Rosa, et al., 2020; Lordelo, et al., 2020).

Yolk diameter and Haugh units reported the best discriminating properties (fourth and fifth position in the rank) after weight-related traits (albumen, eggshell, and yolk weights). The relevance of these traits may be ascribed as suggested by Ukwu et al. (2017), who reported significant differences in yolk weight and albumen height among light (less than 49.99 g), medium (50-55 g), and heavy eggs (more than 55 g) of Isa Brown egg layer chickens in Nigeria. This has also been reported by Alkan et al. (Alkan et al., 2015), who addressed a parallel increase in yolk diameter as egg

weight increases in partridge eggs. However, no differences between Utrerana and Leghorn breeds were detected in previous studies (González Ariza, et al., 2019b).

Haugh units are used as an indicator of internal egg quality (Eisen, et al., 1962). Time of storage and storage conditions affect Haugh units values (Roberts, 2004). However, the strains or breed of the hen have been reported to quantitatively affect them. For instance, several authors have reported higher values for Haugh units in local breeds than in commercial hybrid strains (Lordelo, et al., 2017; Hussain et al., 2018; Lordelo, et al., 2020). In any case, albumen height is correlated with the percentage of albumen (Sreenivas et al., 2013). Hence, commercial strains could present a certain advanced position, provided a larger percentage of albumen is found in hybrid strains in comparison to that in native breeds.

Values for pH-related traits showed the lowest values of F and highest for Wilks' lambda. Egg pH allows the assessment of the egg's freshness (Dong et al., 2017; Nematinia and Abdanan Mehdizadeh, 2018). The loss of $CO_2$ and $H_2O$ inside the egg produces an increase in albumen pH. The time of storage and high temperatures condition this loss of $CO_2$ and $H_2O$ and promote a decrease in albumen viscosity and flavor with detrimental effects for egg quality (Lakins et al., 2009; Samiullah, et al., 2016). Albumen and yolk pH can be slightly influenced by the hen strain (Feddern et al., 2017; González Ariza, et al., 2019b; Lordelo, et al., 2020). Nevertheless, in the present study, when all egg pH values were measured during the 24 h following oviposition, it was found that albumen pH and yolk pH have a low discriminating power between different groups of eggs, which may derive from the low variability in pH found. Such lack of variability may stem from the fact that the eggs considered in this study were fresh enough for those eggs presenting slightly lower values not to be detrimental on egg quality. Additionally, this finding may evidence a patent lack of importance provided to quality traits (such as the pH of the components of the egg) against quantitative traits among the current criteria that are considered for egg quality classification, as the quantity of the product may be better commercially valued than its quality. However, this commercial strategy may be erroneous given it may not match the current general trend of the customers preferring egg quality over quantity (Gangnat et al., 2018).

Figure 2 reports that egg quality classification clusters are mainly grouped depending on their commercial size. In addition, the Leghorn's egg groups differed from the rest of those from the Utrerana varieties, except for those of White Utrerana XL and Leghorn XL eggs, which reported a certain closeness. This may be indicative of the hybridization of the White Utrerana with the Leghorn breed, both with white plumage, which may have been historically performed by breeders as an attempt to decrease the consanguinity of the white variety, which is the Utrerana variety accounting with the smallest number of animals and the one which faces the highest endangerment risk.

Additionally, the present study may confirm the fact that product differentiation could be a feasible opportunity for the eggs of Utrerana varieties, which could constitute a favorable point when compared to eggs from other breeds that have traditionally been sold in the market (Johnston et al., 2011).

The present discriminating tool allows to efficiently classify eggs based on quality-related traits as supported by the 73.2% of observations being correctly classified within their group. In this regard, weight traits play a pivotal role in the determination of the commercial quality of eggs.

All eggs belonging to the S category in White, Franciscan and Partridge, and L category of Franciscan and Partridge were correctly classified (Supplementary Table S1). However, 45.5% of M category Partridge eggs were classified as Franciscan M. Previous research suggested Partridge and Franciscan varieties present a significantly heavier yolk and slightly lower weight than the rest of the varieties or the Leghorn breed (González Ariza, et al., 2019b), hence similarities between egg quality-related traits of these two varieties could be expected. Moreover, 26.7% of XL category Leghorn eggs were classified as Leghorn L eggs, which may be explained as commercial genotypes have been selected to produce rather homogenous eggs, which may translate to a reduction in differences (Boxall et al., 2007; Rath et al., 2015). Furthermore, it may be worth mentioning that 23.8% of M category White Utrerana eggs were classified as M category Leghorn ones, with the likely hybridization between these two strains being the potential source for these similarities.

## 5. Conclusions

The present discriminating method has been proved and validated as an efficient tool to correctly classify eggs considering both external and internal traits. Additionally, this research confirms the fact that product differentiation could be a feasible opportunity for the eggs of Utrerana varieties, which could constitute a favorable point when compared to eggs from other breeds that have traditionally been sold in the market. Weight traits play a pivotal role in the determination of the commercial quality of eggs. This may evidence a patent lack of commercial attention provided to quality traits in favor of quantitative traits. However, this commercial strategy may be erroneous given it may not match the current general trend of customers preferring egg quality over quantity. Partridge and Franciscan classification confusion may derive from the fact that these varieties present significantly heavier yolks and slightly lower weights. Similarities between the eggs of White Utrerana and Leghorn hens may evidence reminiscences of hybridization.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/2304-815 8/10/3/632/s1, Table S1: Appropriately classified eggs (%) according to the commercial size and genotype of the laying hen; Table S2: Leave-one-out cross-validation (%) of eggs according to the commercial size and genotype of the laying hen.

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**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data will be made available from the corresponding author upon reasonable request.

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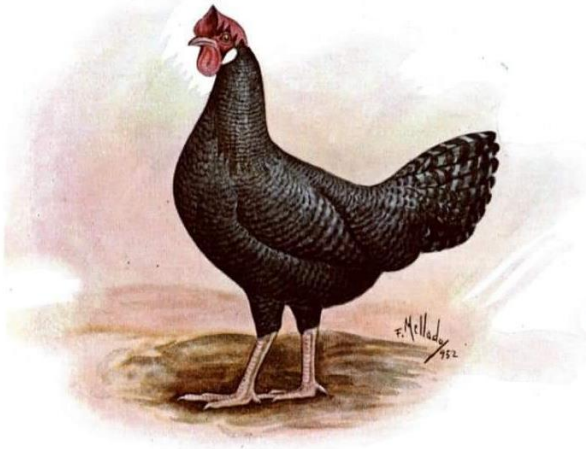
**Table S1.** Appropriately classified eggs according to the commercial size and genotype of the laying hen.

| | White L | White M | White S | White XL | Franciscan L | Franciscan M | Franciscan S | Franciscan XL | Leghorn L | Leghorn M | Leghorn S | Leghorn XL | Black L | Black M | Black S | Black XL | Partridge L | Partridge M | Partridge S | Partridge XL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| White L | 80 | 0 | 0 | 0 | 6.7 | 0 | 0 | 0 | 6.7 | 0 | 0 | 0 | 3.3 | 0 | 0 | 0 | 3.3 | 0 | 0 | 0 |
| White M | 4.8 | 47.6 | 0 | 0 | 0 | 14.3 | 0 | 0 | 0 | 23.8 | 4.8 | 0 | 0 | 4.8 | 0 | 0 | 0 | 0 | 0 | 0 |
| White S | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| White XL | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Franciscan L | 25 | 0 | 0 | 0 | 33.3 | 8.3 | 0 | 0 | 8.3 | 0 | 0 | 0 | 8.3 | 0 | 0 | 0 | 16.7 | 0 | 0 | 0 |
| Franciscan M | 0 | 0 | 2.4 | 0 | 0 | 76.2 | 0 | 0 | 0 | 11.9 | 0 | 0 | 0 | 4.8 | 0 | 0 | 2.4 | 2.4 | 0 | 0 |
| Franciscan S | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Franciscan XL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leghorn L | 2.1 | 0 | 0 | 0 | 0 | 2.1 | 0 | 0 | 87.5 | 8.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leghorn M | 0 | 0 | 0 | 0 | 0 | 2.1 | 2.1 | 0 | 4.2 | 89.6 | 0 | 0 | 2.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leghorn S | 0 | 0 | 0 | 0 | 0 | 12.5 | 0 | 0 | 0 | 0 | 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12.5 | 0 |
| Leghorn XL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 26.7 | 0 | 0 | 73.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Black L | 12.5 | 0 | 0 | 0 | 0 | 6.3 | 0 | 0 | 12.5 | 0 | 0 | 6.3 | 50 | 0 | 0 | 0 | 12.5 | 0 | 0 | 0 |
| Black M | 0 | 0 | 0 | 0 | 0 | 12.5 | 0 | 0 | 0 | 6.3 | 0 | 0 | 0 | 75 | 6.3 | 0 | 0 | 0 | 0 | 0 |
| Black S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 75 | 0 | 0 | 0 | 0 | 0 |
| Black XL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14.3 | 0 | 0 | 0 | 85.7 | 0 | 0 | 0 | 0 |
| Partridge L | 11.1 | 0 | 0 | 0 | 16.7 | 5.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 61.1 | 0 | 0 | 5.6 |
| Partridge M | 9.1 | 9.1 | 0 | 0 | 0 | 45.5 | 0 | 0 | 0 | 9.1 | 0 | 0 | 0 | 9.1 | 0 | 0 | 0 | 18.2 | 0 | 0 |
| Partridge S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 |
| Partridge XL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |

**Table S2.** Leave-one-out cross-validation of eggs according to the commercial size and genotype of the laying hen.

| | White L | White M | White S | White XL | Franciscan L | Franciscan M | Franciscan S | Franciscan XL | Leghorn L | Leghorn M | Leghorn S | Leghorn XL | Black L | Black M | Black S | Black XL | Partridge L | Partridge M | Partridge S | Partridge XL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| White L | 73.3 | 3.3 | 0 | 0 | 6.7 | 3.3 | 0 | 0 | 6.7 | 0 | 0 | 0 | 3.3 | 0 | 0 | 0 | 3.3 | 0 | 0 | 0 |
| White M | 9.5 | 23.8 | 0 | 0 | 0 | 14.3 | 0 | 0 | 0 | 33.3 | 4.8 | 0 | 0 | 9.5 | 0 | 0 | 0 | 4.8 | 0 | 0 |
| White S | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| White XL | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Franciscan L | 33.3 | 0 | 0 | 0 | 8.3 | 8.3 | 0 | 0 | 16.7 | 0 | 0 | 0 | 8.3 | 0 | 0 | 0 | 25 | 0 | 0 | 0 |
| Franciscan M | 0 | 0 | 2.4 | 0 | 0 | 69 | 0 | 0 | 0 | 14.3 | 0 | 0 | 0 | 4.8 | 0 | 0 | 2.4 | 7.1 | 0 | 0 |
| Franciscan S | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Franciscan XL | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leghorn L | 2.1 | 0 | 0 | 4.2 | 0 | 2.1 | 0 | 0 | 81.3 | 8.3 | 0 | 2.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leghorn M | 0 | 2.1 | 0 | 2.1 | 0 | 4.2 | 2.1 | 0 | 4.2 | 83.3 | 0 | 0 | 2.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leghorn S | 0 | 0 | 0 | 0 | 0 | 12.5 | 0 | 0 | 0 | 0 | 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12.5 | 0 |
| Leghorn XL | 6.7 | 0 | 0 | 6.7 | 0 | 0 | 0 | 0 | 26.7 | 0 | 0 | 46.7 | 0 | 0 | 0 | 13.3 | 0 | 0 | 0 | 0 |
| Black L | 18.8 | 0 | 0 | 0 | 0 | 6.3 | 6.3 | 0 | 25 | 0 | 0 | 6.3 | 25 | 0 | 0 | 0 | 12.5 | 0 | 0 | 0 |
| Black M | 0 | 0 | 0 | 0 | 0 | 31.3 | 0 | 0 | 6.3 | 12.5 | 0 | 0 | 0 | 37.5 | 6.3 | 0 | 0 | 6.3 | 0 | 0 |
| Black S | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 25 | 0 | 0 | 0 | 0 | 0 |
| Black XL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 42.9 | 0 | 0 | 0 | 57.1 | 0 | 0 | 0 | 0 |
| Partridge L | 11.1 | 0 | 0 | 0 | 16.7 | 5.6 | 0 | 0 | 5.6 | 0 | 0 | 0 | 0 | 5.6 | 0 | 0 | 50 | 0 | 0 | 5.6 |
| Partridge M | 9.1 | 9.1 | 9.1 | 0 | 0 | 45.5 | 0 | 0 | 0 | 18.2 | 0 | 0 | 0 | 9.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Partridge S | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 60 | 0 |
| Partridge XL | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33.3 | 0 | 0 | 0 | 33.3 | 0 | 0 | 33.3 |



# *Conclusions*

Of chapter 1:

I. The consideration of Utrerana avian breed, as a multivariety breed, is productively advantageous since a broader scope of market demands could be covered in terms of carcass organoleptic characteristics.

II. Discriminant canonical analysis was validated as a tool to perform individual selection and breed adscription through easily measurable traits such as ocular index and phaneroptics.

Of chapter 2:

III. Nonlinear growth models are suitable to describe the biological growth and laying curve of the Utrerana avian breed, with Logistic and Von Bertalanffy models standing out as the best growth fitting models across the different Utrerana varieties. Narushin-Takma and Quadratic Logarithmic models showed acceptable goodness-of-fit and flexibility criteria to describe biological laying curves of Utrerana breed, a census-limited endangered breed and therefore, from which small numbers of observations are available.

IV. Utrerana is a slow-growing breed characterized by a clear body weight sexual dimorphism which becomes evident from 45 days onwards. Low maturity weight of the individuals makes it compulsory to estimate genetic parameters of growth and to differentiate carcass characteristics in this breed.

Of chapter 3:

V. Although Leghorn produces heavier eggs, yolk/albumen proportion is higher in Utrerana eggs, mainly in partridge and franciscan varieties. Laying hen modern line selection may have induced such an increase in egg weight. However, this may have also translated into a simultaneous decrease in the energy content in the egg, as a direct consequence of a decrease in the percentage of egg yolk.

VI. A nonlinear canonical correlation analysis-based tool allows to determine how eggs external quality indicators may indirectly report information on internal quality traits. This could mean a great advancement in the identification and typification of specific products, which may cover the currently increasing demand from markets for non-conventional quality products, such as Utrerana breed eggs.

VII. Some external characteristics, such as eggshell chromaticity and egg shape index, which are easily measurable without the need to break the eggshell, can provide us with a large amount of information which enables the correct classification of eggs from the different genotypes that coexist in southern Spain.

VIII. Among the different internal quality-related traits, albumen related features, such as Haugh units and albumen weight, play a pivotal role in the determination of differences across egg groups. Wide differential quality properties occur when Spanish native breeds are compared to commercial hybrid lines or other foreign native breeds such as the Araucana hen.

IX. The chemical characterization of Utrerana breed eggs has revealed that their eggs could be considered functional foods. Pieces of evidence suggest their physiological benefits on human health may mainly ascribe to their higher content in proteins, in some MUFA and in the total content of polyunsaturated fatty acids.

X. Ca and Mg concentration in Utrerana eggshells suggests that Utrerana eggshells are stronger and stiffer than Leghorn's ones, despite these present a lower eggshell weight than those of Leghorn's.

Of the chapter 4:

XI. A sensory preference test with professionally-instructed panelists suggested sensory attributes in Utrerana breed eggs are rather highly appreciated than those from commercial and ecologic categories. The

differentiated quality of this product could be key to improve the profitability of Utrerana egg production systems.

XII. The definition of an affine profile of professional consumers of Utrerana eggs allows to outline potential strategies for the design and implementation of marketing campaigns and to identify those sectors in which a greater effort should be made to cover the increasing demand for non-conventional quality autochthonous breeds linked products.

XIII. Weight traits play a pivotal role in the determination of the commercial quality of eggs. This may evidence a historically lack of commercial attention provided to quality traits in favor of quantitative traits.

Cross-sectional conclusions:

XIV. Utrerana hen showed optimal adaptability characteristics to anti-predator behaviour and rusticity. Utrerana enhanced ability to lay eggs must be mainly considered in the process of configuration of its breeding program. For this, the use of certain parameters such as peak yield and persistence, can efficiently be used to select best individuals in the first month of laying.

XV. Although Utrerana breed's egg production rates are low, white, franciscan and black Utrerana egg performance was acceptable until the end of summer. Furthermore, Utrerana may have an enhanced tolerance to heat stress and adaptation to alternative productions, such as free-range and ecological systems, given no significant differences for weight were found across the first six months of the year.

XVI. The combination between discriminant canonical analysis and data mining CHAID decision trees may constitute an efficient classification tool to sort eggs from different varieties of Utrerana hen between across national and international genotypes and to classify them depending on their commercial quality as a function of egg quality traits. This in turn may reveal the existence of hybridization or potential mixing across breeds.

General conclusion:

- The study of physical, chemical, and sensory qualities of Utrerana varieties revealed that commercially, product differentiation could be a feasible strategy for Utrerana-derived products, which could constitute a favorable point when compared to other breeds' eggs which have traditionally been sold in the market.

# *Conclusiones*

Del capítulo 1:

I. La consideración de la raza aviar Utrerana, como raza multivariedad, es productivamente ventajosa, ya que podría cubrirse una gama más amplia de demanda del mercado en términos de características organolépticas de la canal.

II. El análisis discriminante canónico fue validado como una herramienta para realizar la selección individual y la adscripción a la raza considerando rasgos fácilmente medibles como el índice ocular y la faneróptica.

Del capítulo 2:

III. Los modelos no lineales son adecuados para describir el crecimiento biológico y la curva de puesta de la raza aviar Utrerana, destacando los modelos logístico y Von Bertalanffy como los mejores modelos de ajuste de crecimiento en las diferentes variedades de Utrerana. Los modelos Narushin-Takma y cuadrático logarítmico mostraron criterios aceptables de bondad de ajuste y flexibilidad para describir las curvas biológicas de puesta de la Utrerana, una raza en peligro de extinción con censos limitados y, por lo tanto, con un bajo número de observaciones disponibles.

IV. La Utrerana es una raza de crecimiento lento que se caracteriza por un claro dimorfismo sexual en términos de peso corporal, que se hace evidente a partir de los 45 días. El bajo peso adulto de los individuos hace preceptivo estimar los parámetros genéticos de crecimiento y diferenciar las características de la canal en esta raza.

Del capítulo 3:

V. Aunque la línea Leghorn produce huevos más pesados, la proporción yema/albúmina es mayor en los huevos de Utrerana, principalmente en las variedades de perdiz y franciscana. La selección de líneas modernas de gallinas ponedoras ha inducido a un aumento de peso del huevo. Sin

embargo, esto se ha traducido en una disminución simultánea del contenido energético del huevo, como consecuencia directa de una disminución del porcentaje de yema.

VI. La herramienta de análisis de correlación canónica no lineal permite determinar cómo los indicadores de calidad externa de los huevos pueden reportar indirectamente información sobre rasgos de calidad interna. Esto podría significar un gran avance en la identificación y tipificación de productos específicos, que puedan cubrir la demanda actual creciente de productos de calidad no convencional en los mercados, como pueden ser los huevos de la raza Utrerana.

VII. Algunas características externas, como la cromaticidad de la cáscara y el índice de forma del huevo, que son fácilmente medibles sin necesidad de romper la cáscara, pueden aportarnos una gran cantidad de información que permite la correcta clasificación de los huevos de los diferentes genotipos que conviven en el sur de España.

VIII. Entre los diferentes rasgos internos relacionados con la calidad, la calidad del albumen, como las unidades Haugh y el peso, juegan un papel fundamental en la determinación de las diferencias entre los grupos de huevos. Los resultados evidenciaron grandes propiedades diferenciales de calidad cuando se compararon razas autóctonas españolas con las de líneas híbridas comerciales u otras razas nativas foráneas, como la gallina Araucana.

IX. La caracterización química de los huevos de la raza Utrerana ha revelado que estos huevos podrían considerarse alimentos funcionales. Hay pruebas que sugieren que sus beneficios fisiológicos para la salud humana pueden atribuirse principalmente a su mayor contenido en proteínas, en algunos MUFA y en el contenido total de ácidos grasos poliinsaturados.

X. La concentración de Ca y Mg en las cáscaras de huevo de Utrerana sugiere que las estas son más fuertes y rígidas que las de Leghorn, a pesar de que presentan un peso de cáscara menor que la Leghorn.

Del capítulo 4:

XI. Un test de preferencia sensorial con panelistas profesionales instruidos sugirió que los atributos sensoriales en los huevos de la raza Utrerana son más apreciados que los de las categorías comerciales y ecológicas. La calidad diferenciada de este producto podría ser la clave para mejorar la rentabilidad de los sistemas de producción de huevos de Utrerana.

XII. La definición de un perfil afín a los huevos de Utrerana permite delinear estrategias potenciales para el diseño e implementación de campañas de marketing e identificar aquellos sectores en los que se debe hacer un mayor esfuerzo, con el fin de cubrir la creciente demanda de productos ligados a razas autóctonas de calidad no convencional.

XIII. Los rasgos de peso juegan un papel fundamental en la determinación de la calidad comercial de los huevos. Esto puede evidenciar una falta histórica de atención comercial a los rasgos de calidad a favor de los rasgos cuantitativos.

Conclusiones transversales:

XIV. La gallina Utrerana mostró características óptimas de adaptabilidad al comportamiento anti-depredador y rusticidad. La raza debe seguir un programa de cría considerando principalmente su capacidad para poner huevos. Para ello, mediante el uso de ciertos parámetros como el pico de puesta y la persistencia, los animales pueden ser seleccionados de manera eficiente en el primer mes de puesta.

XV. Aunque las tasas de producción de huevos de la raza Utrerana son bajas, el índice de puesta de las variedades blanca, franciscana y negra de Utrerana fue aceptable hasta el final del verano. Además, la Utrerana puede presentar una mayor tolerancia al estrés térmico y adaptación a producciones alternativas, como los sistemas campero y ecológico, dado que no se encontraron diferencias significativas en el peso de la cáscara de huevo durante los primeros seis meses del año.

XVI. La combinación entre el análisis discriminante canónico y los árboles de decisión CHAID de extracción de datos puede constituir una herramienta de clasificación eficiente para clasificar huevos de diferentes variedades de gallina Utrerana entre genotipos nacionales e internacionales y clasificarlos de acuerdo con su calidad comercial considerando los rasgos de calidad del huevo. Esto, a su vez, puede revelar la existencia de hibridación o posible mezcla entre razas.

Conclusión general:

- El estudio de las cualidades físicas, químicas y sensoriales de las variedades Utrerana reveló que comercialmente, la diferenciación de productos podría ser una oportunidad factible para los productos derivados de Utrerana y podría constituir un punto favorable al compararlos con huevos de otras razas que tradicionalmente se han vendido en el mercado.

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# *Appendix I*

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SHORT COMMUNICATION



# Characterisation of biological growth curves of different varieties of an endangered native hen breed kept under free range conditions

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**ABSTRACT**

The aim of this study is to model the growth samples of four varieties (White, Black, Partridge, Franciscan) of Spanish Utrerana hen breed, which is endangered, by using Brody, Von Bertalanffy, Verhulst, Logistic and Gompertz models. For this purpose, a total of 16,235 weight data observations from 2004 animals reared in free range system were collected. Logistic was the best suited model for predicting the biological growth curve of White variety in both sexes, while Von Bertalanffy was the best fitting model for the rest of individuals of the breed, based on the 5 goodness-of-fit and flexibility criteria: Pseudo-$R^2$, mean squared error, Akaike information criterion, Bayesian information criterion and the biological coherence of the estimated parameters. Black variety was the heaviest, with values of 2605.96 and 2032.61 g (for males and females, respectively) for *a* parameter, while White variety presented the lowest maturity weight ($a = 2442.99$ and 1874.24 g, for males and females, respectively). Conclusively, this growth characterisation is essential for the conservation of the Utrerana hen, to search for new market niches and a greater profitability to this differentiated product.

**HIGHLIGHTS**

- Non-linear models can explain the Utrerana hen growth.
- Females reach maturity earlier than males.
- Utrerana hen shows a strong sexual dimorphism.

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Growth curves; non-linear models; growth parameters; local breeds; variety

## Introduction

Utrerana hen is an endangered Mediterranean light breed created during the first half of the 20th century in Andalusia (southern Spain) (Orozco 1989). It has four different varieties: White, Black, Partridge and Franciscan (Figure 1). Its initial productive orientation was as a laying hen, raised on family farms, but the birth of approximately 50% of males on each incubation batch has promoted the traditional use of the meat carcase of this breed for self-consumption (Campo 2007). Utrerana poultry breed has shown moderate genetic diversity among the individuals that compose the breed (Macrì et al. 2019). Moreover, Utrerana hen and its varieties can be considered as differentiated populations from other Spanish poultry breeds (Vega-Plá et al. 2019).

There is a growing interest among consumers in animal products obtained through sustainable production systems, the purpose of which is obtain quality food, with less impact on the environment and human health, considering animal welfare (Barba et al. 2016). Alternative forms of farming, involving local breeds, are necessary to avoid the loss of biodiversity, the disappearance of animal genetic resources, the search of economic sustainability and the linkage of population to rural areas (Alderson 2018; Toalombo et al. 2019). Utrerana breed is adapted to these systems due to its high rusticity and low disease prevalence (Del Castillo

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Supplemental data for this article can be accessed here.


**Figure 1.** Hen and rooster of each Utrerana variety. A: White; B: Black; C: Partridge; D: Franciscan.

1951). Native poultry breeds have a genome that makes them more resistant than commercial hybrid lines to conditions caused by climate change in a specific geographical area (Mpenda et al. 2019).

Within the cycle life of animals, the total growth duration can be divided in three phases: an acceleration phase, a deceleration phase, and a stabilisation phase for ripening (Nogales et al. 2017). So, growth pattern usually is typically fitted by models with sigmoidal structure. The study of the growth of a breed and how certain factors such as variety or sex influence it, is necessary to establish the potential for meat productivity (Sariyel et al. 2017). Growth can be explained through mathematical functions, which could also predict for the age of sexual maturity or the suitable age for commercial slaughter, while helping monitoring general health conditions and nutritional requirements (Kaplan and Gürcan 2018).

There are previous bibliographic references on the productive performance of the growth of some local Spanish breeds (Francesch 1998; Cubiló et al. 1999; Sánchez et al. 2000; Muriel 2003; Miguel et al. 2009; Franco et al. 2012; Cajal and Francesch 2014). Non-linear growth models (Brody, Von Bertalanffy, Verhulst, Logistic, Gompertz, and others) have been studied in other native breeds, geographically and genetically separated (Yang et al. 2006; Rizzi et al. 2013; Osei-Amponsah et al. 2014; Mata-Estrada et al. 2020). In the Utrerana poultry breed, research with the aim to describe the quality-related characteristics and the sensory preference of eggs has been performed (González Ariza, Arando Arbulu, et al. 2019, González Ariza, Navas González, et al. 2019, González Ariza et al. 2021).

Consequently, the present study aimed to determine the best fitting non-linear growth curve models for growth performance of the Utrerana poultry breed, and the characterisation of their biological curve while evaluating the relationship between body weight and age.

## Materials and methods

### *Chicken flock and environmental conditions*

The weight-age data for this study were obtained from 2004 individuals reared under free-range conditions during the years 2018 and 2019 in a public hatchery located at the Agropecuary Provincial Centre of Diputación of Córdoba, Spain.

The chickens were hatched during the first half in both years, since this breed describes seasonal laying patterns regarding the period of eggs (González Ariza, Navas González, et al. 2019). The chickens were sorted into incubation batches according to age in 20 batches. Feed and water were available *ad libitum* in all rearing phases (Table 1). At hatching, the chicks

**Table 1.** Chemical composition of the compound feed used for feeding the chicken batches in the study.

| Chemical composition (%) | 0–4 weeks | 4–32 weeks | >32 weeks |
|---|---|---|---|
| Crude protein | 20.50 | 18.00 | 15.70 |
| Crude fat and oils | 2.10 | 3.00 | 4.60 |
| Crude fibre | 2.80 | 3.00 | 3.20 |
| Crude ashes | 6.30 | 5.70 | 14.00 |
| Calcium | 0.90 | 1.00 | 4.10 |
| Phosphorus | 0.69 | 0.50 | 0.46 |
| Sodium | 0.15 | 0.18 | 0.19 |
| Methionine | 0.48 | 0.32 | 0.31 |
| Lysine | 1.13 | 0.90 | 0.72 |

were placed, sorted per incubation batch, in rearing rooms (5 birds/m$^2$ with electric heaters (Copele LGA, Copele, Murcia, Spain) in each room). Animals had access to the outside (1 bird/m$^2$) from 2 month of age on.

## Recording for the biological growth curve

The weights were individually measured: on hatching day, weekly during the first month of life, every 2 weeks from 1 to 3 months and every 28 days from the age of 3 months on. An electronic scale (measurement precision = 0,01 g; CSB-600C, Cobos, Barcelona, Spain) was used to measure weights below 600 g, while a suspended electronic scale (measurement precision = 5 g; Kern CH50K100, Kern & Sohn, Balingen, Germany) was used for animals which exceeded 600 g.

## Curve fitting

The data file was purged as described by Lupi et al. (2015). Finally, data of 98.5% of the total of animals were retained for this study. A total of 16,235 weights were kept. A slightly higher number of observations were sampled in females (56.04%) compared to males (44.96%), due to the fact that 749 males were used versus 1255 females in the present study (Table 2).

Five non-linear functions were evaluated in the present study: Brody, Von Bertalanffy, Verhulst (a variation of Logistic model; frequently called as Logistic model in the literature), Logistic and Gompertz (Table 3). Data were processed with the non-linear regression procedure from the SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016). The results for best-fitted model in each variety were compared with real weight data and animal ages.

The parameter $a$ is defined as the asymptotic or maximum growth response of the adult bird. The parameter $b$ is related to initial weight (hatch weight). The parameter $k$ represents the relative growth rate (exponential growth rate) and indicate the maturity of individuals. Finally, the parameter $m$ shapes the growth curve, thus determining its inflection point (Loaiza-Echeverri et al. 2013; Tariq et al. 2013; Lupi et al. 2015; Iqbal et al. 2019). For the choice of the best fit models for each variety and sex, the following criteria were used (Lupi et al. 2015; Pizarro Inostroza et al. 2020):

**Table 2.** Number of animals ($n$) and weight observations ($N$) used for each variety in both sexes in the study.

| | Females | | Males | | Total | |
|---|---|---|---|---|---|---|
| Variety | $n$ | $N$ | $n$ | $N$ | $n$ | $N$ |
| White | 108 | 967 | 76 | 757 | 184 | 1724 |
| Black | 421 | 3456 | 277 | 2874 | 698 | 6330 |
| Partridge | 373 | 2123 | 175 | 1442 | 548 | 3565 |
| Franciscan | 353 | 2552 | 221 | 2064 | 574 | 4616 |
| Total | 1255 | 9098 | 749 | 7137 | 2004 | 16,235 |

1. The use of the coefficient of determination ($R^2$) in linear regression models determine the quality of the fit of the used model, but in non-linear regression models could overestimate higher values. So, the mathematical approach is Pseudo-$R^2$ and is determined by:

$$\text{Pseudo} - R^2 = 1 - \frac{\text{SSResidual}}{\text{SSTotal}_{\text{corrected}}}$$

   where SS is the sum of squares.
2. The lowest mean square of the error (MSE) of the studied equation, as a measure that includes the variability of factors not considered by research.
3. The lowest value of the Akaike information criterion (AIC). This tool can consider changes in the fitness quality and the number of parameters between models:

$$\text{AIC} = N\ln\left(\frac{\text{SSResidual}}{N}\right) + 2K$$

   where $N$ is the numbers of observations; SSResidual is the sum of squares of the residuals; and K is the number of parameters.
4. The lowest value of Bayesian information criterion (BIC), that is a model-order selection criterion:

$$\text{BIC} = N\ln\left(\frac{\text{SSResidual}}{N}\right) + K*\ln(N)$$

   where $N$ is the numbers of observations; SSResidual is the sum of squares of the residuals; and $K$ is the number of parameters.
5. Biological coherence of the estimated parameters.

The five fitting models used in the present study were ranked considering the goodness-of-fit and flexibility criteria individually. The highest score in the rank was

given to the model obtaining the most desirable value for each particular criterion.

Afterwards, as goodness-of-fit and flexibility criteria may differ in terms of which their most desirable values are and what their magnitude is, a combined selection index (ICO) was developed following the premises in Van Vleck (1993) to summarise the position in the rank for each of the goodness-of-fit and flexibility criteria determined for each model. The combined index used (ICO) was as follows:

$$ICO = \frac{\begin{array}{c}Pseudo{-}R^2RankPosition * W1 + \\ MSERankPosition * W2 + AICRank\end{array}}{\underline{Position * W3 + BICRankPosition * W4}}{4},$$

All criteria were given the same relevance in the ICO, hence, no coefficient was used, that is the proportion of 1:1:1:1 was followed. As a result, the models presenting greater ICO values were those presenting the best-fitting, explanatory and predictive properties for each variety and sex (Supplementary Tables S1, S2, S3, S4 and S5).

## Results

A summary of biological curve shape parameters, fitness and accuracy statistics for the different models that were tested across the Utrerana breed and its varieties are shown in Supplementary Tables S1, S2, S3, S4 and S5. The White genotype, reported the best fitting values as verified with all models, excluding Brody model, which presents, in a generalised way, lower values for Pseudo-$R^2$ across all varieties. Brody model overestimates asymptotic weight, in both sexes, however the rest of models showed convergence and very similar adjustment values. Except for White genotype, Von Bertalanffy model fitted growth data better than the rest of studied models. In the White variety, the Logistic model was the most suitable model. Supplementary Tables S6, S7, S8, S9 and S10 show the observed and predicted weights for the best fitting model for both sexes of Utrerana poultry breed and its varieties (Table 3).

Table 4 shows the estimated parameters for each variety and sex using the best fitting model in the study of the biological growth curve in Utrerana breed. Males showed a higher body weight in all growth stages, however these differences become clearly noticeable from 45 days of life, as can be seen in Figures 2 and 3, where growth of Utrerana and its varieties are graphically represented.

**Table 3.** Mathematical description of growth models, biological parameters and growth evaluators.

| | Mathematical expression | Inflection weight | Inflection age | Growth rate | Age to maturity ($y \approx a$) | Maturity degree |
|---|---|---|---|---|---|---|
| Brody | $y = a*(1-b*exp(-k*t))$ | – | – | $v_c = ka\left(1-\frac{y}{a}\right)$ | $-\frac{ln\left(\frac{a-y}{ba}\right)}{k}$ | $u = \frac{y}{a}$ |
| Von Bertalanffy | $y = a*(1-b*exp(-k*t))**3$ | $y_i = \frac{8a}{27}$ | $t_i = \frac{\ln(3b)}{k}$ | $v_c = 3ky\left[\left(\frac{a}{y}\right)^{1/3}-1\right]$ | $-\frac{ln\left(\frac{1-\sqrt[3]{\frac{y}{a}}}{b}\right)}{k}$ | |
| Verhulst | $y = a/(1+b*exp(-k*t))$ | $y_i = \frac{a}{2}$ | $t_i = \frac{\ln(b)}{k}$ | $v_c = ky\left(1-\frac{y}{a}\right)$ | $-\frac{ln\left(\frac{a-y}{y*b}\right)}{k}$ | |
| Logistic | $y = a*(1+\exp(-k*t))**(-m)$ | $y_i = \frac{a}{2}$ | $t_i = \frac{-\ln\left(2^{1/m}-1\right)}{k}$ | $v_c = mk\frac{y_c}{2}\left(\frac{e^{-kt}}{1+e^{-kt}}\right)$ | $-\frac{ln\left[\left(\frac{a}{y}\right)^{1/m}-1\right]}{k}$ | |
| Gompertz | $y = a*\exp(-b*\exp(-k*t))$ | $y_i = \frac{a}{e}$ | $t_i = \frac{\ln(b)}{k}$ | $v_c = kyln\left(\frac{a}{y}\right)$ | $-\frac{ln\left(\frac{\ln\left(\frac{y}{a}\right)}{-b}\right)}{k}$ | |

$y$ = weight, in kg, at age t; $t$ = age in days; $a$, $b$, $k$ and $m$ = parameters.

**Table 4.** Estimated parameters for the best-fitting model for both sexes of Utrerana poultry breed.

| Breed/Variety | Sex | Model | $a$ (s.e.) | $k$ (s.e.) | $b/m$ (s.e.) | Pseudo-$R^2$ | MSE | AIC | BIC |
|---|---|---|---|---|---|---|---|---|---|
| Utrerana | F | Von Bertalanffy | 1979.00 (4.87) | 0.014 (0.000) | 0.786 (0.004) | 0.971 | 26,591.00 | 92,696.41 | 92,717.76 |
| | M | Von Bertalanffy | 2560.30 (10.00) | 0.014 (0.000) | 0.854 (0.004) | 0.976 | 19,613.76 | 70,535.13 | 70,555.75 |
| White | F | Logistic | 1874.24 (11.12) | 0.022 (0.000) | 5.525 (0.138) | 0.953 | 25,213.26 | 9803.69 | 9818.28 |
| | M | Logistic | 2442.99 (25.92) | 0.021 (0.000) | 5.864 (0.110) | 0.963 | 18,097.62 | 7424.27 | 7438.16 |
| Black | F | Von Bertalanffy | 2032.61 (8.74) | 0.013 (0.000) | 0.788 (0.006) | 0.943 | 22,839.18 | 34,688.22 | 34,706.66 |
| | M | Von Bertalanffy | 2605.98 (18.01) | 0.014 (0.000) | 0.862 (0.006) | 0.958 | 16,114.04 | 27,844.72 | 27,862.61 |
| Partridge | F | Von Bertalanffy | 1937.83 (9.84) | 0.014 (0.000) | 0.781 (0.011) | 0.933 | 32,957.96 | 22,055.54 | 22,105.52 |
| | M | Von Bertalanffy | 2484.10 17.46) | 0.015 (0.000) | 0.845 (0.009) | 0.958 | 19,922.59 | 14,277.23 | 14,293.05 |
| Franciscan | F | Von Bertalanffy | 2024.45 (9.25) | 0.012 (0.000) | 0.752 (0.007) | 0.945 | 24,677.40 | 25,813.02 | 25,830.55 |
| | M | Von Bertalanffy | 2595.94 (19.50) | 0.013 (0.000) | 0.822 (0.007) | 0.947 | 22,827.04 | 20,740.70 | 20,733.58 |

F: female; M: male; s.e.: standard error; Pseudo-$R^2$: non-linear determinative coefficient; MSE: mean square error; AIC: Akaike information criteria; BIC: Bayesian information criteria.

## Discussion

Hatching weight predicted by the Logistic model in the White genotype (41.95 and 40.71 g for males and females, respectively) have higher accuracy respect to the measured hatching weight that the hatching weight predicted by Von Bertalanffy model in the rest of varieties (Table 5). Predictions of hatching weight were between 6.90 and 41.95 g, so slightly some authors supported constraining hatching weight in order to improve fitting ($R^2$) of the data. Barbato (1990) reported that hatching weight should be measured, but not estimated, while others authors suggested to make a data correction: Mignon-Grasteau et al. (1999) suggested to constrain hatching weight within two standard deviations of the mean and Pasternak and Shalev (1994) suggested weighting hatching weight by the inverse of the variance.

Utrerana is a slow-growing breed characterised by clear sexual dimorphism which became evident on the base of the body weight from 45 days onwards. Utrerana can be defined as a light breed because the early age of inflection in the growth curve, the high precocity and the low maturity weight of the individuals, compared with other chicken breeds. Males and females of the White genotype reported lower maturity weights, while for the rest of the varieties (Partridge, Franciscan and Black), both sexes reported more similar values (Table 4). Maturity was reached later in the Franciscan genotype ($k = 0.013$ in males and $k = 0.012$ in females), while an earliest growth was reported for the White variety ($k = 0.021$ in males and $k = 0.022$ in females). A negative correlation between parameters $a$ and $k$ can be observed across the different varieties of the Utrerana breed. Some authors have suggested that there is a high probability that

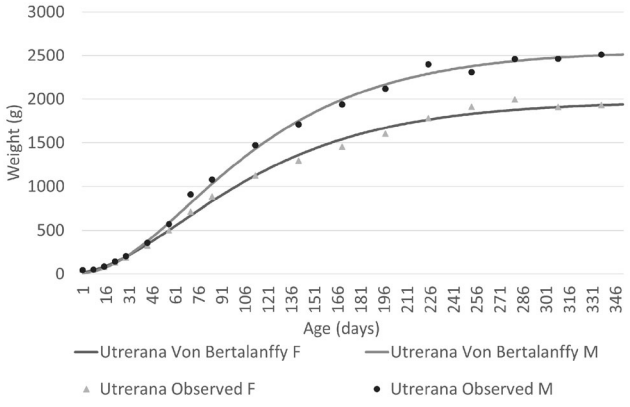


**Figure 2.** Growth curves for both sexes of Utrerana poultry breed predicted with the best fitting model in comparison with the observed data.

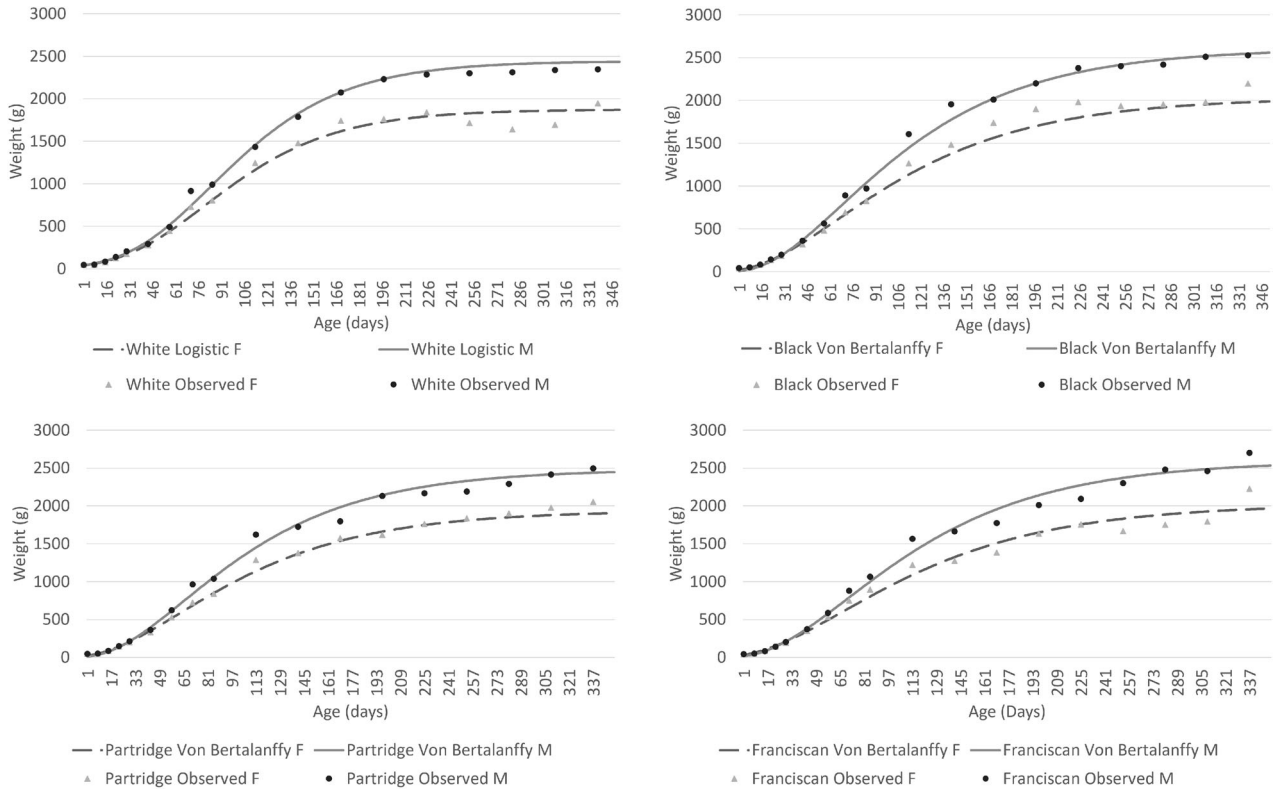


**Figure 3.** Growth curves for both sexes of Utrerana poultry breed predicted with the best fitting model in each variety in comparison with the observed data.

**Table 5.** Estimated hatching weight, inflection weight and inflection age for the best-fitting model for both sexes of Utrerana poultry breed.

| Breed/variety | Sex | Model | Hatching weight (g) | Inflection weight (g) | Inflection age (days) |
|---|---|---|---|---|---|
| Utrerana | Female | Von Bertalanffy | 19.23 | 586.37 | 61.27 |
| | Male | Von Bertalanffy | 8.00 | 758.61 | 67.20 |
| White | Female | Logistic | 40.71 | 937.12 | 79.50 |
| | Male | Logistic | 41.95 | 1221.49 | 83.20 |
| Black | Female | Von Bertalanffy | 19.48 | 602.25 | 64.47 |
| | Male | Von Bertalanffy | 6.90 | 772.14 | 67.49 |
| Partridge | Female | Von Bertalanffy | 20.49 | 574.17 | 60.73 |
| | Male | Von Bertalanffy | 9.29 | 736.03 | 63.59 |
| Franciscan | Female | Von Bertalanffy | 30.90 | 599.84 | 65.52 |
| | Male | Von Bertalanffy | 14.74 | 769.17 | 68.58 |

larger and heavier animals are less precocious than smaller and lighter ones (Bathaei and Leroy 1998).

Regarding other Spanish breeds, Francesch (1998) collected weights of chickens of Empordanesa Roja, Penedesenca Negra and Prat Leonada breeds, from hatching to 20 weeks of age, obtaining values of 2840, 2660 and 2675 g, respectively. In addition, Cubiló et al. (1999) reported values of 2482 g in Penedesenca Negra breed at 16 weeks. These weights are above those estimated for Utrerana breed for the same age. Although Utrerana poultry breed has been classified as a dual-purpose hen, a lower potential for meat-production than these other breeds has also been reported (Fernández et al. 2009). Sánchez et al. (2000) using the Mos hen, a native breed with a clear orientation towards meat production reported higher values for chicken growth (4434 and 3641 g in males and females at 300 days of life).

Contrastingly, it has been reported weights of 1752.60 g (Black variety) and 1740.90 g (brunette variety) of weight in females at adulthood in Sobrarbe hen breed, weights of 2491.96 g in cocks at 32 weeks in Extremeña Azul breed, and values of $k$ of 0.153 and $a$ of 2660.91 g using the Gompertz model for cocks of Castellana Negra breed (Muriel 2003; Miguel et al. 2009; Cajal and Francesch 2014). These results agree with the findings of the present study. Sobrarbe, Extremeña Azul, Castellana Negra and Utrerana hen breeds have been genetically selected towards the egg production in extensive systems, great rusticity and resistance to extreme weather situations. Besides, Castellana Negra and Utrerana poultry breeds have a great geographical proximity and literature indicates that have a common genetic origin (Orozco 1989).

Predicted $k$ parameters in the present study agree with those by other authors who used Von Bertalanffy models in slow-growing broilers and native creole chickens (Narinç et al. 2010; Mata-Estrada et al. 2020). Still these are slightly lower when compared with local breeds or slow-growing genotypes using Logistic and Gompertz models, respectively (Rizzi et al. 2013; Aksoy et al. 2021). However, Topal and Bolukbasi (2008) obtained much higher values of $k$ in fast-growing broilers using Von Bertalanffy model. The relative growth could be slower in native breeds than in fast-growing lines due to the lower productive selection and the environmental conditions of these breeds.

Inflection age estimated by Logistic model in local chicken of Ghana (Osei-Amponsah et al. 2014) are close to the values reported in the present study. Both sexes of Utrerana hen breed present similar results ($<$4 days between males and females) at the estimated inflection age, in contrast with the results obtained by Mata-Estrada et al. (2020), who reported a difference between males and females of approximately 10 and 8 days for Von Bertalanffy and Logistic models. Therefore, the slaughter age for Utrerana breed must be similar in both sexes. In any case, in native breeds with low inflection weight, the slaughter age must be delayed until the birds reach a weight close to the weight at maturity, seeking in the chicken carcase a differentiated product for the market (Franco et al. 2012).

Regarding to the best models for describing growth on poultry, some authors suggested that Von Bertalanffy growth model was the best model to fit growth in local breeds (Yang et al. 2006; Mata-Estrada et al. 2020). Nevertheless, Atil et al. (2007) suggested that logistic growth model reports the best fit in broilers when compared to Von Bertalanffy and Gompertz models. In any case, the results obtained in the present research are also agree with those reported by Narinç et al. (2017), since they reported that Gompertz was a suitable model to fit the growth curve in slow-growing chickens.

## Conclusions

The non-linear growth models used in this study are suitable to describe the biological growth of the

Utrerana breed, with Logistic and Von Bertalanffy models standing out as the best fitting models in different Utrerana varieties, in accordance to the goodness-of-fit and flexibility criteria. Utrerana growth curves are very similar to the rest of light breeds, with a clear sexual dimorphism, hence, males of this breed could be profitable from a meat production point of view. The obtained results can be useful for making zootechnical decisions like determine a slaughter age, the nutritional requirements and control the health status of the batch and may support the breeding program for these endangered breeds. Finally, further studies are needed to estimate genetic parameters of the growth curve of this breed and make a genetic selection of individuals based on growth characteristics.

## Ethical approval

The study follows the national guidelines and premises described in the Declaration of Helsinki. Protocols applicated were permitted by the regulations of the European Union (2010/63/EU) in their transposition to the Royal Decree-Law 53/2013 and its credited entity the Ethics Committee of Animal Experimentation from the University of Córdoba.

## Disclosure statement

The authors declare no conflict of interest.

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# Non-Parametrical Canonical Analysis of Quality-Related Characteristics of Eggs of Different Varieties of Native Hens Compared to Laying Lineage

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**Simple Summary:** The development of new more productive lines of laying hens has displaced native breeds to second place; therefore, new lines of research that ensure the conservation of local breeds and biodiversity are increasingly necessary. The aim of the present study is to characterize the productive capability of Utrerana and to compare the relationships among parameters determining the internal and external quality of the egg, through canonical correlation analysis. We used a flock of 68 Utrerana hens with animals of each of its four varieties (white, black, Franciscan and partridge), and a group of 17 Leghorn hens as a control group. The breed and variety significantly affected egg characteristics. The external and internal quality parameters of the egg were evaluated and reported results providing consistent data for the characterization of the products from this breed. This productive characterization could benefit the conservation of the Utrerana breed, the establishment of livestock models that adapt to it and the search for a market in which this product could be used.

**Abstract:** The aim of the present study is to characterize the productive capability of Utrerana and to compare the relationships among parameters determining the internal and external quality of the egg, through canonical correlation analysis. A flock of 68 Utrerana hens and a control group of Leghorn hens (n = 17) were housed individually to allow individual identification of eggs and for the assessment of egg quality characteristics. Almost all variables showed differences when both breeds were compared, except for white height, yolk diameter, yolk$^{L^*}$ and yolk pH ($p > 0.05$). Only minor diameter, white height, yolk$^{L^*}$, yolk$^{a^*}$, and shell weight reported significant differences between laying age groups. White height, yolk color, and almost all yolk color coordinates were significantly different ($p < 0.05$) for period and month. Egg and white weight reached highest significantly different levels for the fourth and fifth time that the hens laid an egg. External quality-related traits are better predictors of internal quality-related traits than vice versa, enabling the implementation of an effective noninvasive method for internal quality determination and egg classification aimed at suiting the needs of consumers.

**Keywords:** Egg quality; color coordinate decomposition; internal quality traits; external quality traits; DSM color fan

## 1. Introduction

Along with other food such as milk, eggs represent a great contribution of proteins of animal origin to the human diet [1]. In 2017, the world production of eggs exceeded 1416 trillion tons of eggs, equivalent to 80 million metric tons, 30% higher than production in 2000 [2]. In the European Union, the production of food in alternative production systems is on the rise; in 2017, free range and organic egg production accounted for 20% of the total production of eggs [3].

Currently, almost all of the consumed eggs are produced by commercial hybrid lines, which are characterized by high productive performance and a good feed conversion index [4,5]. However, the exploitation of these highly productive lines causes a decrease in the genetic variability of the species and has negative effects on the development of sustainable practices based on local breeds [6].

The emergence of new commercial lines of laying hens with a much greater productive capacity throughout the twentieth century caused the displacement of the autochthonous breed. In many cases, their hybridization with more productive lines relegated autochthonous breeds, including the Utrerana avian breed, to a form of ornamental poultry farming, based on the morphological selection of breeding animals. As a result, a reduction in the census of animals of this breed occurred in addition to a decrease in the productive indices [7].

The Utrerana hen breed was created in the first half of the 20th century, starting with the selection of a heterogeneous population of chickens from the Andalusian countryside [8]. Its initial productive orientation was to be a laying hen, with an annual output of 120–180 eggs, white in color and with an average weight of 62–64 g. It has four different varieties, characterized by the color of the plumage and the legs: white, Franciscan, black, and partridge [9].

The need for the characterization of the products of the Utrerana hen breed is largely due to the situation that it faces. This breed is classified as an endangered breed, according to the Royal Decree Law 2129/26 December, 2008, which establishes the national program of conservation, breeding, and promotion of livestock breeds, and presented a census of 1309 animals on 31 December, 2018 [10]. Therefore, facing this alarming situation, the implementation of programs for the recovery, conservation and productivity improvement of the breed, trying to provide it with an identity and, again, a productive role able to satisfy the demands of the market is required. Thus, the assessment of local products may be a strategy for the conservation of local breeds, for instance, avoiding the loss of linkage between local products and their area of production, as is the case of industrial products [11].

Biodiversity must not only be considered as the genetic conservation of animal resources but also the search economic sustainability and the maintenance of the hen population in rural areas [12]. The increasing concern of the society about animal welfare has allowed the development of alternative forms of livestock, including extensive local farms [13]. The Utrerana hen breed, as a local breed, is perfectly suited to this operating system, since it presents great rusticity and resistance to extreme weather situations, with great ability to search for food in the wild [14].

In general, the quality of the egg is related to characteristics that affect the acceptability of eggs by the consumer [15]. Among the considerable number of characteristics of egg quality that can be measured, external factors such as egg weight are the most important [16–19]. The internal egg quality is also an important aspect to consider, especially when approaching the marketing opportunities of the product. A dense albumen height is among the most important determinants of the internal quality [20,21]. In addition to these factors, other parameters such as the major and minor diameters of the egg, eggshell, yolk color and the weight and pH of the white and yolk allows a more complete characterization of the quality of the egg [22–24]. It has been shown that breed genotype can significantly affect most of these features: egg shape, yolk and albumen quality, shell and egg weight and amount of yolk [25].

The first objective of this study is to characterize the productive capacity of the four varieties of Utrerana hens compared to a globally distributed laying lineage, as a means of demonstrating the benefits of greater genetic diversity on the quality of products derived from sustainable native breeds. In addition, we quantified the explanatory power of the variance by factors such as the laying month,

laying order, period, laying age, variety, and breed found in two sets of parameters of external and internal egg quality. Secondly, we compared the relationships among determining parameters of the internal and external quality of the egg of endangered native hens through a canonical correlation analysis to develop a predictive tool that may enable indirect scoring of the internal quality of the egg from the set of external quality variables.

## 2. Materials and Methods

### *2.1. Animal Sample and Diets*

A total of 85 hens were used in the present study, distributed depending on their age and variety as shown in Table 1. The birds were housed in individual cages (50 × 62 × 41 cm) following Council Directive 1999/74/EC of 19 July, 1999, laying down minimum standards for the protection of laying hens at the Centro Agropecuario Provincial de Cordoba (Spain), for 6 months (January to June 2018). All the animals were fed on the same commercial feed (15.2% crude protein, 4.1% calcium, 0.66% available phosphorus) for the whole experimental period. Feed and water were available ad libitum. All the birds were reared according to the regulations of the European Union (2010/63/EU) in their transposition to the Spanish law (RD 53/2013).

**Table 1.** Flock management information. All cages were chosen according to Council Directive 1999/74/EC of 19 July, 1999, laying down minimum standards for the protection of laying hens.

| Flock Management Parameter | Utrerana | | | | Leghorn (Control) |
|---|---|---|---|---|---|
| | White | Franciscan | Black | Partridge | |
| Breeding hens | 17 | 17 | 17 | 17 | 17 |
| Hens (70 weeks old) | 12 | 12 | 12 | 12 | 12 |
| Pullets (28 weeks old) | 5 | 5 | 5 | 5 | 5 |
| Stocking density | 4 animals per each $m^2$ | | | | |
| Nest box density | 29 animals per each $m^2$ | | | | |
| Waterer allotment/space | Circle waterers of 5 cm of diameter per animal | | | | |
| Feeder allotment/space | 41 cm per animal | | | | |
| Floor substrate | Wood shavings covering the floor at a depth of approximately 1 cm | | | | |
| Nest box substrate | Plastic turf mats covering the floor at a depth of approximately 1 cm | | | | |

### *2.2. Work Sample*

All statistical tests were carried out using an egg sample comprising 194 eggs laid from March to June 2018 by the animal sample described above. A total of 147 eggs had been laid by Utrerana hens, while 47 belonged to Leghorn laying hens. The same information registration protocol was followed for all the eggs comprising the sample except for yolk and white pH determination. Due to economic reasons, 97 eggs were chosen at random to perform yolk and white pH analysis.

### *2.3. External and Internal Quality-Related Traits Set Description*

Two sets of variables were measured. The first set of variables comprised external quality-related traits, those characteristics that can be measured externally without the need to break the eggs. This first set comprised the variables of egg weight, length and breadth, shell color lightness and shell color coordinates (Shell L*, Shell a*, Shell b*, lightness, red/green and yellow/blue coordinates, respectively). In contrast, the second set of traits, considered internal quality-related variables, required the egg to be broken so as to be scored. This second set comprises albumen height, yolk color, yolk lightness and color coordinate decomposition (YolkL*, Yolka*, Yolkb*), yolk diameter, shell weight, yolk weight, albumen weight, yolk pH and white pH.

### *2.4. Information Registration*

Laying lasted for 120 days. All eggs were divided into three periods of 40 days with a mean number of 64.67 eggs per period. Periods ran from second half March to first half April, second half April to first half May, and second half May to first half June. Egg temperature at the time of egg quality assessment was 22 °C ± 1 °C. Individual collection of the eggs of each hen was carried out and all the required variables for the external characterization of the egg were studied daily during the 24 hours following oviposition. Every egg was weighed with a weighing scale (Cobos, CSB-600C, Barcelona, Spain). Major and minor diameters of the egg were measured following a Vernier scale (Electro DH M 60.205, Barcelona, Spain). The color of the shell was determined using a portable spectrophotometer (CM 700d, Konica Minolta Holdings Inc., Tokyo, Japan), and the results were expressed using the International Commission on Illumination (CIE) L*a*b* system color profile (CIE, 1976).

The traits measured to describe the internal quality of the eggs were as follows: weight of the egg, shell, egg yolk and egg white, white height, the diameter of the yolk, pH of the white and yolk and the color of the yolk. These measurements were taken every fifteen days in all the eggs that the flock of hens laid on the day of collection, evaluating a total of 194 eggs. Then, a sample of 97 eggs was tested at random for yolk and white pH.

To determine internal quality-related traits, the eggshell was broken and the egg contents were deposited on a glass surface. The diameter of the yolk was measured with a Vernier scale. The intensity of the yellow color of the yolk of the egg was measured with the portable spectrophotometer and with a DSM® fan (formerly Roche color fan). The pH of the yolk and white was measured using reactive strips. The height of the white was computed as the mean of three measurements obtained with a Haugh digital micrometer (Baxlo, Barcelona, Spain). Finally, eggshell, egg white, and the yolk were weighed separately using a precision balance.

### *2.5. Statistical Analysis*

All variables recorded were separated into two variable sets. The first set included external egg quality-related parameters, such as egg weight, major diameter, minor diameter, shell$^{L^*}$, shell$^{a^*}$, shell$^{b^*}$ and white height, respectively. The second set was internal egg quality-related parameters, such as yolk color, yolk$^{L^*}$, yolk$^{a^*}$, yolk$^{b^*}$, yolk diameter, shell weight, yolk weight, white weight, yolk pH, and white pH.

Levene's test for equality of error variance was run to test for homoskedasticity. Mauchly's W Test was run to test for sphericity. All assumptions except for Shapiro Wilk Francia's normality tests were carried out using SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016). Shapiro Wilk Francia's normality tests were carried out with the sfrancia routine of StataCorp Stata version 14.2. Skewness and Kurtosis statistics were tested to support the reports by Shapiro Wilk Francia's normality tests. As the factors (month of laying, laying order, controlled period, laying age, variety, and breed) and variables in the model had violated most of the common parametric assumptions, the decision to follow a non-parametric approach was made. The Mann–Whitney U test was used to compare differences between the two independent groups of the laying age (laying hens and laying pullets) and breed (Utrerana and Leghorn) variables (Supplementary Tables S1 and S2). Similarly, a Kruskal–Wallis H test was performed to study the potentially existing differences between-levels of the same factor when three or more groups existed within the same independent variable (the rest of independent variables) in Table 2 and Supplementary Table S3.

**Table 2.** Summary of the results for the Kruskal–Wallis H test and the determinative coefficient through r or partial eta squared ($\eta p^2$), for the fixed effects for internal and external egg quality traits from the model, excluding yolk and white pH in Utrerana hens (n = 194).

| Variable | Parameter | Egg Weight | Major Diameter | Minor Diameter | Shell$^{L*}$ | Shell$^{a*}$ | Shell$^{b*}$ | White Height | Yolk Color | Yolk$^{L*}$ | Yolk$^{a*}$ | Yolk$^{b*}$ | Yolk Diameter | Shell Weight | Yolk Weight | White Weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Month | $\chi^2$ | 5.156 | 6.423 | 1.139 | 15.831 | 1.415 | 10.753 | 8.854 | 52.954 | 67.718 | 82.856 | 89.812 | 1.056 | 7.675 | 3.071 | 4.411 |
| | dfn | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | *p*-value | 0.161 | 0.093 | 0.768 | 0.001 | 0.702 | 0.013 | 0.031 | 0.000 | 0.000 | 0.000 | 0.000 | 0.788 | 0.053 | 0.381 | 0.220 |
| | dfd | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 | 192 |
| | F | 1.719 | 2.141 | 0.380 | 5.277 | 0.472 | 3.584 | 2.951 | 17.651 | 22.573 | 27.619 | 29.937 | 0.352 | 2.558 | 1.024 | 1.470 |
| | $\eta p^2$ | 0.026 | 0.032 | 0.006 | 0.076 | 0.007 | 0.053 | 0.044 | 0.216 | 0.261 | 0.301 | 0.319 | 0.005 | 0.038 | 0.016 | 0.022 |
| Order | $\chi^2$ | 13.672 | 8.945 | 10.682 | 5.844 | 3.418 | 1.777 | 9.268 | 2.178 | 1.245 | 1.702 | 6.035 | 4.371 | 7.241 | 4.262 | 25.574 |
| | dfn | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | *p*-value | 0.018 | 0.111 | 0.058 | 0.322 | 0.636 | 0.879 | 0.099 | 0.824 | 0.940 | 0.889 | 0.303 | 0.497 | 0.203 | 0.512 | 0.000 |
| | dfd | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 |
| | F | 2.734 | 1.789 | 2.136 | 1.169 | 0.684 | 0.355 | 1.854 | 0.436 | 0.249 | 0.340 | 1.207 | 0.874 | 1.448 | 0.852 | 5.115 |
| | $\eta p^2$ | 0.067 | 0.045 | 0.053 | 0.030 | 0.018 | 0.009 | 0.047 | 0.011 | 0.007 | 0.009 | 0.031 | 0.022 | 0.037 | 0.022 | 0.119 |
| Period | $\chi^2$ | 1.366 | 2.360 | 0.350 | 1.211 | 0.022 | 5.345 | 7.700 | 6.149 | 10.135 | 11.999 | 4.589 | 2.684 | 7.185 | 3.296 | 0.422 |
| | dfn | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | *p*-value | 0.505 | 0.307 | 0.840 | 0.546 | 0.989 | 0.069 | 0.021 | 0.046 | 0.006 | 0.002 | 0.101 | 0.261 | 0.028 | 0.192 | 0.810 |
| | dfd | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 | 193 |
| | F | 0.683 | 1.180 | 0.175 | 0.606 | 0.011 | 2.673 | 3.850 | 3.075 | 5.068 | 6.000 | 2.295 | 1.342 | 3.593 | 1.648 | 0.211 |
| | $\eta p^2$ | 0.007 | 0.012 | 0.002 | 0.006 | 0.000 | 0.027 | 0.038 | 0.031 | 0.050 | 0.059 | 0.023 | 0.014 | 0.036 | 0.017 | 0.002 |
| Laying Age | $\chi^2$ | 3.666 | 1.657 | 4.491 | 3.065 | 0.15 | 0.291 | 1.078 | 2.879 | 6.163 | 4.382 | 0.831 | 0.379 | 11.707 | 0.323 | 3.754 |
| | dfn | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | *p*-value | 0.056 | 0.198 | 0.034 | 0.08 | 0.699 | 0.590 | 0.299 | 0.09 | 0.013 | 0.036 | 0.362 | 0.538 | 0.001 | 0.57 | 0.053 |
| | dfd | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 |
| | F | 3.666 | 1.657 | 4.491 | 3.065 | 0.150 | 0.291 | 1.078 | 2.879 | 6.163 | 4.382 | 0.831 | 0.379 | 11.707 | 0.323 | 3.754 |
| | r | 0.019 | 0.008 | 0.023 | 0.016 | 0.001 | 0.001 | 0.006 | 0.015 | 0.031 | 0.022 | 0.004 | 0.002 | 0.057 | 0.002 | 0.019 |

**Table 2.** *Cont.*

| Variable | Parameter | Egg Weight | Major Diameter | Minor Diameter | Shell$^{L*}$ | Shell$^{a*}$ | Shell$^{b*}$ | White Height | Yolk Color | Yolk$^{L*}$ | Yolk$^{a*}$ | Yolk$^{b*}$ | Yolk Diameter | Shell Weight | Yolk Weight | White Weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variety | $\chi^2$ | 34.300 | 39.881 | 29.270 | 36.567 | 65.585 | 98.046 | 15.421 | 15.238 | 5.696 | 16.295 | 15.891 | 21.296 | 62.090 | 54.267 | 59.063 |
| | dfn | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | *p*-value | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.004 | 0.004 | 0.223 | 0.003 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 |
| | dfd | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 | 191 |
| | F | 8.575 | 9.970 | 7.318 | 9.142 | 16.396 | 24.512 | 3.855 | 3.810 | 1.424 | 4.074 | 3.973 | 5.324 | 15.523 | 13.567 | 14.766 |
| | $\eta p^2$ | 0.152 | 0.173 | 0.133 | 0.161 | 0.256 | 0.339 | 0.075 | 0.074 | 0.029 | 0.079 | 0.077 | 0.100 | 0.245 | 0.221 | 0.236 |
| Breed | $\chi 2$ | 22.546 | 8.765 | 21.607 | 31.945 | 49.3 | 92.019 | 11.455 | 8.597 | 0.873 | 7.845 | 10.748 | 3.502 | 55.027 | 23.651 | 29.992 |
| | dfn | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | *p*-value | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.003 | 0.350 | 0.005 | 0.001 | 0.061 | 0.000 | 0.000 | 0.000 |
| | dfd | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 | 194 |
| | F | 22.546 | 8.765 | 21.607 | 31.945 | 49.300 | 92.019 | 11.455 | 8.597 | 0.873 | 7.845 | 10.748 | 3.502 | 55.027 | 23.651 | 29.992 |
| | r | 0.104 | 0.043 | 0.100 | 0.141 | 0.203 | 0.322 | 0.056 | 0.042 | 0.004 | 0.039 | 0.052 | 0.018 | 0.221 | 0.109 | 0.134 |

$\chi^2$: Chi squared; dfn: degrees of freedom numerator; dfd: degrees of freedom denominator. $\eta p^2$ can be benchmarked against the Cohen [26] criteria of small (0.01), medium (0.09), and large (0.25) effects as suggested in Richardson [27]. In Cohen's terminology, a small effect size is one in which there is a real effect but which you can only see through careful study. By contrast, a 'large' effect size is an effect which is big enough, and/or consistent enough, that you may be able to see it 'with the naked eye'. Cohen's guidelines for *r* are that a small effect is 0.1, a medium effect is 0.3, and a large effect is 0.5.

After conducting the Mann–Whitney U test, we assessed the relationship between the factors of laying age and breed and the internal and external quality-related variables tested. Simultaneously, we used the Kruskal–Wallis H test to assess the relationship with the same variables and those factors with three or more categories or groups (k). Then, we computed the strength of the effects of these factors using $r$ and partial eta squared ($\eta p^2$) as quantification measures depending on whether Mann-Whitney U or Kruskal-Wallis H tests had been carried out beforehand (Table 2 and Supplementary Table S3). According to Fritz, et al. [28], $r$ can be calculated as an effect size for the Mann–Whitney U test using the formula:

$$r = \frac{z}{\sqrt{N}} \quad (1)$$

Cohen's guidelines [29] for $r$ are that a small effect is 0.1, a medium effect is 0.3, and a large effect is 0.5 [30]. Calculation of $r$, $r^2$, or $\eta^2$ from these z values is possible because

$$r = \frac{z}{\sqrt{N}} \text{ and } r^2 \text{ or } \eta^2 = z^2/N \quad (2)$$

The literature recommends the use of partial eta square instead of classical eta square when using a multifactor design. The reason for this is that, through the use of partial eta square, we report an index of the strength of association between an independent variable and a dependent variable that excludes the variance produced by other variables [31]. The Kruskal–Wallis H test produces $chi^2$ values with $k - 1$ degrees of freedom. We can transform $chi^2$ into an F value with $k - 1$ numerator degrees of freedom (dfn) and N-k denominator degrees of freedom (dfd) using the expression $F(dfn,dfd) = chi^2/(k - 1)$, modified from Murphy et al. [20].

In Cohen's terminology, a small effect size (0.01) is one in which there is a real effect but which you can only see through careful study. By contrast, a 'large' effect size (0.25) is an effect which is big enough, and/or consistent enough that one may be able to see it 'with the naked eye'.

As almost all the variables have been previously reported to be non-normally distributed (Table 1) (Shapiro-Wilk Francia's tests ($p < 0.001$), an independent-sample median test was carried out to assess the differences in the median between categories within the same factor (Supplementary Tables S4 and S5). Supplementary Table S6 shows descriptive statistics for external and internal egg quality-related traits in Utrerana hens compared to the laying lineage in two models, including (n = 97) and excluding (n = 194) yolk and white pH.

Afterward, we studied the pairwise comparisons for any dependent variables for which the Kruskal–Wallis test is significant, aiming at assessing whether there were statistically significant differences between groups of the same factor concerning the external and internal quality-related variables using Dunn's test (Supplementary Tables S1 and S2). Then to provide a quantifiable measure of such differences, we provide within-group (level) medians in Supplementary Table S7.

We estimated the Pearson product-moment correlation coefficient among variables from both sets using a bivariate procedure from the Correlate package of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016) [32] to avoid the severe multicollinearity or linear dependency between several variables, aiming at excluding those with multiple correlation coefficients higher than 0.80 according to Montgomery, et al. [33] (Supplementary Table S8). Canonical correlation analysis was performed to analyze the relation between the two sets of traits (internal quality and external quality) [34,35]. Therefore, it is possible to define the linear combination of the two sets of variables as [36]:

$$U_1 = a_{11}X_1 + a_{12}X_2 + \ldots + a_{1p}X_p \quad (3)$$

$$V_1 = b_{11}Y_1 + b_{12}Y_2 + \ldots + b_{1q}Y_q \quad (4)$$

Canonical variables $U_1$ and $V_1$ belong to the ith canonical pair associated with the first canonical correlation, expressed for:

$$r_i = \frac{c\hat{o}v(U_i, V_i)}{\sqrt{\hat{V}(U_i) \cdot \hat{V}(V_i)}} \quad (5)$$

The percentage of variance explained by the canonical variable $U_x^2$ and its opposite $V_y^2$ is determined by:

$$U_{x_i}^2 = \frac{\sum_{j=1}^{p} a_{ij}^2}{p} \quad (6)$$

$$V_{y_i}^2 = \frac{\sum_{j=1}^{q} b_{ij}^2}{q} \quad (7)$$

where $p$ and $q$ are the number of variables from X and Y, respectively. To check for the significance of canonical correlation, maximum likelihood ratio test was performed, considering Lambda ($\Lambda$) from Wilk's statistics, following the equations reported in Khattree and Naik [37].

All non-parametric tests were carried out using the independent samples package from the non-parametrical task of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016). Canonical correlation analysis was carried out using the Canonical correlation procedure from the Correlate package of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016).

### *2.6. Publication Ethics Statement*

All farms included in the study followed specific codes of good practices and, therefore, the animals received humane care in compliance with the national guidelines for the care and use of laboratory and farm animals in research. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki. The Spanish Ministry of Economy and Competitivity through the Royal Decree-Law 53/2013 and its credited entity the Ethics Committee of Animal Experimentation from the University of Córdoba permitted the application of the protocols present in this study as cited in the fifth section of its second article, as the animals assessed were used for credited zootechnical use. This national Decree follows the European Union Directive 2010/63/UE, from the 22 September 2010.

## 3. Results

### *3.1. Parametric Nature Assumption Testing*

The data was non-normally distributed (Shapiro Wilk's Francia W, $p < 0.001$) in all cases except for egg weight, major diameter, and white weight. Skewness statistics reported values between $-1/2$ and $1/2$, which suggested that almost all variables were approximately symmetric, except for egg weight and white weight which were moderately skewed. All variables presented a distribution with kurtosis <3 (excess kurtosis <0) or platykurtic. Compared to a normal distribution, the central peak of the data distribution is lower and broader, and its tails are shorter and thinner.

Levene's test for equality of error variance reported that the error variance around the predicted scores was not the same for all the predicted values ($p < 0.05$), except for minor diameter, thus there was no homogeneity of variances for each combination of the levels of the independent variables (species, month, year, and pathology diagnosed); hence, the assumption of homoscedasticity was violated. Mauchly's W Test of Sphericity (Mauchly's W = 0.001), $\chi^2(104) = 2985.402$, $p < 0.05$) indicated that the variances of the differences were not equal; hence, the assumption of sphericity was also violated.

### *3.2. Factor Variance Explanatory Power and Within Between-Level Differences*

The Mann–Whitney U test was used to compare differences between the two independent groups of the laying age (Laying hens and Laying pullets) and breed variables (Utrerana and Leghorn). Almost all variables showed differences when the two breeds were compared, except for white height, yolk diameter, yolk$^{L^*}$ and yolk pH. However, only minor diameter, white height, yolk$^{L^*}$, yolk$^{a^*}$,

and shell weight reported a significant difference between the different laying age groups (Table 2 and Supplementary Tables S1–S3).

The study reports the results from the Kruskal–Wallis H test for all the variables and levels considered in the study and *r* and partial eta squared ($\eta p^2$) as a measure of the strength of the factors the variables tested (Table 2 and Supplementary Table S3). Supplementary Tables S4 and S5 show the differences between the median of the categories of the factors; month of laying, laying order, controlled period, laying age, variety, and breed reported by the independent-sample median test. Supplementary Tables S1 and S2 show the results for Dunn's tests pairwise comparisons between the different levels of the factors and variables.

Dunn's test pairwise comparisons and the independent-sample median test showed the white variety of the Utrerana hen and Leghorn were not significantly different ($p > 0.05$) for all variables except for egg weight, minor diameter (breadth) and eggshell, with Leghorns reporting the highest median for all the three variables and varieties. The same tests reported a generalized significant difference between eggs from the first lay and the rest of the lays regarding egg and albumen/white weight. There were significant differences between March, April, May and June for almost all the variables measured except for shell$^{L^*}$ (between March–May and April–June), shell$^{b^*}$ (among any of the months compared), white height (between March and May themselves and between the months of March and May and April), Yolk color (March-June) and Yolk$^{L^*}$ (between June and May themselves and between the months of June and May and April). Minor diameter, yolk$^{L^*}$, yolk$^{a^*}$, and shell weight were significantly different ($p < 0.05$) when hens and pullets were compared, with hens having a significantly higher median than pullets for all the variables except for yolk$^{L^*}$.

### 3.3. External and Internal Quality-Related Variables Canonical Correlation Analysis

The results of the canonical correlation produced three significant canonical correlations, when yolk and white pH were included and four significant correlations when such factors were not considered as shown in Table 3 and Supplementary Table S9, respectively.

**Table 3.** Standardized canonical coefficients of variables, canonical correlations between two sets of variables (r), squared canonical correlation ($r^2$) and their probabilities (F) for internal and external egg quality-related traits, excluding yolk and white pH, in Utrerana hens compared to laying lineage (n = 194).

| Canonical Pairs | 1st | 2nd | 3rd | 4th | 5th | 6th |
|---|---|---|---|---|---|---|
| r ($R_c$) | 0.936 | 0.616 | 0.394 | 0.314 | 0.260 | 0.068 |
| $r^2$ $(R_c^2)$ | 0.876 | 0.379 | 0.155 | 0.099 | 0.068 | 0.05 |
| F | 12.999 | 4.071 | 2.348 | 1.856 | 1.382 | 0.214 |
| Degrees of Freedom | 54 | 40 | 28 | 18 | 10 | 4 |
| Sig. | 0.000 | 0.000 | 0.000 | 0.017 | 0.187 | 0.931 |
| Standardized canonical coefficients of external quality-related traits | | | | | | |
| Egg weight | **−0.972** | 0.139 | −0.065 | 0.240 | −0.934 | 1.888 |
| Major diameter | 0.025 | −0.345 | −0.337 | **0.575** | 0.606 | −1.464 |
| Minor diameter | −0.066 | 0.091 | **0.520** | **−0.859** | 0.597 | −1.059 |
| Shell$^{L^*}$ | −0.021 | 0.134 | −1.327 | **−0.787** | 0.763 | 0.167 |
| Shell$^{a^*}$ | −0.076 | 0.251 | −0.322 | −0.104 | −1.092 | −0.443 |
| Shell$^{b^*}$ | 0.015 | **−0.967** | **−0.929** | **−0.838** | 0.993 | 0.545 |
| Standardized canonical coefficients of internal quality-related traits | | | | | | |
| White height | −0.037 | 0.229 | −0.219 | −0.311 | −0.194 | 0.462 |
| Yolk color | −0.030 | −0.189 | 0.139 | **−0.543** | 0.224 | −1.428 |
| Yolk$^{L^*}$ | −0.010 | **0.616** | **−0.944** | **0.670** | 0.136 | −0.308 |
| Yolk$^{a^*}$ | 0.040 | 0.198 | **−1.370** | **0.449** | −0.206 | 1.537 |
| Yolk$^{b^*}$ | 0.093 | −0.031 | **0.543** | **0.479** | −0.630 | −1.367 |
| Yolk diameter | −0.026 | 0.010 | −0.09 | −0.139 | −0.089 | 0.102 |
| Shell weight | −0.305 | **0.551** | 0.188 | **−0.405** | −0.607 | −0.595 |
| Yolk weight | **−0.400** | **−0.470** | **−0.538** | −0.098 | −0.132 | 0.313 |
| White weight | **−0.702** | −0.277 | 0.306 | **0.694** | 0.446 | −0.164 |

**Bold**: Given the sample size of 194, a criterion of $\geq |0.39|$ was considered for variable loadings to be significant [38].

For the model that did not include pH values for yolk and white, the first, second and third significant canonical correlations produced Wilk's Lambda values that were found to be highly significant through the use of a chi-square test that yielded $p < 0.001$. The fourth canonical correlation also proved significant at the $p < 0.05$ level. However, only the first and second canonical correlations were highly significant ($p < 0.001$) and the third canonical correlation was significant ($p < 0.05$) when yolk and white pH were not considered. All other canonical correlations were found to be non-significant.

When we did not consider yolk and white pH, the first, second, third and fourth canonical correlations produced an r ($R_c$) of 0.936 which indicates that the four variates have a shared variance ($r^2$ or $R_c^2$) of 87.6%, respectively (Table 3 and Supplementary Table S9). The literature proposes three methods to determine the relative importance of each original variable in each function: (1) canonical weights (standardized coefficients), (2) canonical loadings (structural correlations) and (3) canonical cross-loadings. As the canonical weights, are vulnerable to multicollinearity, the use of canonical loadings or cross-loadings is recommended (Table 4 and Supplementary Table S10).

**Table 4.** Correlations between the variables and related canonical variables (canonical loadings) and between the variables and the other set of canonical variables (canonical cross-loadings) for internal and external egg quality-related traits, excluding yolk and white pH, in Utrerana hens compared to laying lineage (n = 194).

| Variable | $U_1$ | $U_2$ | $U_3$ | $U_4$ | $U_5$ | $U_6$ | $V_1$ | $V_2$ | $V_3$ | $V_4$ | $V_5$ | $V_6$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Egg weight | **−0.996** | **−0.014** | **0.003** | **0.070** | **0.046** | **0.032** | −0.932 | −0.009 | 0.001 | 0.022 | 0.012 | 0.002 |
| Major diameter | **−0.745** | **−0.211** | **−0.222** | **0.448** | **0.168** | **−0.349** | −0.697 | −0.130 | −0.088 | 0.141 | 0.044 | −0.024 |
| Minor diameter | **−0.742** | **0.060** | **0.347** | **−0.497** | **0.114** | **−0.255** | −0.695 | 0.037 | 0.136 | −0.156 | 0.030 | −0.017 |
| Shell$^{L*}$ | **−0.087** | **0.817** | **−0.471** | **−0.018** | **0.319** | **0.039** | −0.081 | 0.503 | −0.185 | −0.006 | 0.083 | 0.003 |
| Shell$^{a*}$ | **0.013** | **−0.351** | **−0.292** | **−0.362** | **−0.753** | **−0.305** | 0.012 | −0.216 | −0.115 | −0.114 | −0.196 | −0.021 |
| Shell$^{b*}$ | **0.044** | **−0.934** | **−0.029** | **−0.293** | **−0.194** | **0.031** | 0.041 | −0.575 | −0.011 | −0.092 | −0.050 | 0.002 |
| White height | −0.364 | 0.177 | 0.063 | −0.088 | −0.010 | 0.023 | **−0.389** | **0.288** | **0.160** | **−0.281** | **−0.038** | **0.344** |
| Yolk color | −0.039 | −0.260 | −0.132 | −0.122 | 0.016 | −0.034 | **−0.042** | **−0.422** | **−0.335** | **−0.389** | **0.062** | **−0.505** |
| Yolk$^{L*}$ | −0.011 | 0.372 | −0.157 | 0.066 | 0.126 | −0.006 | **−0.011** | **0.604** | **−0.399** | **0.209** | **0.486** | **−0.093** |
| Yolk$^{a*}$ | 0.038 | −0.243 | −0.091 | 0.034 | −0.149 | −0.010 | **0.040** | **−0.394** | **−0.232** | **0.109** | **−0.573** | **−0.145** |
| Yolk$^{b*}$ | 0.201 | −0.249 | 0.022 | 0.134 | −0.189 | −0.011 | **0.215** | **−0.405** | **0.057** | **0.426** | **−0.728** | **−0.156** |
| Yolk diameter | −0.288 | −0.079 | −0.028 | −0.027 | −0.050 | 0.003 | **−0.308** | **−0.129** | **−0.072** | **−0.087** | **−0.193** | **0.048** |
| Shell weight | −0.582 | 0.340 | 0.025 | −0.079 | −0.100 | −0.009 | **−0.622** | **0.553** | **0.064** | **−0.250** | **−0.384** | **−0.132** |
| Yolk weight | −0.390 | −0.313 | −0.194 | −0.044 | −0.037 | 0.003 | **−0.417** | **−0.508** | **−0.492** | **−0.139** | **−0.143** | **0.051** |
| White weight | −0.800 | −0.015 | 0.102 | 0.082 | 0.039 | −0.001 | **−0.855** | **−0.024** | **0.260** | **0.262** | **0.151** | **−0.010** |

U1, U2, U3, U4, U5, U6: canonical variates containing external quality-related traits; V1, V2, V3, V4, V5, V6: canonical variates containing internal quality-related traits. **Bold**: canonical loadings; Regular: canonical cross-loadings.

Significance was determined by using factor loading guidelines commonly found in the literature considering the sample sizes of 194 (when yolk and white pH were not considered) and 97 (when yolk and white pH had been included) [38–41]. We used both loadings and cross-loadings; however, there is no established cutoff. There is a rule of thumb that if any variable loading is $\geq |0.30|$, then it can be considered to be an important contributing variable in the function. However, this is only for explanatory studies. Hair, Black, Babin and Anderson [38] discuss the ideal case for each factor loading, i.e., the common variance should be greater than the unique one (Wilk's Lambda $\geq$0.72 in order to have a variance $\geq$0.50), but mainly for the average; that is the reason why we use the average variance attracted (AVE $\geq$ 0.50). In some cases, especially a new measure, lambda $\geq$0.5 (AVE > 0.25) can be considered to be acceptable (but we have to address the limitation of this low AVE measure). In our case, loadings $\geq |0.53|$ were used considering the sample size (n = 97) that included yolk and white pH among the variables, while the greater sample when both variables were excluded (n = 194) permitted considering loadings $\geq |0.39|$ following Hair, Black, Babin and Anderson [38] criteria.

Egg weight showed a strong negative loading on the first canonical variate of external quality-related traits. Hence the first canonical variate was given the title "external lightness", reflecting upon the negative values associated with the pool of internal quality-related variables.

In addition, only white and yolk weight were found to have significant negative loadings on the second canonical variate of internal quality-related traits. Because of this, the first canonical variate on internal quality-related traits was given the title of "internal lightness".

A negative loading means that eggs scoring high on the canonical variate will tend to score low on the variable, and vice versa. Hence, the heavier the egg, white and yolk weight is, the lower the score it will receive on external and internal lightness canonical covariates, respectively.

Shell$^{b*}$ (Shell b*, shell yellow/blue coordinate) showed a strong negative loading on the second canonical variate of external quality-related traits. Hence the second canonical variate was given the title "external yellow/blue coordinate absence", reflecting upon the negative and positive values associated with the pool of internal quality-related variables.

Interestingly, while yolk lightness (Yolk$^{L*}$, Yolk L*) and shell weight were found to have significant positive loadings on the second canonical variate of internal quality-related traits, yolk weight loading, significantly scored negatively. Because of this, the second canonical variate on internal quality-related traits was given the title of "internal brightness". This means those eggs presenting a high internal brightness present high yolk lightness and shell weight values and low yolk weight.

The minor diameter showed a moderate positive loading on the third canonical variate of external quality-related traits. By contrast, shell$^{b*}$ (Shell b*, shell yellow/blue coordinate) showed a strong negative loading on the third canonical variate of external quality-related traits. Hence the third canonical variate was given the title "egg wideness", reflecting upon the negative and positive values associated with the pool of internal quality-related variables. Eggs with a higher wideness presented lower values for the shell yellow/blue coordinate.

Yolk$^{L*}$ and Yolk$^{a*}$ color decompositions (Yolk L* or lightness and Yolk a* or red/green coordinate, respectively) and yolk weight were found to have significant moderate negative loadings on the third canonical variate of internal quality-related traits. However, yolk$^{b*}$ (Yolk b* or yellow/blue coordinate) significantly scored positively. Due to this, the third canonical variate on internal quality-related traits was given the title of "yolk dullness and yellow dominance". This means that those eggs presenting a high yolk lightness and yellowness present high values for the yolk yellow/blue coordinate and low values for the yolk lightness color coordinate, yolk red/green coordinate and yolk weight.

The minor diameter, Shell$^{L*}$ (Shell L*, shell lightness) and Shell$^{b*}$ (Shell yellow/blue coordinate) showed a high negative loading on the fourth canonical variate of external quality-related traits. By contrast, the major diameter showed a moderate positive loading on the fourth canonical variate of external quality-related traits. Hence, the fourth canonical variate was given the title "egg length and external dullness", reflecting upon the negative and positive values associated with the pool of internal quality-related variables. This means that the eggs which were longer were also duller and reported lower values for the shell yellow/blue coordinate, thus they were orangish.

The Yolk$^{L*}$, Yolk$^{a*}$ and Yolk$^{b*}$ color lightness and coordinates (Yolk L* or lightness, Yolk a* or red/green coordinate, and Yolk b* or yellow/blue coordinate, respectively) and yolk weight were found to have significant moderate positive loadings on the fourth canonical variate of internal quality-related traits with Yolk$^{L*}$ or Yolk lightness reporting the highest loading (0.670). However, Shell weight and yolk color measured with the DSM Yolk Color Fan (formerly Roche Yolk Color Fan) significantly scored negatively. Due to this, the fourth canonical variate on internal quality-related traits was given the title of "yolk yellowness and lightness, color coordinates balance, shell lightness". This means those eggs presenting high yolk lightness and scoring low in the DSM Yolk Color Fan (yellowish) present balanced values for the yolk yellow/blue and yolk red/green coordinates and low shell weights.

Table 5 and Supplementary Table S11 show the imbalance between the proportion of variance explained by each of the canonical variates of the two sets of variables (external and internal quality-related traits) and their opposites. The proportion of the variance of external quality-related variables explained by its own canonical variate (38.2% to 35.1%, when yolk and white pH were included and excluded respectively) was slightly different to the proportion of variance of internal

quality-related variables explained by opposite canonical variate (35.5% to 28.5% when yolk and white pH were included and excluded respectively). By contrast, the proportion of variance of external quality-related variables explained by its own canonical variate (15.6% to 8.0%, when yolk and white pH were included and excluded respectively) was similar to the proportion of variance of internal quality-related variables explained by opposite canonical variate (14.5% to 11.2% when yolk and white pH were included and excluded respectively).

**Table 5.** Proportion of explained variance, eigenvalues and percentages of explained common variance associated with each factor of internal and external egg quality-related trait, excluding yolk and white pH, in Utrerana hens compared to laying lineage (n = 194).

| Canonical Variable | 1st | 2nd | 3rd | 4th | 5th | 6th |
|---|---|---|---|---|---|---|
| Eigenvalue | 7.056 | 0.610 | 0.184 | 0.110 | 0.072 | 0.05 |
| Wilk's Λ Statistic | 0.054 | 0.439 | 0.707 | 0.836 | 0.928 | 0.995 |
| Proportion of variance of external quality-related variables explained by its own canonical variate $(U_e^2)$ | 0.351 | 0.308 | 0.176 | 0.154 | 0.351 | 0.308 |
| Proportion of variance of external quality-related variables explained by opposite canonical variate $(V_e^2)$ | 0.285 | 0.108 | 0.169 | 0.064 | 0.285 | 0.108 |
| Proportion of variance of internal quality-related variables explained by its own canonical variate $(U_i^2)$ | 0.080 | 0.012 | 0.075 | 0.012 | 0.080 | 0.012 |
| Proportion of variance of external quality-related variables explained by opposite canonical variate $(V_i^2)$ | 0.112 | 0.011 | 0.069 | 0.007 | 0.112 | 0.011 |

## 4. Discussion

The demand for products deriving from non-industrial production systems has triggered and increased the interest in more sustainable farming practices, enabling the introduction of products stemming from native breeds in the common production systems and commercial chains [14]. This context lays the basis for the characterization of the quality of differentiated products linked to sustainable production involving autochthonous breeds. The differences in the values obtained in this study for egg quality-related parameters may promote the definition of products depending on which and at which level egg components are present across the different varieties and breeds studied. For instance, eggs with greater egg yolk proportions may make for richer and softer baked final products and better quality pasta, while egg whites provide the resulting products with lighter and airier textures and are richer in lysozyme [41], which is currently, the only lysozyme industrially applied for food applications.

Among external egg quality-related traits, the Leghorn hen breed's eggs were heavier than those from the Utrerana hen breed, due to the higher weight of their shell and white. By contrast, although the Franciscan variety presented eggs with lower weight, the eggs of the Utrerana breed generally presented similar or heavier weights than other Spanish breeds [42–44].

The eggs of laying pullets presented a significantly lower weight than the eggs of laying hens. In addition, egg weight was observed to increase with the age of the flock hens (in consecutive months), except for March, when a higher weight of eggs was observed in the flock than that reported in April or May. The fact that first laying hens had not yet started laying eggs in March may be one of the main reasons for this finding. These results are supported by other studies in which hens of different laying periods were compared [45–47]. Some authors report a simultaneous increase in egg weight while there is a decrease in shell weight, which may be attributable to such parameters being conditioned by the weight of egg components (yolk and albumen). Simultaneously, egg weight has been reported to increase as the age of hens increases, while eggshell quality deteriorates, which translates in greater quality larger chicks [46].

The major diameter of the eggs was often related to the weight of the eggs. As results showed, the Leghorn eggs had significantly longer major diameters and minor diameters. However, the partridge variety reached the same major diameter as the Leghorn eggs. In addition, as previously described by Saatci et al. [46], a smaller size of the eggs of the first laying hens was observed. Variety or plumage color has been reported to significantly affect egg weight in other local bird breeds such as in Native Turkish Geese ($p < 0.05$). However, such differences were not observed regarding shape index ($p > 0.05$), or length or breadth (parameters involved in the calculation of shape index) as opposed to our results [45].

Another important characteristic of the commercialization of the product is the eggshell color profile that represents an important trait for consumer's perception. Almost equal numbers of brown and white eggs are sold in the markets of some countries such as Spain, Germany, and Holland [48]. In the present study, a significant increase in lightness (Shell$^{L^*}$ values) on Leghorn eggs regarding Utrerana breed was observed. However, in terms of redness (Shell$^{a^*}$ values) and yellowness (Shell$^{b^*}$ values), the Utrerana breed showed higher values. These results could be due to a large amount of genetic variation for eggshell characteristics [49].

An increased value of shell$^{a^*}$ was observed in the Franciscan variety, suggesting the hybridization with the Plymouth Rock breed (a breed with barred feathers and brown eggs), which was carried out while aiming at defining the barred feather characteristic in the Utrerana, also added to the appearance of the undesirable characteristic of darker shell eggs. No significant differences were observed to shell color between the white variety of Utrerana and Leghorn. When Shell$^{L^*}$ value was considered to measure for eggshell lightness [50], both the white Utrerana variety and Leghorn breed reported the brightest shell tone of all remaining varieties studies.

According to other authors, the month of laying did not have a significant effect on shell weight; although egg size increases with the hen's age, the shell weight mantains values around the same range [45,51,52]. Heat stress reduces the shell thickness and the shell quality in laying hens [53,54]. However, the Utrerana eggshell weight showed no significant differences in all the studied months. It is well known that the south of Spain is influenced by Mediterranean weather—maximum temperatures of 40 °C were reached in June 2018 in Cordoba, as reported by the State Meteorological Agency (AEMET) from Spain, with very high temperatures since late spring and summer. Taking into account that this study occurred during this period, these results suggest that the Utrerana breed tolerates high temperature-induced stress, so this might be an interesting alternative to commercial production systems with fewer adapted animals.

The Utrerana breed showed a lower eggshell weight in comparison with the Leghorn breed. Modern commercial birds showed clear differences in terms of shell weights in regard to traditional breeds [49,55]. Nevertheless, the selection of breeds for one characteristic such as egg weight can affect others such as the quality of the eggshell [56]. By contrast, Sreenivas et al. [57] suggested that Leghorn eggshell contributes a lower proportion to overall egg weight when compared to native poultry.

Some authors have reported the characteristics of the egg white to be conditioned by the strain of bird and genetic selection [58–60]. In this study, the Leghorn white weight was significantly heavier than those of the Utrerana varieties. In the Franciscan variety, the white weight was significantly lower than in the rest of the varieties. This could explain the lower weight of the eggs of this variety. No significant differences were observed in white weight in all the studied months, neither between the laying pullets, nor the laying hens. However, there were significant differences between June and the rest of the months, which suggests that the white height reduces as age increases, supported by the findings of Renden et al. [61].

Although the yolk diameter did not differ between breeds, the yolk weight was significantly higher in the Utrerana breed. The selection of the modern lines of laying hens induced an increase in egg weight, which translated into a simultaneous decrease in the energy content of the egg as a direct consequence of a decrease in the percentage of egg yolk. The egg white contains a larger amount of water than the yolk which results in heavier eggs. This greater contribution to egg weight is produced

at a lower energetic cost as its synthesis is energetically more efficient, on a weight for weight basis, than deposition of yolk which contains proportionally 0.5 of solids with equal proportions of fat and protein [24,49].

At the same time, as consumers begin to demand and consider egg energy as a quality criterion, egg selection for a higher percentage of yolk will be necessary [62]. The yolk weight of the partridge and Franciscan varieties was significantly higher than the rest of the varieties or even the Leghorn breed, which may make them profitable alternatives.

An important aspect for the commercialization of the eggs is yolk color as European consumers tend to prefer darker ones, given the psychological misattribution of a healthier origin [14]. The strain of the laying hen has been suggested to determine egg pigmentation [63]. In addition, the yolk darkness is determined by the Yolk$^{a^*}$ value [50]. In the present study, yolk$^{a^*}$ and yolk$^{b^*}$ values were observed to be higher in Utrerana eggs than in Leghorn ones. On the other hand, in April and May, the yolk$^{a^*}$ value decreased, in comparison to what happened in March and June. This finding suggests that yolk$^{a^*}$ value, which accounts for the darker yolk in Utrerana, significantly decreased when the laying of the hen was higher. This suggests that the reduction in the yolk$^{a^*}$ color coordinate could be a consequence of the dilution effect originated by the increase in egg production [49].

A higher Yolk$^{a^*}$ value was found for the Franciscan variety, which could be linked to the higher Shell$^{a^*}$ value observed for the same variety. However, Aygun [50] reported that there was no significant correlation between eggshell color and that of the yolk. Besides, no significant differences were detected between the color of the eggshell and yolk color between white variety and Leghorn, suggesting that there was a great resemblance in the egg color of the two breeds when their plumage was white.

White quality is affected by the age of the laying hens, the strain of the birds and the storage time of the eggs [58]. The Leghorn hen's eggs showed higher values of white height than the Utrerana breed's eggs. The Leghorn breed laid heavier eggs with a higher proportion of white too. These results could suggest that the white height is correlated with the percentage of white, in accordance with similar observations in earlier reports [57]. When months were compared, the white height presented lower values in June, when the temperature increased. During the storage of eggs, a decrease in white height at higher temperatures has been reported by Keener et al. [64].

The Utrerana breed's eggs reported higher white pH values in comparison to the Leghorn breed's eggs. The white pH has been suggested to increase as $CO_2$ decreases inside the egg. Factors related to this loss of $CO_2$ such as the time of storage and high temperatures have been suggested to promote such a pH increase and a subsequent decrease in white viscosity and flavor, hence directly depreciating egg quality [47].

According to our results for the canonical correlation analysis (Table 5 and Supplementary Table S11), the higher the value an egg scores on the external lightness variable of egg weight, the more likely this egg will also score a higher value on internal lightness variables such as white and yolk weight, as it was also reported for the same phenotypical correlations in Japanese quails, exotic Isa brown layers and naked neck, normal and dwarf strains of Tswana chickens [65–67].

Similarly, those scoring a low value on the external yellow/blue coordinate are more likely to report higher values for internal brightness. Those eggs presenting high egg wideness presented higher yolk dullness and yolk yellow dominance. By contrast, the longer and duller the egg was externally, the more yellowish and lighter it was, the more balanced their color coordinates and the less heavy their shells. These results contradict some previous studies in which a weaker statistical analysis is performed [50,68], and in which it is stated that the external color of the egg is not related to its internal color. Given our results, the Utrerana breed eggs allow consumers to associate the external appearance of the egg with their internal characteristics. The relationship between the outer shape of the egg and the internal color of the egg may suggest that there could be a dilution effect of pigments depending on the shape and size of the egg as reported by other studies [49].

The moderately high values for the proportion of variance of external quality-related variables explained by its own and opposite canonical variable, which doubles the explanatory power of variance

of the internal quality-related traits set, suggest external quality-related traits may have a remarkably 2-fold higher predictive power of internal quality-related traits than vice versa. Interestingly, the reduction in the proportion of variance of internal quality-related variables, explained by its own canonical variate from 15.6 to 8.0% when pH was excluded, suggests these variables (yolk and white pH) may be relevant traits to consider for the determination of internal egg quality. Furthermore, external egg quality-related traits may act as better predictors of internal quality-related traits, which is desirable as it permits not having to break the eggs to classify them, relying on their internal quality to enable the implementation of an effective noninvasive method for internal quality determination.

## 5. Conclusions

Involving autochthonous breeds in common production systems and commercial chains seeking the characterization of the quality of differentiated products could be the key to future poultry sustainable productions. Leghorn eggs are heavier than those from Utrerana hens; however, these generally presented similar or heavier weights than other Spanish breeds. There is a simultaneous increase in egg weight and a decrease in shell weight, which may be conditioned by the weight of egg components (yolk and albumen). Egg weight increases with age, while eggshell quality deteriorates. The variety or plumage color affects egg weight and egg length or breadth. Utrerana hybridization with the Plymouth Rock breed (a breed with barred feathers and brown eggs) added to the appearance of the undesirable characteristic of darker shell eggs, while the possible hybridization between the white Utrerana variety and Leghorn breed may account for the increased values for shell brightness reported. The Utrerana breed may tolerate high temperature-induced stress better than the Leghorn breed, so this might be an interesting alternative to commercial production systems with fewer adapted animals. The Leghorn breed's white weight was significantly heavier than those of the Utrerana varieties. The white height reduces as age increases. The modern line selection of laying hens has induced an increase in egg weight, which translates into a simultaneous decrease in the energy content of the egg, as a direct consequence of a decrease in the percentage of egg yolk. As white pH increases, $CO_2$ content decreases inside the egg. Simultaneous to this decrease, there is a subsequent decrease in white viscosity and flavor, which directly depreciates egg quality. The canonical correlation analysis addresses the possibility to develop a tool comprising external indicators that may indirectly report information on certain determinants of the internal quality of these eggs. This could mean a great advancement in the identification and typification of specific products, which may cover the currently increasing demand from markets for non-conventional quality products linked to specific breeds or production systems, or even settle new commercialization niches linked to local defined traceable products.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2076-2615/9/4/153/s1. Table S1: Summary of the significant ($p < 0.05$) pairwise differences (Green) obtained after Dunn's test for the levels of the factors of month, order, period, and variety and Mann–Whitney U Test ($p < 0.05$) differences between groups (Green) on internal and external egg quality-related traits, excluding yolk and white pH, in Utrerana hens compared to laying lineage (n = 194); Table S2; Summary of the significant ($p < 0.05$) pairwise differences (Green) obtained after Dunn's test for the levels of the factors of month, order, period, and variety and Mann–Whitney U Test ($p < 0.05$) differences between groups (Green) on internal and external egg quality-related traits including yolk and white pH in Utrerana hens compared to laying lineage (n = 97); Table S3: Summary of the results for the Kruskal–Wallis H test and the determinative coefficient through r or partial eta squared ($\eta p^2$), for fixed effects for internal and external egg quality traits from the model including yolk and white pH in Utrerana hens (n = 97); Table S4: Summary of the results of the independent sample median test of the factors month, order, period, laying age, variety and breed on internal and external egg quality-related traits, excluding yolk and white pH, in Utrerana hens compared to laying lineage (n = 194); Table S5: Summary of the results of the independent sample median test of the factors month, order, period, laying age, variety and breed on internal and external egg quality-related traits including yolk and white pH in Utrerana hens compared to laying lineage (n = 97); Table S6: Descriptive statistics for external and internal egg quality-related traits in Utrerana hens compared to laying lineage in two models, including (n = 97) and excluding (n = 194) yolk and white pH; Table S7: Median for external and internal egg quality-related traits in Utrerana hens compared to laying lineage in two models, including (n = 97) and excluding (n = 194) yolk and white pH for each of the levels of the factors of month of laying, laying order, controlled period, laying age within study, variety and breed (the greener the higher, the redder the lower);

Table S8: Pearson product-moment correlation coefficient between external and internal egg quality-related traits in Utrerana hens compared to laying lineage in two models, including (n = 97) and excluding (n = 194) yolk and white pH; Table S9: Standardized canonical coefficients of variables, canonical correlations between two sets of variables (r), squared canonical correlation (r2) and their probabilities (F) for internal and external egg quality-related traits including yolk and white pH in Utrerana hens compared to laying lineage (n = 97); Table S10: Correlations between the variables and related canonical variables (canonical loadings) and between the variables and the other set of canonical variables (canonical cross-loadings) for internal and external egg quality-related traits including yolk and white pH in Utrerana hens compared to laying lineage (n = 97); Table S11: Proportion of explained variance, eigenvalues and percentages of explained common variance associated with each factor of internal and external egg quality-related traits including yolk and white pH in Utrerana hens compared to laying lineage (n = 97).

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Original Research Article

# Hen breed and variety factors as a source of variability for the chemical composition of eggs

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ABSTRACT

Eggshell, white and yolk chemical composition was compared across Utrerana breed varieties (black, partridge and franciscan) and Leghorn Lohmann LSL-Classic lineage. An Utrerana hens flock (n = 51) and a control group of Leghorn hens (n = 17) were housed individually allowing egg identification and quality characteristics assessment. Eggshell, yolk and white macroelements and microelements, carbohydrates, moisture, ashes, protein, fat (polyunsaturated and saturated), sugars, cholesterol, and α-tocopherol contents were analyzed. Simultaneously, itemized yolk fatty acids composition was evaluated. Calcium contents were higher in Utrerana eggshell (358.53 g/kg vs. 337.01 g/kg) and white (593.75 mg/kg vs. 584.31 mg/kg), while protein contents were higher in Utrerana yolk (17.40 % vs. 16.90 %) and white (10.60 % vs 10.30 %). Utrerana yolks reported higher α-tocopherol (102.00 mg vs. 88.00 mg), total polyunsaturated fatty acids (19.80 % vs. 16.60 %), and some monounsaturated fatty acids content (C18:1 n9: 42.68 % vs. 41.31 % ; C16:1 n9: 0.60 % vs. 0.50 %). Black variety and Leghorns reported higher linoleic acid contents (13.72 % and 13.27 %, respectively) than the rest of the Utrerana varieties. Conclusively, detailed knowledge on differentiated properties of eggs depending on the animals originating them enables a correctly approaching market needs to improve the profitability and find a competitive niche for local products.

## 1. Introduction

Since its creation during the first half of the 20th century, Utrerana avian breed has been productively oriented towards a laying aptitude. Utrerana's eggs weight around 64 g and present a characteristic white eggshell color. The breed's annual egg output ranges from 120 to 180 eggs. Despite its traditional link to egg production, the use of surplus males for meat production has also provided the breed with a collateral dual purpose nature (Campo, 2007). This breed is characterized by a high environmental adaptability to the conditions of the extensive family farms found in the Andalusian countryside from southern Spain (Campo, 2007).

Not only the four varieties of Utrerana breed present particular features in regards the colour of their feathers, tarsus, and skin (namely, partridge, franciscan, black and white), but also, the eggs from Utrerana physically and organoleptically differ among varieties and from those of commercial laying lineages (González Ariza et al., 2019a, c).

Avian eggs represent an important source of nutrients which contains all the proteins, lipids, minerals, and vitamins that enable the development of the embryo. Some of these constituents, such as enzymes or immune proteins, may have multibiological functions (Nowaczewski et al., 2013). As a result, egg is present in a great fraction of the human diet across the different cultures in the world. This added value of eggs is supplemented by its wide cuisine applicability as a product. They can be used for breakfast or home meal preparations, baking and as an ingredient in many culinary recipes.

In this context, the emergence of commercial hybrid lines of laying hens, brought about an increase in the productive capacity of egg and meal from the middle of the last century. As a result, local genotypes were replaced and with them the genetic diversity that such resources implied (Cardellino, 2003). Commercial hybrid genotypes present homogeneous product characteristics that may no longer fulfil market demands considering the need of customers for new products. Under this framework, some authors have addressed that differences among native

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breeds and varieties may promote the increase and diversification of egg cuisine opportunities, at the same time that they open new commercial niches, as it has been reported for the Utrerana breed (González Ariza et al., 2019a).

Despite the dual-purpose of some of these native breeds, which decreases their productive performance in comparison to egg or meat specialized breeds (Castellini et al., 2010), they are usually more resistant to pathologies, thermal stress, environmental conditions and have a great ability to search for food in the wild (Lordelo et al., 2017; Palacios et al., 2016). Additionally, the comparatively reduced productivity of these animals may be counteracted by the greater quality of their products, which helps widening the variety of foods offered to the market.

As a way to better respond to the necessities of the market, recent research has focused on scientifically proving the superior organoleptic and sensorial attributes of Utrerana breed eggs when compared to the eggs of a laying lineage among cuisine professionals (González Ariza et al., 2019a). Such attributes may base on the difference in the chemical composition between eggs produced by different strains.

Conservation of animal genetic resources cannot be understood without considering these animals as productive units within a sustainable framework (Lordelo et al., 2020). With this in mind, biodiversity conservation plays a pivotal role as it may allow the conservation of animal genetic resources and the maintenance of local populations in the rural areas where they originated through the promotion of their products (Barba et al., 2016). Farm animal sustainability and welfare are intertwined concepts which increasingly concern a greater part of society every day. Therefore, alternative forms of livestock, such as free range and organic farms become necessary (Taylor et al., 2017).

Seeking an increased productivity, commercial genotypes are usually produced under free range and organic production systems, as barn systems. However, these genotypes might have a lower ability to adapt to these organic and free-range systems than local breeds, which in turn translates into a decrease in profitability and lower survival and resistance to animal diseases (Alderson, 2018). Oppositely, local breeds, and among them, the Utrerana hen, are perfectly adapted to thrive under the conditions of these aforementioned production systems.

The conditioning effect of nutrition has been reported to be more influential than genetics in regards to the chemical composition of eggs. However, there are some relevant pieces of evidence for a heredity influence on some aspects of egg composition; namely, relative proportion of yolk and white, white quality, qualitative protein polymorphism, total protein content, contents of cholesterol, vitamin A, thiamine, riboflavin, fatty acids, enzymes and deposition of metabolic products in the eggs (Washburn, 1979).

Another important aspect regarding the chemical composition of the egg of laying hens is the composition of heavy metals in eggs, which usually derives from the fishmeal that is supplied to animals in their diets (Farahani et al., 2015). Feed provided to laying hens may often be contaminated with mercury or arsenic, among others. However, the ability of these animals to metabolize such substances, and the heritable component of this ability may become relevant, as they may translate into the reduction of the accumulation of residues in the final products that reach the market, which minimizes the potential harms to humans (Ding et al., 2019).

Egg components are not only quantitatively conditioned by genetic factors, but can also be qualitatively affected. Certain egg quality parameters, such as the proportion of yolk and albumen and albumen quality have been previously studied in Utrerana hens given their implications with the final value of the products (González Ariza et al., 2019c). Contextually, chemical composition concerning the metabolism and concentration of diverse elements of nutritive interest in laying hens, like fatty acid composition have been reported to be affected by other factors such as age, genotype and environmental (Rizzi and Chiericato, 2010). Furthermore, apart from the influence of genetic factors themselves, an interaction between strain and environmental conditions has been reported in literature and has been suggested to condition the absorption and use of dietary components by laying hens. This genetically dependent interaction may play a decisive role in the determination of the ability to incorporate some diet nutrients like fatty acids to the products to which they give origin (Rizzi and Chiericato, 2010).

Among other important nutritional resources that can be conditioned by heritable components, albumen quality widely reflects the variation in protein polymorphism, which have been shown to present high heritability values (Washburn, 1979). This high heritabilities contribute to the greater possibilities to perform effective selection strategies based on a wider genetic variability among individuals.

To this aim, the first goal of this study was to compare the chemical composition (carbohydrates, moisture, raw ashes, crude protein, crude fat, polyunsaturated fatty acid (PUFA), saturated fatty acid (SFA), sugars, cholesterol, and vitamin E) of the yolk and the white of eggs produced by hybrid commercial strain hens to those of Utrerana hen breed reared under the same production system. Secondly, we assessed the ability of these hen breeds to metabolize heavy metals and to accumulate them in different parts of the egg (yolk, white and eggshell). Finally, we determined the concentration of fatty acids that deposits in the yolk of the three varieties of the Utrerana avian breed (black, partridge, and franciscan) in comparison to those from the eggs produced by a Leghorn Lohmann LSL-Classic lineage (Leghorn further on the text).

## 2. Material and methods

### 2.1. Institutional animal care and use committee statement

Animals were housed following specific codes of good practices and therefore, the receiving humane care in compliance with the national guide for the care and use of laboratory and farm animals in research. The study was conducted in accordance with the Declaration of Helsinki. The Spanish Ministry of Economy and Competitivity through the Royal Decree Law 53/2013 and its credited institution the Ethics Committee of Animal Experimentation from the University of Córdoba permitted the application of the protocols present in this study as cited in the 5$^{th}$ section of its 2$^{nd}$ article provided the animals considered in the study were used for credited zootechnical use. This national Decree follows the European Union Directive 2010/63/UE, from the 22$^{nd}$ of September of 2010.

### 2.2. Layer flock and environmental conditions

The experiment was carried out in the public hatchery located at the Agropecuary Provincial Centre of Diputación of Córdoba, Spain (37°54'50.9"N-4°42'40.4"W). The eggs used in the study were obtained from a layer flock of 51 autochthonous Utrerana hens and 17 Leghorn Lohmann LSL-Classic lineage hens at 70 weeks of age. The breeding flock comprised 17 hens of each Utrerana variety (black, franciscan and partridge).

The layer flock, from which the eggs were collected, was reared in individual cages (50 × 62 × 41 cm) following Council Directive 1999/74/EC of 19 July 1999, laying down minimum standards for the protection of laying hens at the Agropecuary Provincial Centre of the Council Office of Córdoba (Spain), for 6 months (January to June 2018). All the animals were fed on the same commercial feed (15.2 % crude protein, 4.1 % calcium, 0.66 % available phosphorus) for the whole experimental period. Table S1 describes the composition of the feed supplied to the animals. Feed and water were available *ad libitum*. The birds were subjected to the same prophylaxis procedures (Marek's disease, Newcastle disease, Gumboro disease, infectious bronchitis, coccidiosis, and fowl pox vaccines, Table S2) and rearing conditions (temperature and photoperiod) until the end of the experimental period.

All the birds were reared according to the regulations of the European Union (2010/63/EU) in their transposition to the Spanish law (RD

53/2013). Further information regarding the rearing system used can be consulted in González Ariza et al. (2019c).

### 2.3. Egg pool

A total of 5090 eggs (3678 egg from Utrerana hens and 1412 from Leghorn Lohmann LSL-Classic hens) were collected in three different phases of the hen laying cycle (at the beginning (25–36 weeks of hen age), in the middle (47–52 weeks) and at the end (70–73 weeks), respectively). This was performed following the premises described in Campo (2007), with the aim to perform a complete sampling of the different stages of the laying curve as maturity in this breeds is reached around 25 weeks of age.

Whole eggs and shell, albumen and yolk were individually weighted. Albumen height was measured as referred in González Ariza et al. (2019c). All eggs collected were analyzed and the outputs derived from chemical analyses were considered for the statistical analysis. At 52 weeks of age, a total of 48 eggs (36 egg from Utrerana hens; 12 per variety and 12 from Leghorn Lohmann LSL-Classic hens) were collected per group during the production period going from March to June. Defective eggs (double yolk and/or abnormal shell) were excluded.

The yolk was manually separated from the albumen 3 times (by means of a yolk/white separator composed of the top cup designed to retain the yolk while letting the albumen slide to the bottom cup) to obtain 12 samples per Utrerana variety and 12 from Leghorn Lohmann LSL-Classic. Samples were frozen at −20 °C up to 20 days for further laboratory analyses as described in Rizzi and Chiericato (2010).

### 2.4. Basic chemical composition

The moisture, ash, carbohydrates, sugars, protein, lipid and α-tocopherol contents in albumen and yolk were quantified. Egg samples were separately homogenized by a laboratory blender (Moulinex A327, France). Carbohydrate content was computed from total sum of moisture, fat, protein, ash percentage and subtracted from 100. Moisture was determined by drying the samples in a conventional oven at 98 °C for 24 h according to AOAC (2000). The ash content was analyzed by ashing the samples using a muffle furnace oven at 525 °C for 12 h (Hui and Sherkat, 2005). The total fat content in the albumen and yolk was assessed using $CO_2$ in the supercritical state (TFE-2000 analyzer, LECO, USA) (Domagała et al., 2010). In order to assess protein content, the nitrogen amount was determined by the Dumas method, by means of the TruSpec N LECO Company Analyzer. Values of N% were multiplied by 6.25 to calculate the protein percentage.

The sugar composition in eggs was measured using Megazyme assay kits (Megazyme International Ireland Ltd, Wicklow, Ireland). Albumen and yolk were homogeneized separately (Vitamix, Olmsted Township, Ohio). The amount of individual sugars in the egg samples was determined by reading the absorbance of sample solutions on a 96-well plate at 340 nm using a microplate reader (SpectraMax 190; Molecular Devices, Sunnyvale, California), and calculation of their concentration was performed using formulas described in the kit procedures. Samples were weighed and values normalized per 100 g weight. α-tocopherol analysis was conducted by saponification and liquid extraction of organic solvents with HPLC-Fluorescence detection.

As part of the quality control of the above methods, Certified Reference Materials were tested. The laboratory competences in all analytical methods were confirmed in interlaboratory/international test.

### 2.5. Multielemental composition

The elements analyzed were aluminum (Al), arsenic (As), boron (B), calcium (Ca), cadmium (Renden et al., 1984), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Farahani et al.), iodine (I), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), selenium (Se) and zinc (Zn). Approximately 0.2–0.5 g of sample were digested in screw-cap Teflon bombs with concentrated high purity nitric acid. Bombs were heated from 2 to 8 h and opened three times to release $CO_2$ buildup. Most elements in the digestate were analyzed with a Perkin- Elmer, model ELAN 5000, inductively coupled plasma spectrometer. As, Se, Pb, and Cd were analyzed with a Varian VGA-76 hydride generation accessory mounted to an atomic absorption spectrophotometer, AA Perkin-Elmer, model 3030. Hg was analyzed by the standard cold vapor atomic absorption method. The lowest detection limits were calculated by a standard procedure which is based on the analysis of seven samples of the matrix with the analyte. Percent recoveries of spiked samples and certified reference materials were above 90 % in most cases. Mean relative percent differences between duplicates were <10 %.

### 2.6. Fatty acid composition

Total egg yolk lipids were extracted using the FAT Extractor TFE 2000 Leco, St. Joshep USA, with liquid carbon dioxide as a solvent. After the extraction of fat, the lipids were esterified according to the method described by De Man (1964). 0.1 mL of extracted fat was placed in the glass test tube of 2 mL capacity, and 0.5 mL of 0.025 M solution of sodium methylate was added. The mixture was heated in a closed tube at 60 °C until the mixture was clear. The analysis of fatty acids was carried out using gas chromatograph Trace GC Ultra (Thermo Electron Corporation, USA) with a Supelcowax 10 column (dimensions 30 m x 0.25 mm x 0.25 mm). Helium as a gaseous phase was applied with the flow rate of 5 mL/min. The feeder was set at the temperature of 220 °C. The temperature of the column was kept for 3 min at 60 °C, then increased at a rate of 7 °C/min up to 200 °C and held at this temperature for 20 min. The detector had the temperature of 250 °C and the split flow was 10 mL/min. (Kostogrys et al., 2017). The fatty acid percentage was calculated with respect to the total fatty acid mass expressed as total area. Each fatty acid was identified in the form of a methyl ester by comparing the retention times with the standard sample (F.A.M.E. mix C4-C24, lipid standard, 18919-1AMP, Sigma-Aldrich, St. Louis, MO).

### 2.7. Cholesterol content

Cholesterol content in yolk was determined following the method described in Kostogrys et al. (2017). Cholesterol was extracted from the egg yolks by hexane after saponification by 60 % potassium hydroxide. The extracts were analyzed using a gas chromatograph equipped with a FID detector (Model GC-2010, Shimadzu Corporation, Kyoto, Japan FID: 300 °C). A non-polar column was used for the analysis of cholesterol (Zebron ZB-5, l = 30 m, I.D. = 0.25, df = 0.25). The injector and detector temperatures were set at 300 °C, split 1:10. The internal standard (5a-Cholestan) was used. Helium was used as a carrier gas at a flow rate of 1.9 mL/min, volume of sample 5 μL. Analysis time was 30 min.

### 2.8. Statistical analysis: egg pool descriptive statistics

For eggshell, the components analyzed were as follows, Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Na, Ni, P, Pb, S, Se and Zn. Apart from the components that were previously analyzed for the eggshell, carbohydrates %, moisture (4 h a 103 °C) %, raw ashes (out of fresh matter) %, raw protein (out of fresh matter) %, fat raw (out of fresh matter) %, polyunsaturated fat (PUFA) %, saturated fats %, sugars mg/kg, cholesterol (mg) and vitamin E (α-tocopherol) (mg) were also determined for the yolk and white. Breed (Utrerana and Leghorn Lohmann LSL-Classic lineage) and varieties (black, franciscan and partridge) were considered independent categorical factors.

Although, separate pools for Utrerana varieties were not analyzed for general chemical composition (given the costs involved), fatty acid detailed profile was computed using pools for each variety as follows.

To determine and quantify the effect of the breed factor (Utrerana vs

Leghorn) on the chemical composition of eggs through pooled sampling, we opted for the implementation of pooled-variance t procedures provided their robustness to violations of the normality assumption than their one-sample counterparts. Pooled variances were computed after squaring pooled standard deviations ($s_p$) as follows (McNaught and Wilkinson, 1997):

$$S_p = \sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2 + \ldots + (n_k-1)s_k^2}{n_1+n_2+\ldots+n_k-k}}$$

The subindexes *1, 2, …, k* refer to the different series of measurements. In pooled sampling conditions, a single underlying standard deviation ($\sigma$) is assumed to exist across groups. In this context, pooled standard deviation $s_p$ is a better estimate than the individual calculated standard deviations $s_1$, $s_2$, …, $s_k$. If *k* sets of duplicate measurements are available, the above equation reduces to (McNaught and Wilkinson, 1997),

$$S_p = \sqrt{\frac{\sum(x_{i1}-x_{i2})}{2k}}$$

Afterward, Cohen's d effect size (Cohen, 1977) can be calculated as follows, $d = \frac{x_1-x_2}{s_p}$. Cohen's d works best for larger sample sizes (> 50). A correction factor is available, which reduces effect sizes for small samples as follows, $d = \frac{x_1-x_2}{s_p}\left(\frac{n-3}{n-2.25}\right)\sqrt{\frac{n-2}{n}}$. A d of 1 indicates the two groups differ by 1 standard deviation, a d of 2 indicates they differ by 2 standard deviations, and so on. Standard deviations are equivalent to z-scores (1 standard deviation = 1 z-score). To interpret Cohen's d, "rule of thumb" guidelines should be used cautiously. Ranges found in literature state that a value for Cohen's d of 0.20 to 0.25 should be considered a small effect, a value of 0.5 to 0.75 should be considered a medium effect and a value of 0.8 or above should be considered a large effect. By "small" effects we consider those effects which are difficult to see with the naked eye, "medium" effects may probably be big enough to be discerned with the naked eye, while effects that are "large" can definitely be seen with the naked eye (Cohen calls this "grossly perceptible and therefore large"). Oppositely, values for Cohen's d below 0.2 may denote trivial effects, even if statistically significant differences have been found. A "large" effect is not necessarily better than a "small" effect, especially in settings where small differences can have a major impact. Hence, prior research should be consulted for an idea of where our findings fit into the bigger context.

In small sample contexts, the bias occurring is slightly smaller for Hedges' g alternative method, which uses n-1 for each sample as follows, $g = \frac{d}{\sqrt{\frac{n}{df}}}$, where; n is sample size and df are the degrees of freedom. Additionally, d could also be transformed into *r* correlation coefficient through the following formula, $r = \frac{d}{\sqrt{d^2+4}}$.

Remarkably, Cohen's d is not influenced by the ratio of $n_1$ to $n_2$, but $r_{pb}$ and eta-squared are, hence Cohen's d may be preferable. The magnitude of effect power should be considered bearing the seriousness of Type II to Type I errors in mind. For $P < 0.05$, r are frequently characterized as small (r = 0.10), medium (r = 0.30), and large (r = 0.50) as described by Cohen (1977).

At a laboratory level, even small effects could be detected, as error terms are often attempted to be kept at very small levels. However, the common conditions simultaneously dealt with when a small effect is detected, such as the existence of a small sample, and a binary decisional $P < 0.05$ makes interpreting such small effects difficult. As stated above, Cohen (1977) recommended a value of 0.8 as a convention for the desirable level of power. With a "small" effect (i.e., r = 0.10, d = 0.20), a power of 0.8 would require us to employ a total N of approximately 1000 to detect various effects at p = 0.05, two-tailed (Cohen, 1977). With a "medium" effect (i.e., r – 0.30, d -0.63), it would mean a total N of approximately 115 sampling units, and with a "large" effect (i.e., r -0.50, d = 1.15) a total N of approximately 40 sampling units, to detect various effects at p = 0.05, two-tailed.

### 2.9. Effect power of breed and variety factors on shell, yolk and white chemical composition

The effect power of the factors of breed and variety were also computed following the premises developed by Onderdeel van Slim Academy. According to these guidelines, we must first find out the value of $\bar{x}$ under the wrong null hypothesis to figure out when $H_0$ is rejected under the wrong null hypothesis. Then, according to the following equation, $z = \frac{\overline{x_1}-\mu_2}{\frac{\sigma}{\sqrt{n}}}$, we may possibly calculate the correct probability of rejecting H0 (Ha = $\mu_2$), as follows; $PHa = (\bar{x} > \overline{x_1}) = PHa = \left(\frac{\bar{x}-\mu_2}{\frac{\sigma}{\sqrt{n}}}\right) > \left(\frac{\overline{x_1}-\mu_2}{\frac{\sigma}{\sqrt{n}}}\right)$, solving the equation considering the area under a normally distributed curve is 1, as follows $PHa = \left(Z > \left(\frac{\overline{x_1}-\mu_2}{\frac{\sigma}{\sqrt{n}}}\right)\right) = 1-\alpha_Z$.

At this point, we may be able to determine rejection region for two-tailed Z Test ($H_1$: $\mu \neq \mu_0$) with $\alpha = 0.05$. The decision rule to make is, we either reject $H_0$, if $Z \leq$ -1.960 or $Z \geq 1.960$ and consider mean from first group to be higher than mean from second group, or we accept $H_0$ when $-1.960 \leq Z \leq 1.960$, and determine both means are statistically equal.

### 2.10. Effect power of breed factor on fatty acid detailed composition

The second section of the study aimed at assessing the effects of breed and variety (Utrerana and Leghorn Lohmann LSL-Classic lineage) on fatty acid detailed composition profile. All the data available were tested to check whether data violated the assumptions for regular parametric tests to report valid results.

Those fatty acids in the profile that followed a normal distribution ($P > 0.001$) and were homoscedastic were subjected to one-way ANOVA as a completely randomized design, with breed (Utrerana and Leghorn Lohmann LSL-Classic lineage) and variety (black, franciscan and partridge) as the independent factors, using independent samples *t*-test and One-Way ANOVA tasks and Waller-Duncan posthoc test, respectively, from the Compare Means procedure of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016).

Contrastingly, when a certain fatty acid component did not fit to a normal distribution or were heteroscedastic ($P < 0.001$), we used a Mann–Whitney *U* test to compare differences between the two independent groups of the breed factor (Utrerana and Leghorn Lohmann LSL-Classic lineage). Then, a Kruskal-Wallis H test was performed to study the potentially existing differences across the three levels of the variety factor (black, franciscan and partridge). After conducting Mann–Whitney *U* test (for two groups) and Kruskal-Wallis H with three or more groups (k), we measured the strength of the effect of the factors of breed and variety on fatty acids profile using r and partial eta squared ($\eta p^2$) as quantification measures depending on whether Mann-Whitney U or Kruskal-Wallis H tests had been carried out beforehand. Partial eta squared was computed following the methodology described and reported for non-standard evaluations in research (Li et al., 2019).

Cohen's guidelines for r are that a small effect is 0.1, a medium effect is 0.3, and a large effect is 0.5. Calculation of r, $r^2$, or $\eta^2$ from these z values is possible because partial eta-squared ($\eta p^2$) can be computed as the ratio of variance associated with an effect, plus that effect and its associated error variance. Crosstabs procedure from SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016) were used to calculate $\eta p^2$ and to measure the strength of association between the categorical independent factors of breed and variety and the fatty acids comprised in the profile considered in our study.

Cohen's f allows to analyze the relationship between a quantitative variable and a categorical variable in the case where the latter has more

than two possible levels (k values). SPSS cannot calculate the Cohen's f directly, but they can be calculated using partial eta$^2$ ($\eta p^2$). Cohen analyzes the relationship between the d and f values of Cohen and partial Eta$^2$, through $\eta^2 = \frac{f^2}{(1+f^2)}$ and $f = \sqrt{\frac{\eta^2}{(1-\eta^2)}}$, respectively, where $f^2$ is the square of the effect size, and $\eta^2$ is $\eta p^2$ calculated by SPSS (Cohen, 1977).

Once effect power has been determined, we evaluated pairwise comparisons across the levels of the independent factors of breed and variety for which the Kruskal-Wallis test had resulted significant using the Dunn's test. Then, Bonferroni correction was applied to compensate for the increase in the likelihood of incorrectly rejecting the existence of statistically significant differences between two or more groups.

All nonparametric tests were carried out using the independent samples package from the non-parametrical task of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016).

## 3. Results

A summary of the results for pooled descriptive statistics (mean, mean difference and pooled standard deviation) and effect power (Cohen's d, Corrected d, Hedge's g, r correlation coefficient and Pha (Z)) for the breed factor on general chemical composition of the shell, yolk and white derived from pooled-variance *t*-test procedures is shown in Tables S3, S4 and S5, respectively.

Fatty acids data distribution properties are shown in Table S6 (Shapiro-Wilk's, $P < 0.001$ for significant results). Levene's test for equality of error variance reported that the error variance around predicted scores was not the same for all predicted values ($P < 0.001$), thus there was not homogeneity of variances for each combination of the levels of the independent factors, and the assumption of homoscedasticity was violated. A summary of descriptive statistics for fatty acids profile in egg yolk is shown in Table S7.

The existence of outliers could be a cause for the lack of normality of the variables studied in our population. Walsh's Outlier non-parametric test was used to detect multiple outliers in the data set. Although this test requires a large sample size (n>220 for a significance level α of 0.05), it may be used whenever the data are not normally distributed. However, no outlier was detected. We assessed multicollinearity through tolerance and variance inflation factor (VIF). If the VIF value lies between 1–10, then there is no multicollinearity. However, if the VIF < 1 or >10, then there is multicollinearity. Multicollinearity can also be detected with the help of tolerance, its reciprocal. A tolerance close to 1 means there is little multicollinearity, whereas a value close to 0 suggests that multicollinearity may be a threat. Hence, we used nonparametric tests to statistically assess the information recorded.

For nonnormally distributed fatty acids, a summary of the results for Mann–Whitney *U* test to assess for the effect of the breed factor on the detailed composition of fatty acids and for Kruskal-Wallis H and Dunn tests (nonnormally distributed fatty acids) to assess for the effect of the variety factor on the detailed composition of fatty acids is shown in Table 1. Afterward, Table 2 reports a summary of the results for median *t*-test for independent samples to detect differences in the medians for fatty acids composition between Utrerana and Leghorn breeds and across varieties of Utrerana and Leghorn's egg yolks.

Contrastingly for normally distributed fatty acids, Table 3 shows a summary of the results for one-way ANOVA test to detect differences in the mean across Utrerana varieties and Leghorn's egg yolk fatty acids profile. Then, Table 4 shows a summary of the results for one-way ANOVA to detect differences in the mean between Utrerana breed and Leghorn breed's egg yolk fatty acids profile.

After one-way ANOVA results have been presented in Tables 3, 4 and 5 reports a summary of the results for the Waller-Duncan posthoc test to detect differences across the mean for varieties of Utrerana and Leghorn's egg yolk fatty acids profile.

**Table 1**
Summary of the results for Mann Whitney's U to detect differences in fatty acids composition between Utrerana and Leghorn's eggs and for Kruskal-Wallis H to detect differences in fatty acids composition across eggs from the different Utrerana varieties (black, franciscan and patridge) and Leghorns'.

| Factor | Breed (df = 1) | | Variety (df = 3) | |
|---|---|---|---|---|
| Parameter | Mann Whitney U | Asymp. Sig. | Kruskal-Wallis H | Asymp. Sig. |
| C14:0 | 2.687 | 0.101 | 7.004 | 0.072 |
| C16:1 n7 | 1.212 | 0.271 | 5.300 | 0.151 |
| C17:0 | 1.896 | 0.169 | 2.907 | 0.406 |
| C18:2 n6 | 5.650 | 0.017 | 8.506 | 0.037 |
| C18:3 n3 | 4.419 | 0.036 | 6.317 | 0.097 |
| C20:1 n9 | 1.105 | 0.293 | 1.602 | 0.659 |
| C20:2 n6 | 2.567 | 0.109 | 4.422 | 0.219 |
| C20:4 n6 | 3.712 | 0.054 | 5.019 | 0.170 |
| C22:4 n6 | 0.735 | 0.391 | 3.402 | 0.334 |
| C22:5 n3 | 0.063 | 0.802 | 0.134 | 0.987 |
| C22:6 n3 | 1.053 | 0.305 | 2.110 | 0.550 |

**Table 2**
Summary of the results for median *t*-test for independent samples to detect differences in the medians for fatty acids composition between Utrerana and Leghorn breeds and across varieties of Utrerana and Leghorn's egg yolks.

| Factor | Breed (df = 1) | | | Variety (df = 3) | | |
|---|---|---|---|---|---|---|
| Parameter | Median | Chi-Square | Asymp. Sig. | Median | Chi-Square | Asymp. Sig. |
| C14:0 | 0.282 | 2.310 | 0.129 | 0.282 | 7.527 | 0.057 |
| C16:1 n7 | 2.204 | 0.000 | 1.000 | 2.204 | 4.933 | 0.177 |
| C17:0 | 0.137 | 0.247 | 0.619 | 0.137 | 0.982 | 0.806 |
| C18:2 n6 | 12.605 | 4.059 | 0.044 | 12.605 | 7.267 | 0.064 |
| C18:3 n3 | 0.258 | 1.804 | 0.179 | 0.258 | 3.400 | 0.334 |
| C20:1 n9 | 0.279 | 0.451 | 0.502 | 0.279 | 1.400 | 0.706 |
| C20:2 n6 | 0.145 | 1.804 | 0.179 | 0.145 | 5.267 | 0.153 |
| C20:4 n6 | 2.151 | 0.451 | 0.502 | 2.151 | 1.400 | 0.706 |
| C22:4 n6 | 0.000 | 1.458 | 0.227 | 0.000 | 5.715 | 0.126 |
| C22:5 n3 | 0.169 | 0.031 | 0.861 | 0.169 | 0.247 | 0.970 |
| C22:6 n3 | 0.551 | 1.804 | 0.179 | 0.551 | 2.667 | 0.446 |

## 4. Discussion

### 4.1. Study context and limitations

The present study was developed in the context of a project financer with FEDER funds (Project PP.AVA.AVA201601.16) entitled "Conservation strategy of Utrerana hen: valorization of its products". Utrerana hen is an endangered breed whose population is comprised by a limited number of individuals (MAPA, 2020). According to Gonzalez Ariza et al., 2019b, although the fertility rate of the animals of this breed was 90.68 ± 0.72 %, the percentage of fertilized egg suffering embryonic death was 14.21 ± 0.98 % with the white feather color obtaining the lowest rates (5.88 ± 2.55 %). Therefore, Utrerana eggs must be destined for incubation to ensure the successful accomplishment of the breed's conservation strategies annually (González Ariza et al., 2019b). This situation is common to other endangered hen breeds, and limits the number of eggs used in research.

Several authors have identified reproductive, productive and genetic differences within the varieties of Utrerana hen and when compared to other breeds (González Ariza et al., 2019b, c; Macrì et al., 2019; Zofia et al., 2018). For this reason, and as it was also suggested by our statistical findings, the determination of the chemical basis on which to support the reproductive and productive characterization of the breed and its varieties can help managing and stabilizing new niches for its products.

Strategies based on standardizing the products of the different varieties can extend or diversify the application of the eggs laid by the hens of the different varieties. This in turn suggests the preservation for the breed diversity may be one of the motor elements to ensure the future

**Table 3**
Summary of results for one-way ANOVA test to detect differences in the mean across Utrerana varieties and Leghorn's egg yolk fatty acids profile.

| Fatty acids profile | Parameters | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| C16:0 | Between Groups | 13.809 | 3 | 4.603 | 2.805 | 0.051 |
| C16:0 | Within Groups | 68.925 | 42 | 1.641 | | |
| C16:0 | Total | 82.734 | 45 | | | |
| C16:1 n9 | Between Groups | 0.205 | 3 | 0.068 | 5.345 | 0.003 |
| C16:1 n9 | Within Groups | 0.537 | 42 | 0.013 | | |
| C16:1 n9 | Total | 0.742 | 45 | | | |
| C17:1 | Between Groups | 0.001 | 3 | 0 | 1.183 | 0.328 |
| C17:1 | Within Groups | 0.018 | 42 | 0 | | |
| C17:1 | Total | 0.019 | 45 | | | |
| C18:0 | Between Groups | 6.214 | 3 | 2.071 | 1.95 | 0.136 |
| C18:0 | Within Groups | 44.61 | 42 | 1.062 | | |
| C18:0 | Total | 50.824 | 45 | | | |
| C18:1 n9 | Between Groups | 61.642 | 3 | 20.547 | 5.829 | 0.002 |
| C18:1 n9 | Within Groups | 148.056 | 42 | 3.525 | | |
| C18:1 n9 | Total | 209.698 | 45 | | | |
| C18:1 n7 | Between Groups | 0.177 | 3 | 0.059 | 2.244 | 0.097 |
| C18:1 n7 | Within Groups | 1.107 | 42 | 0.026 | | |
| C18:1 n7 | Total | 1.284 | 45 | | | |
| C20:3 n6 | Between Groups | 0.009 | 3 | 0.003 | 4.733 | 0.006 |
| C20:3 n6 | Within Groups | 0.026 | 42 | 0.001 | | |
| C20:3 n6 | Total | 0.035 | 45 | | | |
| C22:5 n6 | Between Groups | 0.074 | 3 | 0.025 | 0.998 | 0.403 |
| C22:5 n6 | Within Groups | 1.035 | 42 | 0.025 | | |
| C22:5 n6 | Total | 1.109 | 45 | | | |

**Table 4**
Summary of results for one-way ANOVA test to detect differences in the mean between Utrerana breed and Leghorn breed's egg yolk fatty acids profile.

| Fatty acids profile | Parameters | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| C16:0 | Between Groups | 5.774 | 1 | 5.774 | 3.301 | 0.076 |
| C16:0 | Within Groups | 76.96 | 44 | 1.749 | | |
| C16:0 | Total | 82.734 | 45 | | | |
| C16:1 n9 | Between Groups | 0.125 | 1 | 0.125 | 8.933 | 0.005 |
| C16:1 n9 | Within Groups | 0.617 | 44 | 0.014 | | |
| C16:1 n9 | Total | 0.742 | 45 | | | |
| C17:1 | Between Groups | 0.001 | 1 | 0.001 | 1.689 | 0.2 |
| C17:1 | Within Groups | 0.018 | 44 | 0 | | |
| C17:1 | Total | 0.019 | 45 | | | |
| C18:0 | Between Groups | 0.187 | 1 | 0.187 | 0.162 | 0.689 |
| C18:0 | Within Groups | 50.637 | 44 | 1.151 | | |
| C18:0 | Total | 50.824 | 45 | | | |
| C18:1 n9 | Between Groups | 39.689 | 1 | 39.689 | 10.272 | 0.003 |
| C18:1 n9 | Within Groups | 170.008 | 44 | 3.864 | | |
| C18:1 n9 | Total | 209.698 | 45 | | | |
| C18:1 n7 | Between Groups | 0.022 | 1 | 0.022 | 0.759 | 0.388 |
| C18:1 n7 | Within Groups | 1.263 | 44 | 0.029 | | |
| C18:1 n7 | Total | 1.284 | 45 | | | |
| C20:3 n6 | Between Groups | 0.005 | 1 | 0.005 | 7.78 | 0.008 |
| C20:3 n6 | Within Groups | 0.03 | 44 | 0.001 | | |
| C20:3 n6 | Total | 0.035 | 45 | | | |
| C22:5 n6 | Between Groups | 0.012 | 1 | 0.012 | 0.463 | 0.5 |
| C22:5 n6 | Within Groups | 1.097 | 44 | 0.025 | | |
| C22:5 n6 | Total | 1.109 | 45 | | | |

survival of a breed, through the enhancement of its ability to cover a wider scope of market demands, hence to reach a broader public.

### 4.2. Eggshell chemical composition

Significant differences were found between Utrerana and Leghorn eggs in the concentrations of elements measured in eggshells, such as Ca, Cu, Fe, Mg, Mn, Na, P, Pb and S, in which Utrerana has a statistically significant higher mean than Leghorn, however K, Ni and Zn values were higher in Leghorn (Table S3), despite the fact of Küçükyılmaz et al. (2012) stated that eggshell composition is influenced by the quality of the feed and the production system. In any case, these results are in agreement with those obtained in different avian species by other authors (Dalbeck and Cusack, 2006; Dauphin et al., 2018). Higher Ca deposits were found for the eggshell in Utrerana avian breed. Sun et al. (2016) reported several genes could be involved on the avian eggshell calcification during the course of the egg through the uterus. However, For instance, some authors linked the eggshell strength with calcification-related genes to determinate the eggshell strength (Ahmed et al., 2005). The CALB1 gene is associated with Ca transport, both in uterus and intestine. Hence, the CALB1 expression affects the strength of eggshell and could be variable between breeds or even varieties. In addition, other genes, such as the DMP4 gene, codes for a protein that is used to bind Ca to the eggshell (Wilson, 2017).

Mg is known to inhibit calcite nucleation, favoring the precipitation of aragonite (Rodriguez-Navarro et al., 2002). Therefore, it can be assumed that the Ca-Mg exchange equilibrium depends closely on changes in the concentration of either of these elements. According to our results, Utrerana hens could retain higher Mg and lower Ca in the shell gland mucosa and secrete less Mg and more Ca into the shell gland

**Table 5**
Summary of the results for the Waller-Duncan posthoc test to detect differences across the mean for varieties of Utrerana and leghorn's egg yolk fatty acids profile after One-way ANOVA had been performed.

| Fatty acids profile | Subset for alpha = 0.05 | Leghorn | Black Utrerana | Franciscan Utrerana | Partridge Utrerana |
|---|---|---|---|---|---|
| C16:0 | 1 | N/A | 25.416 | 24.881 | 24.260 |
| C16:0 | 2 | 25.658 | 25.416 | 24.881 | N/A |
| C16:1 n9 | 1 | 0.497 | 0.555 | N/A | N/A |
| C16:1 n9 | 2 | N/A | 0.555 | 0.625 | 0.669 |
| C17:1 | 1 | N/A | N/A | N/A | N/A |
| C17:1 | 2 | N/A | N/A | N/A | N/A |
| C18:0 | 1 | N/A | N/A | N/A | N/A |
| C18:0 | 2 | N/A | N/A | N/A | N/A |
| C18:1 n7 | 1 | N/A | N/A | N/A | N/A |
| C18:1 n7 | 2 | N/A | N/A | N/A | N/A |
| C20:3 n6 | 1 | N/A | 0.157 | 0.160 | 0.137 |
| C20:3 n6 | 2 | 0.175 | 0.157 | 0.160 | N/A |
| C22:5 n6 | 1 | N/A | N/A | N/A | N/A |
| C22:5 n6 | 2 | N/A | N/A | N/A | N/A |

N/A: No significant differences in the mean were found.

lumen for eggshell deposition, in comparison to Leghorn hens. The Mg content in Utrerana hens eggshells was much lower than that for Leghorn hens indicating that the eggshell Mg content might have an effect on eggshell quality (Shen and Chen, 2003).

Significant differences between the concentration of Na and Mg on eggshell from Utrerana and Leghorn hens were found. In general, hens' eggshells Mg concentration decreases after shell nucleation at the mammillary caps, showing a recovery in Mg concentration at termination of growth. The variability between breeds and strains may be attributed to organic distribution and crystal size. The same procedure occurs with Na deposits, which also shows a similar pattern between breeds with some exceptions. These differences may also be attributed to the same factors possibly influencing Mg distribution (Dalbeck and Cusack, 2006).

Several authors have suggested that some metals, primarily divalent cations, such as most trace elements, can replace Ca in both the eggshells and the inside of eggs (Van Dyke et al., 2013). In this context, calcium carbonate in an ionic form binds to membrane proteins on the albumen and begins to crystallize. Physiologically, this process continues until protective eggshell is formed, whereby the terminal cuticle is deposited and acts as a bacterial barrier (Wilson, 2017).

Metals could be harmful to humans, especially when they are taken in excessive quantities. Such toxic substances are eliminated by birds using several methods, including normal excretion, but also through their deposition in eggs and feathers (Burger, 1994). We found significant differences between the two breeds studied with Leghorns showing higher deposits of some heavy metals like Cu, Ni and Zn in most egg structures than Utrerana varieties. In this case, this may be attributed to a greater excretion efficiency, which contrastingly may be detrimental if we consider the egg is the final product to be consumed by humans.

Moreover, the higher concentration found for Mg, Na and K may be responsible for the eggshell's strength. Eggshell with greater concentrations of these micronutrients are usually softer and fragile (Orłowski et al., 2019). According to this authors, Utrerana eggshells should be stronger and stiffer than Leghorn's ones, despite these present a lower eggshell weight than that of Leghorn's (González Ariza et al., 2019c).

In regards Pb concentrations, Patee (1984) reported even if concentrations of Pb were found in the eggshell of certain birds, little or no Pb was transferred to the egg contents (yolk and white). Our results partially agree those reported by Patee (1984) as no significant differences were found between the white of Leghorn's and Utrerana's eggs, even when significant differences had been found between the Pb concentrations in the eggshell of both breeds. The significant differences found between the egg yolks' of Utrerana and Leghorn breed, which contrarily were not reported between the white of both breeds may be supported on the considerably greater ability to capture dietary Pb of yolk in comparison to white when Pb concentrations are 30 mg/kg of diet (Yuan et al. 2013). As shown in Tables S3 to S5, the magnitude of the differences between Pb concentrations in yolk and egg for both breeds is the same. This suggests Pb diet concentrations may be in the limit for which such deposit start to significantly increase in egg white (Yuan et al. 2013).

As suggested by Šály et al. (2004), Pb addition in diet may promote a decrease in egg weight, strength and thickness of eggshell, Ca, Fe and Pb in blood and solidity of the tibiae of layers. This may support our previous results (González Ariza et al., 2019c), which suggested Utrerana's eggshells were significantly lighter ($P < 0.05$) that those of Leghorn's.

### 4.3. Multielemental composition of yolk and white

As for the macroelements in yolk eggs, an important fact is that Ca is lower in Utrerana breed (mean values of 1790.00 mg/kg) than in Leghorn ones (mean values of 2170.00 mg/kg) (Table S4). However, both breeds described higher Ca concentrations than other authors in previous studies (Rubio et al., 2017). Contrastingly, Utrerana hens showed a significantly higher concentration for some toxic metals Pb, with mean values of 0.55 mg/kg, in comparison with Leghorn ones, with mean values of 0.52 mg/kg, that could produce reproductive and developmental issues when consumed in excess. Still, European legislation does not establish any limits on toxic metals in hens eggs, as it has been reported that the magnitude of these percentages in yolk and white does not imply a risk for health (Rubio et al., 2017).

### 4.4. Basic chemical composition of yolk and white

Utrerana hen breed eggs presented higher concentrations of raw proteins (17.40 %) and raw fats (30.50 %) in the yolk in comparison to Leghorn hens (16.90 and 25.60 % for raw proteins and raw fats, respectively) (Table S4), in agreement with other authors (Mori et al., 2020; Yin et al., 2008). The oil-water interface film forming function of egg yolk proteins decreases interfacial tension. In addition, this protein film acts as a mechanical barrier due to its viscoelastic properties which prevents disruption (Anton et al., 2003). According to others authors, yolk lipids and proteins are not influenced by the strain of hens (Ahn et al., 1997).

Moreover, similar values of crude protein and crude fat were observed in the Romagnola hen, a native Mediterranean avian breed (Sirri et al., 2018). Additionally, Utrerana egg yolks showed lower raw ash content than Leghorn's ones (3.20 vs 4.20 %). These results support those previously reported by other authors (Sirri et al., 2018), since lower ash contents have been reported for native breed yolks when compared to those from selected lines of laying hens.

Oppositely, no significant differences were observed for yolk cholesterol content between breeds and across varieties (Table S4). References have addressed the fact that cholesterol deposition in the egg yolk can be affected by nutrition. However, birds can produce 10 times more cholesterol per kg of liver than humans. Faitarone et al. (2013) reported that birds are able to maintain egg cholesterol at levels which are considered essential to ensure embryo development. For this reason, eggs have been historically blamed for causing coronary disease due to their high cholesterol content, which in turn even promoted its consumption to decrease. Contrastingly, clinical trials have demonstrated the absence of any link between egg intake and an increase in serum cholesterol concentrations in humans which may oppose to traditional misconceptions (Djoussé and Gaziano, 2008).

Higher concentrations of polyunsaturated and saturated fats in Utrerana hens' egg yolks were found (19.80 and 10.70 % for polyunsaturated and saturated fats, respectively). This finding was also found for the yolk of some Italian hen breed, such as Ermellinata di Rovigo and Robusta maculate. These breeds showed a higher PUFA and SFA proportion when compared to that of a Hy-Line Brown (Rizzi and Marangon, 2012), suggesting possible similarities between fatty acid metabolism in the Utrerana hens and others unselected Mediterranean avian breeds.

Utrerana egg yolks showed significant higher values for vitamin E than Leghorn ones (102.00 vs 88.00 mg/kg). The mechanism of absorption, transportation, metabolism and deposition of maternal vitamin E in birds was linked to heritable influences (Müller et al., 2012). The major form of yolk vitamin E in domestic birds is α-tocopherol (Speake et al., 1999). The intake of grass by birds produces an increase in the levels of α-tocopherol, since the chloroplast membranes are rich in this substance (Bunea et al., 2017; Speake et al., 1999). In this experiment, both Utrerana and Leghorn breeds were fed on the same diets, hence, we can conclude a higher easiness of Utrerana hen to metabolize this substance may exist. In addition, a positive relationship between α-tocopherol contents and carotenoids concentration has been previously described for hens (Skřivan and Englmaierová, 2014). González Ariza et al. (2019c) reported Utrerana egg yolks presented a higher pigmentation than Leghorn ones. At a previous study, a greater difference was also observed between some hen breeds, with Utrerana hen reporting comparable results to those suggested for Rhode Island and Partridge Brahma breeds as far as α-tocopherol content is concerned

(Bunea et al., 2017).

Carbohydrate levels found for Utrerana yolk (1.30 %) and white (1.10 %) (Tables S4 and S5) supported the data obtained by previous studies (Naderi et al., 2017; Réhault-Godbert et al., 2019). However, in the present study, carbohydrate content was higher for Leghorn yolks (7.10 %) and lower in Leghorn whites (0.01 %), when are compared to the levels found for the Utrerana breed. These results should be regarded as very positive, given Utrerana eggs may present more desirable features for high-protein rich diets, especially indicated for people suffering certain dietary-linked or endocrinological conditions such as diabetes (Fuller et al., 2015).

A lower percentage of moisture (84.80 vs 89.70 %) and a higher concentration of carbohydrates (1.10 vs 0.01 %), raw protein (10.60 vs 10.30 %), fat raw (0.50 vs 0.30 %), and therefore, of raw ashes (3.00 vs 2.00 %) was observed in Utrerana whites, when these were compared to Leghorn ones (Table S5). Egg white characteristics have been suggested to be conditioned by the strain of bird and genetic selection (Bílková et al., 2018; Silversides and Scott, 2001). The selection of modern lines of laying hens may have induced an increase in egg weight, which translated in a larger amount of water, which mainly accumulates in the white of the egg. Hence, this greater contribution of water to egg weight, instead of protein, fat and carbohydrates is produced at a lower energetic cost thus translating into a more efficient energy synthesis (González Ariza et al., 2019c).

### 4.5. Fatty acid composition of yolk

Popiela-Pleban et al. (2013) suggested that the fatty acid composition of eggs depend on the composition of the diet provided to the individuals, the bird's digestive system, and the biosynthetic processes of the laying hens, while Goldberg et al. (2013) reported that diet is the main single determinant of fatty acid composition of yolk. Reductions in SFA levels may be considered beneficial for human nutrition as eggs have been criticized for their high SFA contents. This criticism mainly bases on the traditional association of high SFA dietary intake with the development of cardiovascular diseases (Tang et al., 2015). In the present study, the fatty acid composition obtained for both breeds was in concordance with the levels reported for previous studies (Bunea et al., 2017; Kostogrys et al., 2017).

Eggs are naturally poor in linoleic acid (C18:2 n6). However, a higher concentration of this essential fatty acid could be found in the yolk of the eggs of the Leghorn breed and black Utrerana variety in comparison with the rest of Utrerana varieties (franciscan and partridge). However, our results are opposed to those showed by Grobas et al. (2001), that did not observe significant differences in the linoleic acid deposition in the egg yolk of different lines of laying hens. Such an increase in the levels of this fatty acid would be nutritionally advantageous for humans, since we are unable to naturally produce it, hence, it must be supplied in the diet. Furthermore, essential fatty acid are precursors of hormone-like eicosanoids, such as prostaglandins, leukotrienes, and thromboxanes. These substances are involved in the regulation of heart pressure, heart rate, vascular dilation, blood clotting, immune response, lipolysis, and the central nervous system in humans (Kostogrys et al., 2017).

The changes in the yolk fatty acid composition observed in the current study might be ascribed to differences in the variety metabolism, which translates into changes at several enzymatic processes related to the yolk sac membranes, lipoprotein transport and the activity of the stearoylcoenzime, a desaturase enzyme system in the liver (Latour et al., 1998). As a result, black variety and Leghorn breed eggs could be intended for special human diets with higher requirements of essential fatty acids, as for example in people with skin or cardiovascular diseases (Jandacek, 2017).

A significantly higher content for certain PUFA such as α-linolenic (C18:3 n-3) and dihomo-gammalinolenic (C20:3, n-6) was found in Leghorn hens. However, total PUFA was significantly higher in Utrerana breed yolks. Lordelo et al. (2020) partially agreed with the findings in this study, since a higher content in α-linolenic was observed in egg yolk of hybrid strain when are compared with those of indigenous chicken breed in Portugal, however, similar results in the content of dihomo-gammalinolenic were reported when compared the native breeds with the commercial strain. This type of fatty acids could be effective to prevent and treat chronic diseases, frequently occurring in Europe. As a result, many researchers have focused on enhancing the PUFA content in egg (Kostogrys et al., 2017). In this context, our results suggest Utrerana breed eggs could be considered functional foods, since they provide pieces of evidence that may support their physiological benefits on human health.

Additionally, the Utrerana hen yolks showed a significantly higher content in some monounsaturated fatty acid, specific, oleic acid (C18:1 n9) and 7-hexadecenoic acid (C16:1 n9). These results are in line with Lordelo et al. (2020), who reported differences for the levels of some monounsatured fatty acid, like oleic acid, between the egg yolk of eggs from commercial and native genotypes. The monounsaturated fatty acid (MUFA) percentage is directly dependent of the content of the diet supplied to hens. Considering that all the animals in our study had the same diet, differences could be ascribed to other factors such as a potential inheritable genetic factor behind the metabolism of the MUFA (Bunea et al., 2017). Qian et al. (2016) reported that monounsaturated fatty acids, followed by PUFA are the most desirable fatty acids to be present in human diet. The reason for this, is that this type of fats produces a better mitochondrial functioning, which in turn prevents cardiovascular disease by lowering cholesterol and helps avoid complications associated with diabetes such as kidney damage (Abdullah et al., 2017; Qian et al., 2016).

## 5. Conclusions

The chemical characterization of Utrerana breed eggs, has revealed that these eggs may be quite beneficial for human consumption, mainly due to their high content in proteins, in some MUFA and in the total content of polyunsaturated fatty acids. Fatty acids profile may depend on certain genetic inherent characteristics linked to the differences across breeds but also varieties. However, the fact that no difference in regards fatty acid composition was found between black feathered Utrerana and Leghorn may suggest it may not be linked to colouration genetic factors, but others, as it has often been reported in literature. Standardizing this chemical profile may also evidence new strategies on how to cover the currently increasing demand for non-conventional quality products linked to particular breeds. Further studies are needed to determine the effect that this product would have on the health of people suffering from certain diseases. Conclusively, autochthonous breeds must be introduced in common production systems and commercial chains but always under the scope of sustainable production systems. For this, the principal product of each breed has to be characterized, seeking for a differentiated product focused at satisfying the need of specific sectors within the population.

## CRediT authorship contribution statement

**Antonio González Ariza:** Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing. **Francisco Javier Navas González:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing - original draft, Writing - review & editing. **Ander Arando Arbulu:** Investigation, Resources, Supervision, Writing - review & editing. **Juan Vicente Delgado Bermejo:** Methodology, Validation, Writing - review & editing. **María Esperanza Camacho Vallejo:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing - review & editing.

## Declaration of Competing Interest

The authors report no declarations of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2020.103673.

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# Sensory Preference and Professional Profile Affinity Definition of Endangered Native Breed Eggs Compared to Commercial Laying Lineages' Eggs

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**Simple Summary:** A local breed's particularities may provide eggs with sensory properties which may overcome laying lineage, regardless of their production system characteristics. Hence, methods clarifying what the appreciation of a certain product is like can outline the actions required to improve the market value of that product. Affine and non-affine profiles were defined based on the information provided by sixty-four professionally-instructed panelists on sensory attributes, diet habits, production context awareness, product consciousness, cuisine applicability and panelist attributes. Egg consumption was lower in non-affine profile professionals, as were the scores provided to sensory attributes. The higher the knowledge about Utrerana breed, the greater the importance provided to the ecological and autochthonous nature of the products. The level of study, gender and age are crucial factors to consider when approaching the commercialization of Utrerana hen eggs. Conclusively, defining consumer profiles among professionals of the cuisine sector may improve the profitability of Utrerana eggs and may help educating non-affine profiles, something key to the success in product appreciation.

**Abstract:** This study aimed to compare Utrerana native hen eggs' sensory properties to Leghorn Lohmann LSL-Classic lineage's commercial and ecological eggs through free-choice profiling. Second, affine and non-affine profiles were defined using the information provided by professionally-instructed panelists on six sets (sensory attributes, diet habits, production context awareness, product consciousness, cuisine applicability and panelist attributes) using nonlinear canonical correlation analysis. Sixty-four instructed professional panelists rated 96 eggs on 39 variables comprising the above-mentioned sets. Observers reported a significantly higher appreciation ($p > 0.05$) towards yolk color, odor, flavor, texture, overall score, and whole and on plate broken egg visual value when Utrerana eggs were compared to the rest of categories. Professional Profile A (PPA), or egg non-affine profile, consumed less eggs and provided lower scores to sensory attributes than Professional Profile B (PPB), or affine profile. Additionally, PPB accounted for higher knowledge about the Utrerana breed and provided greater importance to a product's ecological and autochthonous nature. PPA was generally characterized by women under 20 years old with no higher studies, while PPB comprised

21–40 years old men with secondary studies. In conclusion, defining professional profiles enables correctly approaching market needs to improve the profitability of Utrerana eggs, meeting professional demands and educating non-affine profiles.

**Keywords:** professional profile; sensory attributes; academic level; product knowledge; production context awareness; cuisine applicability

## 1. Introduction

The Utrerana hen is a rustic medium-sized Spanish endangered breed with four feather varieties (partridge, Franciscan, black, and white). Initially regarded as a white egg laying hen (120–180 eggs/year, mean ~64 g/egg for the entire laying period) raised on family farms, a traditional meat/egg aptitude has been addressed in literature and is reappearing in the market scene [1].

In 2017, around 92% of eggs were produced by laying hens. The annual growth rate of egg production was approximately 0.6 million tons per year from 2000 to 2016, with a total of 1416 trillion tons of eggs, equivalent to 80 million metric tons [2]. However, consumer tastes constantly evolve towards the obtainment of quality food products with special properties, aimed at a specialized market and obtained through sustainable production systems [3]. In this context, Utrerana eggs have shown to have differentiated internal and external characteristics from commercial line eggs [4]. Hence, the need for animal genetic resources proper conservation has greatly increased, since these resources present economic, scientific and cultural interests [5].

Modern livestock production high specialization increasingly threatens animal genetic resources diversity [5]. For instance, commercial herds are based on exploiting few genetically selected breeds for intensive production. However, specialized breeds do not guarantee a genetic reserve for the future. After long periods of natural selection and evolution, variability loss compromises diversity and characteristics such as adverse conditions adaptation, which conforms an invaluable protein rich source [6].

Although nutrition has been suggested to be more influential than genetics in egg chemical composition, Washburn [7] found evidence for a heredity component. Egg quality is directly related to characteristics determining consumer acceptability. For instance, some traits, such as egg weight and dense white height, are highly valued by consumers [8]. However, there are a number of egg sensory attributes that are more difficult to assess, for which taster panels are used.

Food chemical composition is appreciated through the taste sense. Still, some caution should be taken to transform taste sensations into reliable measures, given the existence of interpanelist variability sources that cannot be eliminated even after training [9].

Panelists have been suggested to be unable to routinely describe the sensory attributes they perceive; hence, profiling methods must be homogenized (same sensory lexicon, among others). To prevent this, techniques such as hedonic scale measurements are used [10].

Developing sensory tools to define potential consumers profiles and attitudes towards products has always concerned food scientists. Tests are diverse and range from those analyzing the process of standardization for food evaluation and perception derived from product/consumer interaction to panelists sensation elaboration and verbalization [11].

Empirically determining panelists discriminating capacity for organoleptic characteristics commonly involves the implementation of linear or logistic regression models used in preference surveys and social epidemiology [12]. However, in the case of multivariate analysis, non-linear canonical correlation analysis more appropriately allows to map a series of explanatory factors in correlation to different sensory attributes and eggs' cuisine applicability [13].

First, we aimed to determine the ability of panelists to discriminate organoleptic characteristics across egg type categories basing on hedonic scales. Second, we inferred different professional profiles

regarding their affinity to eggs and their personal context, as a strategy to plan potential marketing strategies to reinforce affine professionals and to attract non-affine ones, to promote autochthonous breed conservation strategies relying on the improvement of their profitability.

## 2. Materials and Methods

### *2.1. Free-Choice Profiling (FCP)*

#### 2.1.1. Professional Panel Description

Sixty-four professional background instructed panelists (students and teachers of the Hospitality School of Córdoba and Granada), with ages ranging between less than 20 to over 50 years old and a minimum formation of 30 h/week during a two-module professional expertise course (Royal Decree Law 687/2010), were recruited to participate in a short-term sensory evaluation study. The number of panelists included in the hedonic test complies with recommendations for best practice in sensory and consumer science proposed by Hough et al. [14] for primary and processed food.

The recruitment was performed after a filter questionnaire, which included demographic and socio-economic information (gender, age and academic level), food products consumption frequency and egg cuisine applicability willingness. No panelist was removed given the lack of missing values.

#### 2.1.2. Sampling

Ecological and commercial eggs (A category and of the M class (53–63 g) belonged to white shell Leghorn Lohmann LSL-Classic lineage. Utrerana and Leghorn Lohmann LSL-Classic lineage commercial hens were managed in individual cages (50 × 62 × 41 cm) following Council Directive 1999/74/EC of 19 July 1999, setting the minimum standards for the protection of laying hens at the Centro Agropecuario Provincial de Córdoba (Spain). Feed and water were available ad libitum. All birds were reared according to the regulations of the European Union (2010/63/EU) in their transposition to the Spanish law (RD 53/2013). Stocking density was four animals per each $m^2$, nest box density consisted of 29 animals per $m^2$. Circle waterers of 5 cm diameter and 41 cm feeder allotment/space were provided for each animal. Wood shavings were used as a floor substrate covering the floor to a depth of approximately 1 cm. Nest box substrate consisted of plastic turf mats covering the floor at a depth of approximately 1 cm. Further information regarding maintenance system of Leghorn Lohmann LSL-Classic lineage commercial hens and Utrerana native breed can be found in González Ariza et al. [4].

Ecologic/organic eggs were obtained from Leghorn Lohmann LSL-Classic lineage hens. The birds were placed in pens comprising a ceiling covered surface of 41.6 $m^2$ and a free-roaming surface of 1000 $m^2$ following the Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. A total of 20 cm of perch was provided per bird. Food and water were available ad libitum. All birds were reared according to the regulations of the European Union (2010/63/EU) in their transposition to the Spanish law (RD 53/2013). Stocking and nest box density, waterers and feeder space followed the requirements stated at the regulation referenced above. Wood shavings were used as a floor substrate covering the floor at a depth of approximately 1 cm and nest box substrate consisted of plastic turf mats covering the floor at a depth of approximately 1 cm as well.

A description of the recipe and chemical composition of the compound feed used for hen feeding in this study is provided in Table S1. To avoid an effect of storage time on the sensory properties, evaluation of the samples was performed in two locations (Córdoba and Granada) simultaneously. A 10 min cooking time was determined after a preliminary boiling test performed on a random control group of 27 eggs to determine the duration (9, 10 and 11 min) that prevents overcooking. Eggs were first strained in cold water to prevent shelling during cooking. Sixty-four testing stations were set. Eggs were cut following their longitudinal axis. Each panelist tested and scored half an egg from

Utrerana, half an egg from a commercial intensive production Leghorn Lohmann LSL-Classic lineage, and half an egg of the same lineage raised under ecological free-range conditions.

#### 2.1.3. Evaluation Sessions

Participants were received in a conference room and placed at individual blind stations under white lighting (700 lx ± 150 lx), as suggested by Guàrdia et al. [15]. First, panelists were briefed on the methodology and the procedure to allow for acquaintance with the vocabulary to describe the three egg types. Each sample was labeled with random three-digit code matched with the panelist number plus an additional random code to identify samples of the same egg type (Commercial eggs—386; Free-range eggs—745; and Utrerana eggs—639), in a randomized complete-block design. A maximum of three samples were presented to each panelist and were assessed in the same tasting session balancing the first-order and the carry-over effects [16], as suggested by Guàrdia et al. [15].

Eggs were evaluated at room temperature (20 °C) and presented on white ceramic plates covered with a food grade PVC film (oxygen permeability; 20,000 $cm^3/m^2$/24 h; water-vapor transmission rate 2000 $g/m^2$/24 h; Macopal, S.L., Lliçà de Vall, Spain) to prevent drying. Mineral water and 15 g golden delicious variety apple slices [17] at room temperature were provided for mouth rinsing and sense saturation reduction in participants between samples.

#### 2.1.4. Sensory Evaluation and Panelist Contextual Records

Each half egg was rated on six egg sensory attributes (yolk color, white color, odor, flavor, texture and overall score). The visual value of the whole egg and a broken egg on plate visual value were also scored for each egg type separately. Collaterally, panelists provided information on five additional sets comprising a total of 31 variables. These questionnaires later allowed characterization of the overall profile of the panel and the panelists' preferences for the products tested. The panel comprised fairly equal amounts of females (44%) and males (56%).

Panelist context is defined by five sets of variables as follows: Panelist diet habits, production context awareness, product consciousness, cuisine applicability and panelist characterization. The definition for each set and its comprising variables and scales is shown in Supplementary Tables S2 and S3. The scales used follow one unit increases to indicate panelists' ratings. Egg sensory attributes were rated on a 1 to 8 hedonic structured or categorized scoring scale extracted from Anzaldúa Morales [18], except for white color, where panelists only provided answers for seven categories. The sensory attributes set was evaluated using an structured 100 mm line scale anchored with the following ordinal categories: (1) I extremely dislike it, (2) I dislike it a lot, (3) I dislike it moderately, (4) I slightly dislike it, (5) I like it, (6) I slightly like it, (7) I like it moderately, (8) I like it a lot and (9) I extremely like it, adapting the criteria in Anzaldúa Morales [18].

### *2.2. Free-Choice Profiling Interobserver Correlation Coefficient (ICC)*

The intraclass correlation coefficient (ICC), based on multiple paired Cohen's κ tests, was calculated to determine if there was agreement between the sixty-four panelists. Fleiss and Cohen [19] established repeatability guidelines for ICC interpretation as less than 0.4 (low), from 0.4 to 0.59 (reasonable), from 0.6 to 0.74 (good), and from 0.75 to 1.0 (excellent). As we used a random sample of consistent raters for all ratees, we used a "Two-Way Random" model. Then, 95% confidence intervals were computed. The ICC and 95% CI were calculated with the reliability analysis routine of the scale procedure of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016) (Table 1).

**Table 1.** Cronbach's Alpha, Cohen's kappa Intra Class Correlation Coefficient and 95% confidence intervals for interobserver reliability testing and scale consistency sorted by egg type.

| **Egg Type** | | | **Cronbach's Alpha** | | | | |
|---|---|---|---|---|---|---|---|
| Commercial | | | 0.800 | | | | |
| Utrerana | | | 0.826 | | | | |
| Ecologic | | | 0.829 | | | | |
| Egg type | Measure type | Intraclass Correlation | 95% Confidence Interval | F Test | df1 | df2 | Significance |
| Commercial | Single | 0.105 | 0.071–0.158 | 5.003 | 63 | 2079 | 0.00 |
| | Average | 0.800 | 0.723–0.864 | 5.003 | 63 | 2079 | 0.00 |
| Utrerana | Single | 0.122 | 0.084–0.180 | 5.733 | 63 | 2079 | 0.00 |
| | Average | 0.826 | 0.758–0.882 | 5.733 | 63 | 2079 | 0.00 |
| Ecologic | Single | 0.125 | 0.086–0.183 | 5.843 | 63 | 2079 | 0.00 |
| | Average | 0.829 | 0.763–0.884 | 5.843 | 63 | 2079 | 0.00 |

### *2.3. Scale Reliability*

Scale internal consistency was studied using Cronbach's alpha. As a general criterion, George and Mallery [20] suggest the following recommendations for evaluating Cronbach's alpha coefficients: >0.9 is excellent, >0.8 is good, >0.7 is acceptable, >0.6 is questionable, >0.5 is poor and <0.5 is unacceptable. Variables with values over 0.5 were retained as they were able to explain the highest percentage of variance.

### *2.4. Quantitative Descriptive Analysis (QDA)*

Variables and scales use agreement was performed at a preliminary open discussion involving 32 professional panelists, following the premises described in Anzaldúa Morales [18]. The same author reported the references used to illustrate the criteria for the variables on each set. Several training and refresher training sessions were set up to develop the different sensory attributes and normalize the panelists according to common perceptions [18]. Descriptors varied from 8 to 10 for each panelist.

### *2.5. Egg Type Sensory Attributes Difference Analysis*

Descriptive statistics for the variables on each set are reported in Table S1. Variables were not transformed and sorted into six sets considering their common nature, namely, egg sensory attributes, panelist diet habits, production context awareness, product consciousness, cuisine applicability, and panelist characterization. Shapiro–Francia tests were carried out with the .sfrancia routine of StataCorp Stata version 14.2. (Supplementary Table S4). As normality was not found, a Kruskal–Wallis H test was performed to study differences across variables. Afterwards, interlevel distribution and median differences among Kruskal–Wallis H significant variables were tested using the pairwise comparisons Dunn's test and sorting medians respectively. If we test for multiple comparisons, the likelihood of incorrectly rejecting statistically significant differences between two or more levels (Type I errors) increases. The Bonferroni correction was performed to compensate for that increase. All nonparametric tests were carried out using the independent samples package from the non-parametrical task of SPSS Statistics for Windows, Version 24.0, IBM Corp. (2016) and results are provided in Table S4.

### *2.6. Statistical Justification*

Although some authors [21] have suggested Procrustes analysis to be one of the most common and strict techniques to analyze sensory attributes related to other aspects such as free choice profiling, it is only applicable when all variables measurement dimensions (p) have similar scales. Contrarily, this analysis renders inaccurate [22] if we do not only have different scales but also different measurement units.

The same authors suggest alternatives such as the nonlinear version of canonical correlation analysis, report results with a virtually perfect fit, which may be partly attributed to the freedom to choose non-linear transformations, which enables scoring traits on very different scales.

### *2.7. Non-Linear Canonical Correlation between Sets*

A nonlinear canonical correlation analysis (OVERALS) was performed to determine interset similarities to maximize the variance in the relationships among two sets of numerical variables in a low dimensional space. Optimal scaling approach in OVERALS expands the standard canonical analysis as first, it allows more than two sets of variables, accommodating varying scaling standards [13]. Second, variables can be scaled as nominal, ordinal, or numerical in an intervariable integrative analysis of non-linear relations. Finally, instead of maximizing interset correlations, these are compared to an unknown compromise set that is defined by the object scores. OVERALS uses the "alternating least squares (ALS) algorithm", to calculate the "fit function" and the "loss function". The loss function states the difference between the number of chosen dimensions to the best calculated adaptation and shows the lack of fit of a solution, being within a p-dimensional case, the minimum equal to 0 and maximum equal to p. Loss represents the proportion of variation in object scores for each dimension and set in Table 2. The mean of sets is the average loss in sets and gives us the difference between the maximum and actual fits. Summation of average loss and fit is equal to the number of dimensions. Therefore, small loss values indicate large multiple correlations between weighted sums of optimally scaled variables and dimensions [23]. The eigenvalue can be calculated by dividing loss per dimensions, and carrying out 1 minus loss per dimension. The eigenvalue is a goodness of fit measure, which ranges from 0 to 1, indicating the level of relationship shown by each dimension, and the sum of these values is called total fit (Table 2), that is, the statistical index widely used in OVERALS to decide analysis solution dimensionality.

**Table 2.** Eigenvalues for the two-dimensional solution of nonlinear canonical correlation analysis for Utrerana native hen egg sensory attributes (yellow), panelist diet habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and panelist characterization (grey) as perceived by cuisine instructed panelists (n = 192).

| | Egg Sensory Attributes | Panelist Diet Habits | Production Context Awareness | Product Consciousness | Cuisine Applicability | Panelist Characterization | Mean | Eigenvalue |
|---|---|---|---|---|---|---|---|---|
| Dimension 1 | 0.446 | 0.069 | 0.46 | 0.055 | 0.081 | 0.849 | 0.327 | 0.673 |
| Dimension 2 | 0.816 | 0.266 | 0.212 | 0.159 | 0.214 | 0.465 | 0.355 | 0.645 |
| FIT | 1.262 | 0.335 | 0.672 | 0.213 | 0.294 | 1.315 | 0.682 | 1.318 |

For visual mapping of the constructed space, we used the nonlinear canonical correlation analysis with the described six sets and their variables. Component loadings are the correlations between object scores and optimal scaled variables and are sorted in dimensions 1 and 2. These loadings act as coordinates of the variable points on the graph given below in Figures 1–6 and help with illustrating the distribution of variables in a bi-dimensional space. To this aim, quantifications of multiple categories or numerical ranges are used. These quantifications present the center for all respondents belonging to one category and account for the importance of other variables from the set. Variables close to others have more similarities among interviewed persons than variables that are far apart. To interpret the dimensions obtained, attributes with loadings of over 0.5 [24] were the most effective variables in relationships among variable sets because they were positioned far from the origin (denoting the mean) [25] (Supplementary Table S6). The plots of centroids were labeled according to the categories in the scale for each variable and are presented in Figures 1–6, showing how well variables separate groups of objects. Centroids were in the center of gravity of the objects. Matching clusters of categories in centroid plots need to be identified and interpreted to understand intervariable relationships [26].

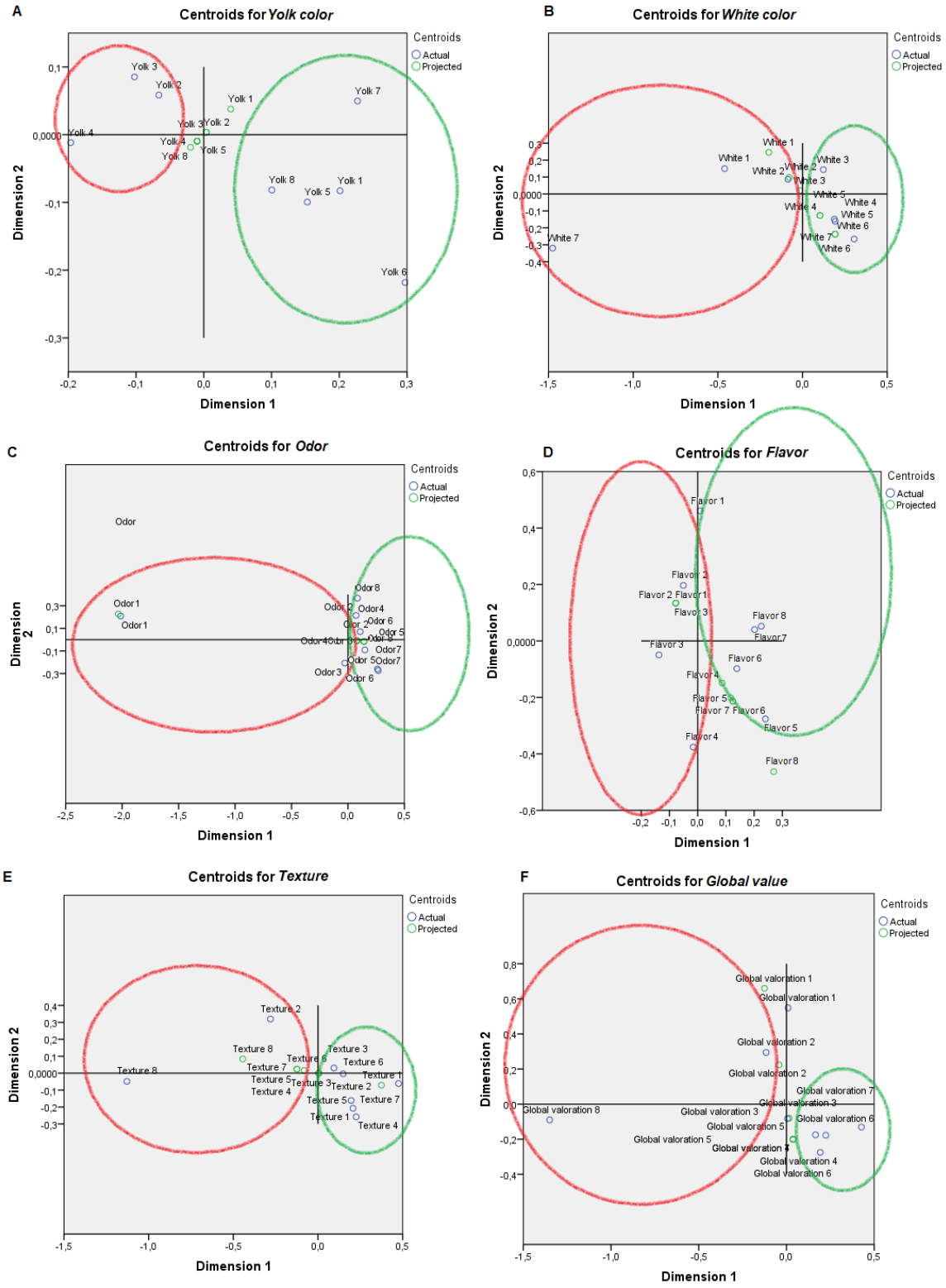


**Figure 1.** *Cont.*

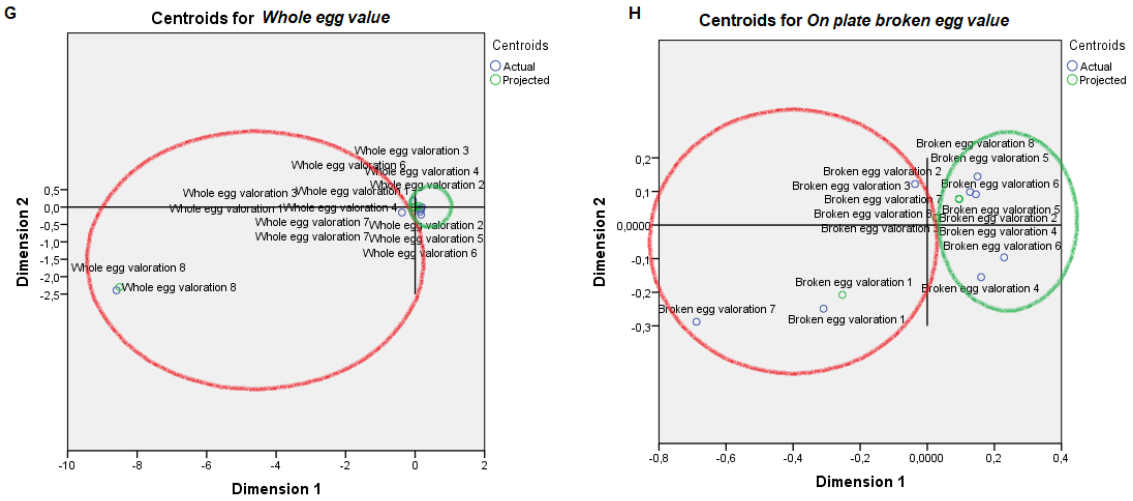


**Figure 1.** Object scores plot visualization of Professional Customer Profiles in regards egg sensory attributes, egg consumption non-affine profile or PPA (red), and affine profile or PPB (green). Egg sensory attributes were as follows: (**A**) Yolk color, (**B**) White color, (**C**) Odor, (**D**) Flavor, (**E**) Texture, (**F**) Global value, (**G**) Whole egg value and (**H**) On plate broken egg value.

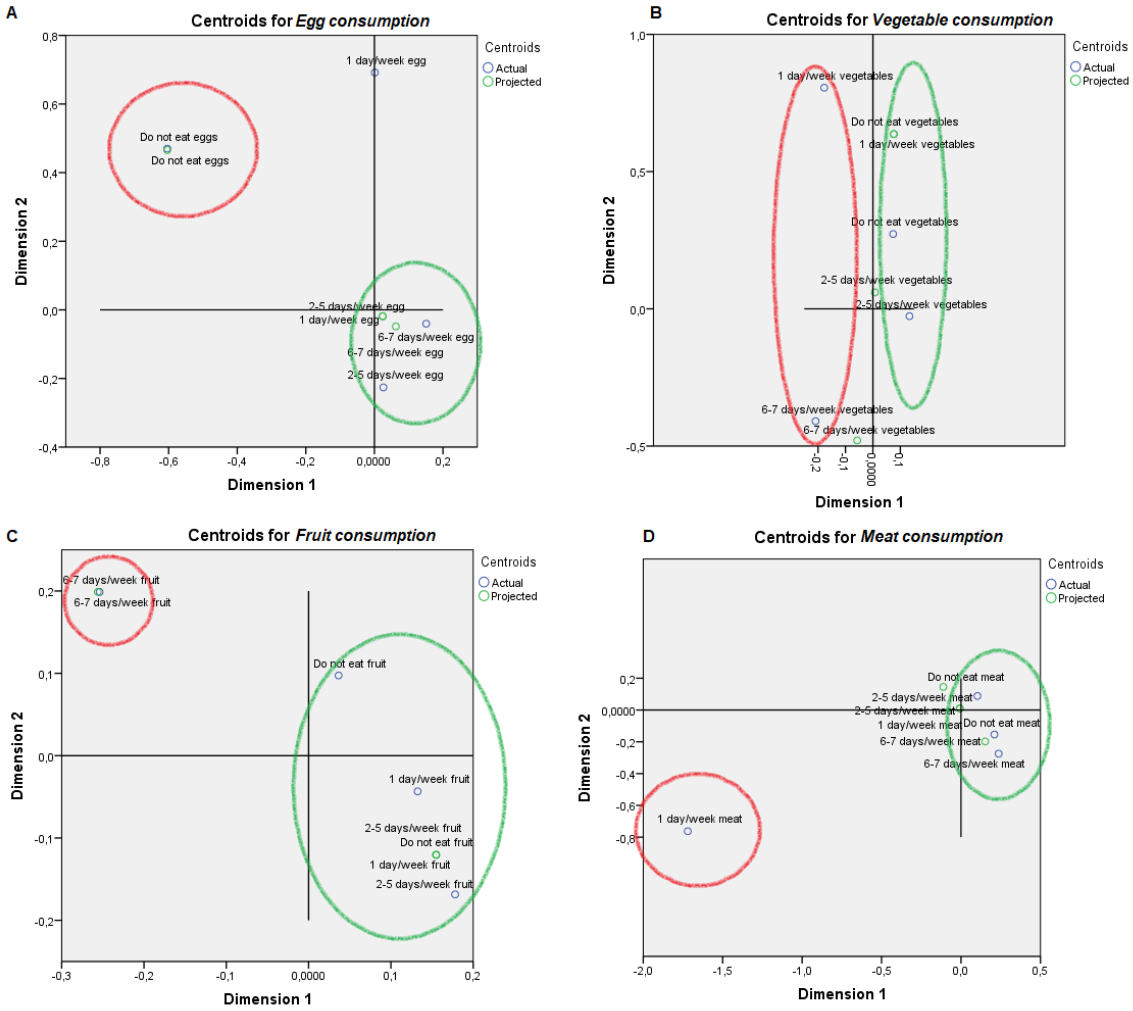


**Figure 2.** *Cont.*

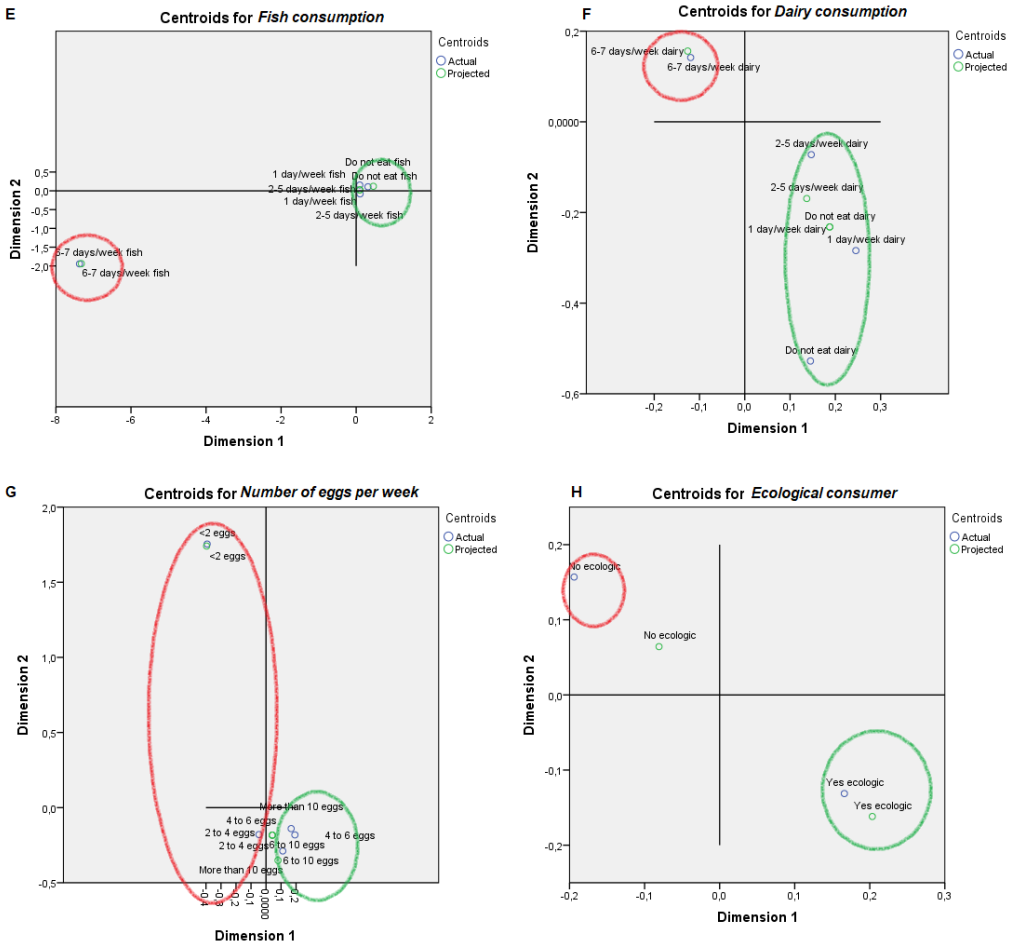


**Figure 2.** Object scores plot visualization of Professional Customer Profiles with regards panelist diet habits, egg consumption non-affine profile or PPA (red), and affine profile or PPB (green). Panelist diet habits were as follows: (**A**) Egg consumption, (**B**) Vegetable consumption, (**C**) Fruit consumption, (**D**) Meat consumption, (**E**) Fish consumption, (**F**) Dairy consumption, (**G**) Number of eggs per week and (**H**) Ecological consumer.

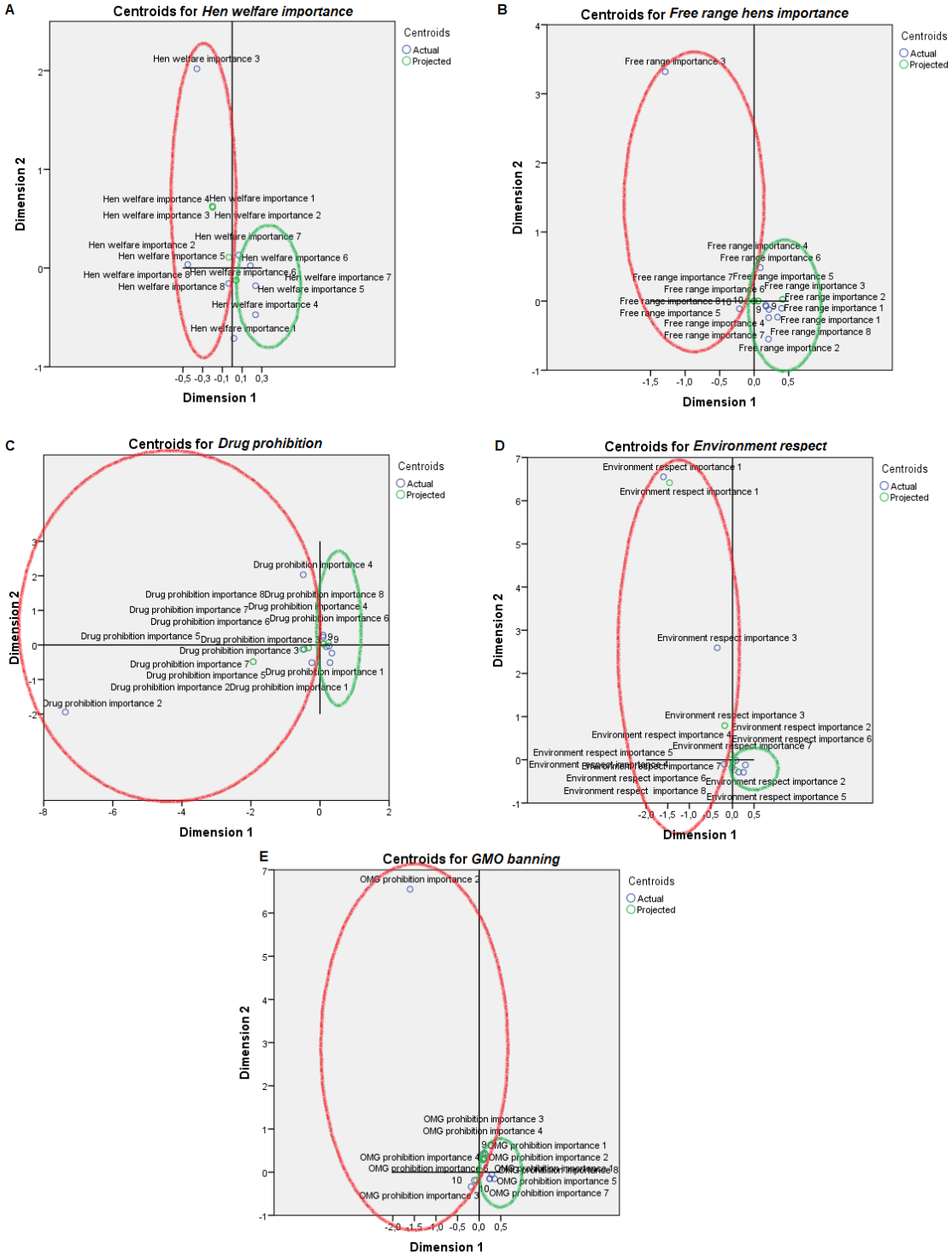


**Figure 3.** Object scores plot visualization of Professional Customer Profiles with regards production context awareness, egg consumption non-affine profile or PPA (red), and affine profile or PPB (green). Production context awareness was scored through: (**A**) Hen welfare importance, (**B**) Free range hens importance, (**C**) Drug prohibition, (**D**) Environment respect and (**E**) GMO banning.

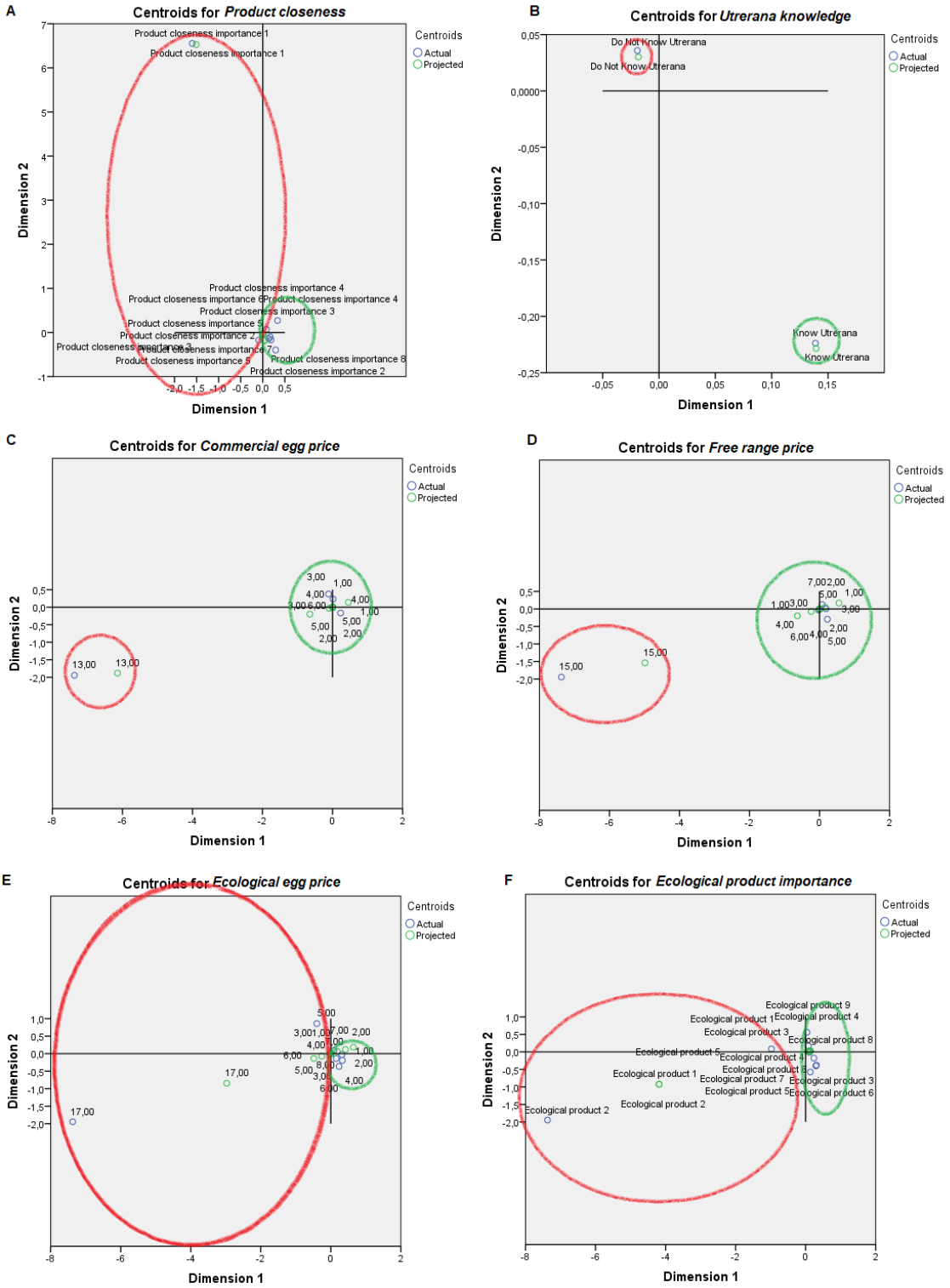


**Figure 4.** *Cont.*

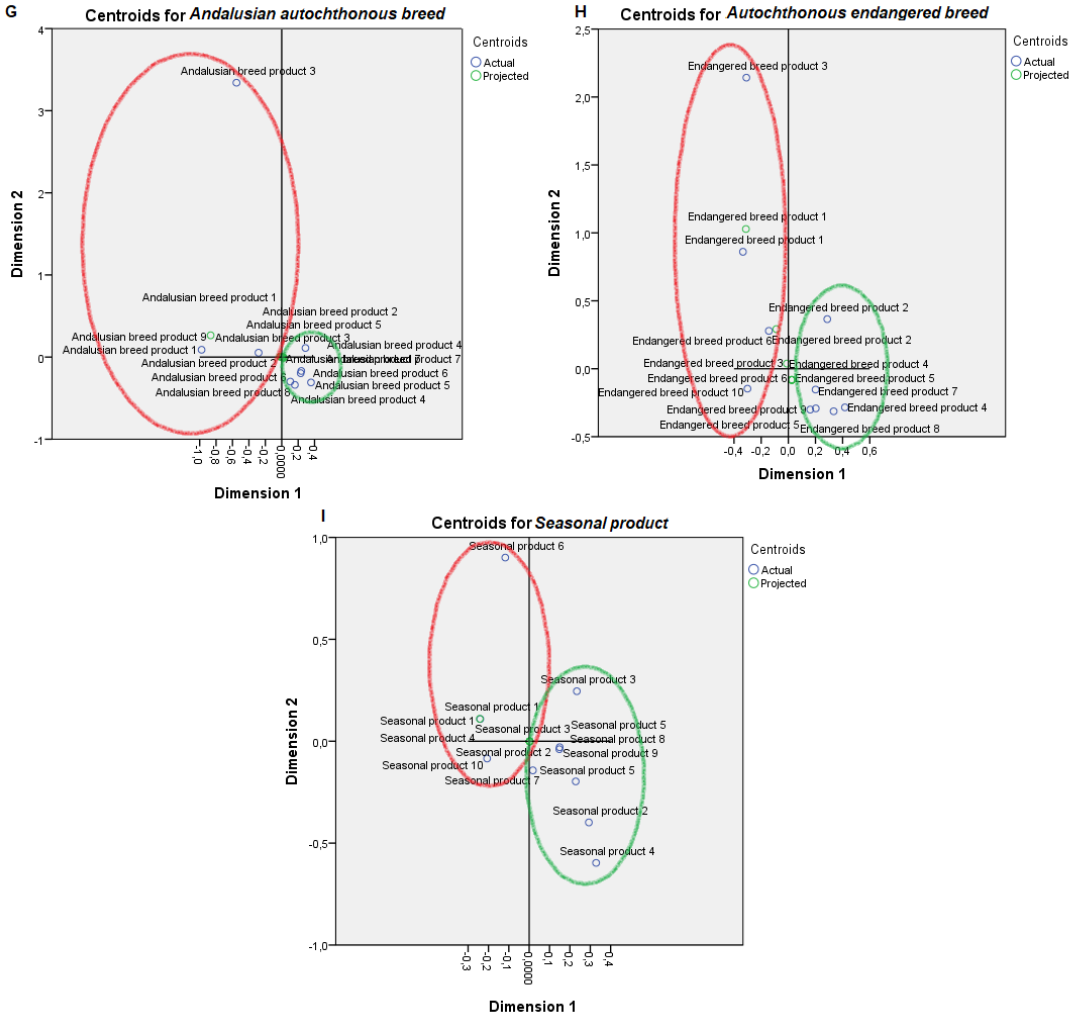


**Figure 4.** Object scores plot visualization of Professional Customer Profiles with regards product consciousness, egg consumption non-affine profile or PPA (red), and affine profile or PPB (green). Product consciousness was scored through: (**A**) Product closeness, (**B**) Utrerana knowledge, (**C**) Commercial egg price, (**D**) Free range price, (**E**) Ecological egg price, (**F**) Ecological product importance, (**G**) Product deriving from an Andalusian autochthonous breed, (**H**) Product deriving from an autochthonous endangered breed and (**I**) Product having a seasonal nature.

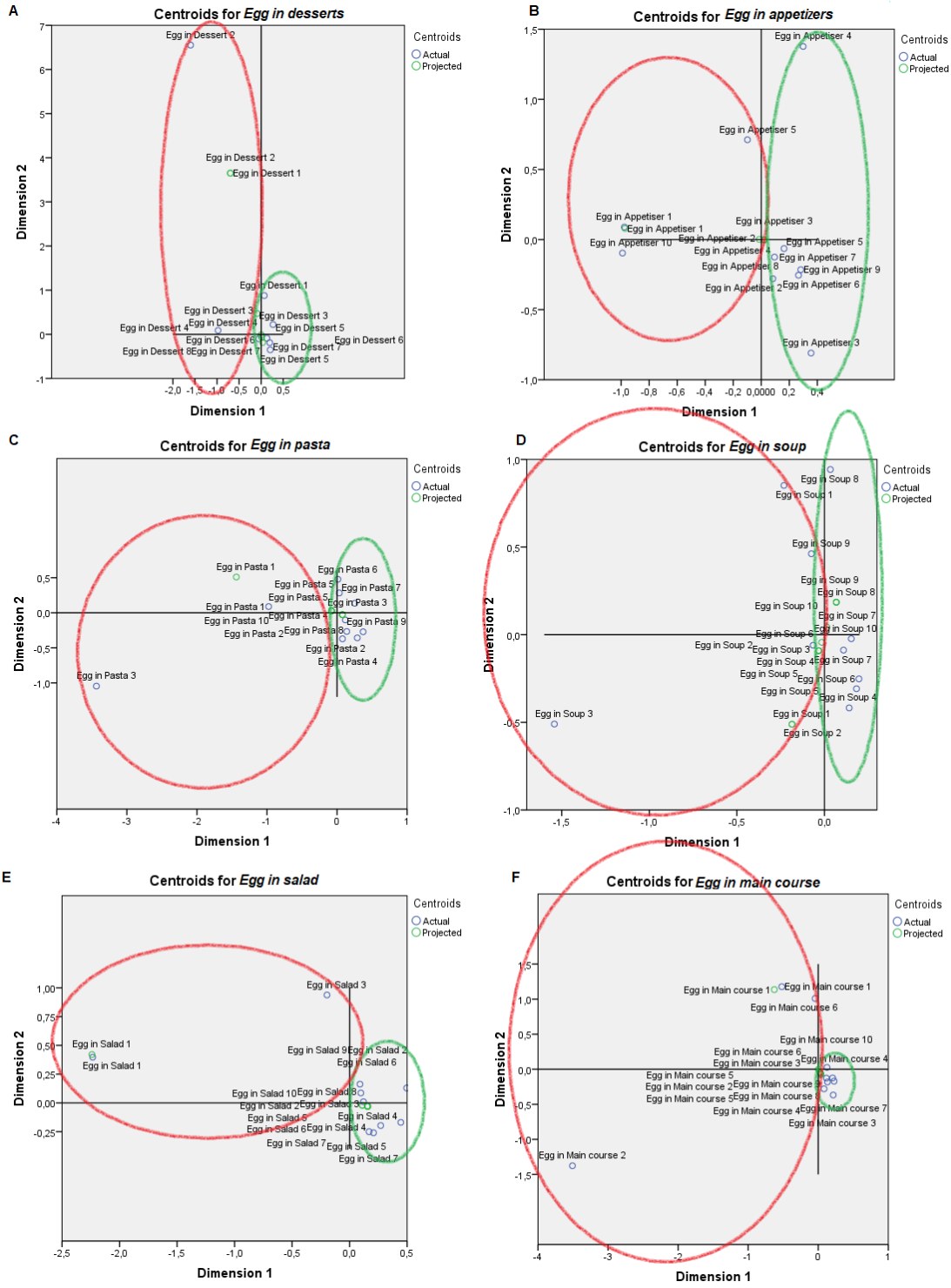


**Figure 5.** Object scores plot visualization of Professional Customer Profiles with regards cuisine applicability, egg consumption non-affine profile or PPA (red), and affine profile or PPB (green). Cuisine applicability was scored through: (**A**) Egg applicability in desserts, (**B**) in appetizers, (**C**) in pasta, (**D**) in soup, (**E**) in salad and (**F**) in main course.

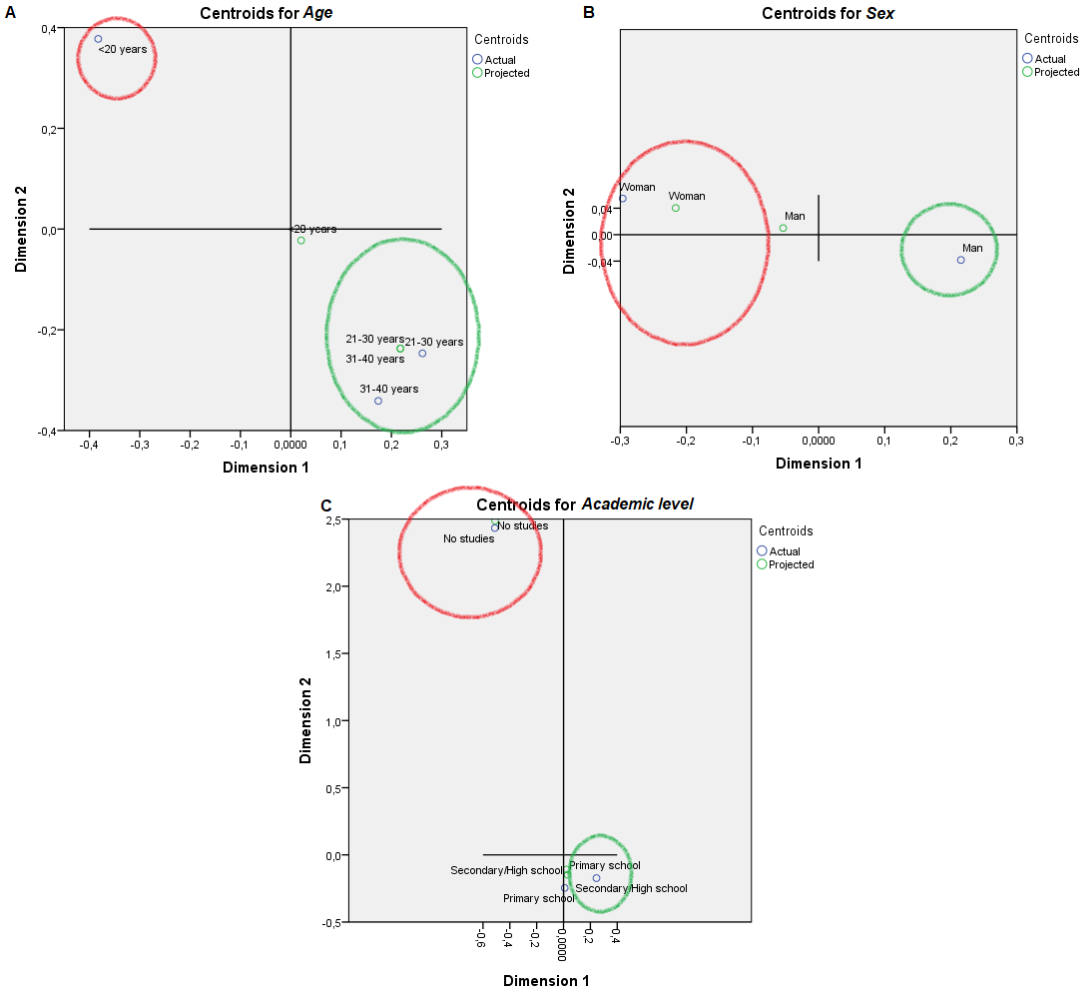


**Figure 6.** Object scores plot visualization of Professional Customer Profiles with regards professional characterization, egg consumption non-affine professional profile or PPA (red), and affine profile or PPB (green). Professional characterization was determined through: (**A**) Age, (**B**) Sex and (**C**) Academic level.

Contrary to what happens in principal component analysis (PCA), for which a dimensionality criterion of explained variance over 80%, is required. When OVERALS is linked to FCP, if all variables are specified as ordinal, single nominal, or numerical, the maximum number of dimensions is the lesser of the following two values: The number of observations (n = 192) minus 1, or the total number of variables [26]. Then, we reduce the dimensions until we reach the maximum number of dimensions that explains the greatest percentage of variance at an acceptable loss level (Table 3). Single variables are only important when containing information independent from information of other variables of the same set [27].

**Table 3.** Model partitioning fit and loss analysis for Utrerana native hen egg sensory attributes (yellow), panelist diet habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and panelist characterization (grey) as perceived by cuisine instructed panelists (n = 192).

| Set. | Variables | Categories | Multiple Fit | | | Single Fit | | | Single Loss | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Dimension 1 | Dimension 2 | Sum | Dimension 1 | Dimension 2 | Sum | Dimension 1 | Dimension 2 | Sum |
| Egg sensory attributes | Yolk color | 8 | 0.060 | 0.015 | 0.074 | 0.052 | 0.013 | 0.065 | 0.007 | 0.002 | 0.009 |
| | White color | 7 | 0.078 | 0.026 | 0.105 | 0.077 | 0.019 | 0.096 | 0.002 | 0.007 | 0.008 |
| | Odor | 8 | 0.088 | 0.022 | 0.110 | 0.085 | 0.005 | 0.090 | 0.003 | 0.017 | 0.021 |
| | Flavor | 8 | 0.048 | 0.030 | 0.078 | 0.039 | 0.024 | 0.062 | 0.009 | 0.006 | 0.015 |
| | Texture | 8 | 0.045 | 0.095 | 0.140 | 0.032 | 0.093 | 0.126 | 0.013 | 0.002 | 0.015 |
| | Overall value | 8 | 0.009 | 0.099 | 0.107 | 0.005 | 0.097 | 0.103 | 0.004 | 0.001 | 0.005 |
| | Whole egg visual value | 8 | 0.311 | 0.068 | 0.379 | 0.302 | 0.047 | 0.349 | 0.010 | 0.021 | 0.030 |
| | On plate broken egg visual value | 8 | 0.017 | 0.042 | 0.059 | 0.012 | 0.041 | 0.053 | 0.005 | 0.002 | 0.006 |
| Panelist diet habits | Egg consumption | 4 | 0.002 | 0.162 | 0.164 | 0.002 | 0.161 | 0.163 | 0.000 | 0.001 | 0.001 |
| | Vegetable consumption | 4 | 0.032 | 0.254 | 0.286 | 0.032 | 0.249 | 0.281 | 0.000 | 0.005 | 0.005 |
| | Fruit consumption | 4 | 0.014 | 0.134 | 0.148 | 0.013 | 0.132 | 0.145 | 0.001 | 0.002 | 0.003 |
| | Meat consumption | 4 | 0.005 | 0.017 | 0.022 | 0.005 | 0.016 | 0.021 | 0.001 | 0.001 | 0.001 |
| | Fish consumption | 4 | 0.906 | 0.067 | 0.974 | 0.906 | 0.055 | 0.961 | 0.001 | 0.012 | 0.013 |
| | Dairy consumption | 4 | 0.006 | 0.116 | 0.122 | 0.004 | 0.101 | 0.105 | 0.002 | 0.015 | 0.017 |
| | Number of eggs per week | 5 | 0.022 | 0.525 | 0.547 | 0.021 | 0.524 | 0.545 | 0.001 | 0.001 | 0.002 |
| | Ecological consumer | 2 | 0.000 | 0.006 | 0.007 | 0.000 | 0.006 | 0.007 | 0.000 | 0.000 | 0.000 |
| Production context awareness | Hen welfare | 8 | 0.040 | 0.025 | 0.065 | 0.003 | 0.008 | 0.011 | 0.038 | 0.017 | 0.054 |
| | Free range hens | 10 | 0.230 | 0.026 | 0.256 | 0.216 | 0.001 | 0.217 | 0.014 | 0.025 | 0.039 |
| | Drug prohibition | 10 | 0.985 | 0.108 | 1.093 | 0.644 | 0.073 | 0.717 | 0.341 | 0.035 | 0.376 |
| | Environment respect | 8 | 0.069 | 0.854 | 0.923 | 0.036 | 0.798 | 0.834 | 0.033 | 0.056 | 0.089 |
| | GMO banning | 10 | 0.048 | 0.024 | 0.072 | 0.045 | 0.012 | 0.058 | 0.002 | 0.012 | 0.014 |
| Product consciousness | Product closeness | 8 | 0.055 | 0.676 | 0.732 | 0.055 | 0.674 | 0.729 | 0.001 | 0.003 | 0.003 |
| | Utrerana knowledge | 2 | 0.000 | 0.002 | 0.002 | 0.000 | 0.002 | 0.002 | 0.000 | 0.000 | 0.000 |
| | Commercial egg price | 13 | 0.021 | 0.051 | 0.072 | 0.020 | 0.050 | 0.071 | 0.001 | 0.001 | 0.001 |
| | Free range egg price | 15 | 0.002 | 0.051 | 0.052 | 0.001 | 0.051 | 0.052 | 0.000 | 0.000 | 0.001 |
| | Ecological egg price | 17 | 0.002 | 0.025 | 0.027 | 0.000 | 0.001 | 0.001 | 0.002 | 0.024 | 0.026 |
| | Ecological product | 9 | 2.097 | 0.080 | 2.177 | 2.094 | 0.056 | 2.150 | 0.002 | 0.024 | 0.027 |
| | Andalusian autochthonous breed product | 9 | 0.803 | 0.009 | 0.812 | 0.803 | 0.001 | 0.804 | 0.000 | 0.008 | 0.009 |
| | Endangered breed product | 10 | 0.011 | 0.140 | 0.150 | 0.008 | 0.127 | 0.135 | 0.003 | 0.013 | 0.016 |
| | Seasonal product | 10 | 0.004 | 0.019 | 0.023 | 0.001 | 0.001 | 0.002 | 0.004 | 0.018 | 0.021 |
| Cuisine applicability | Desserts | 8 | 0.039 | 0.641 | 0.679 | 0.023 | 0.526 | 0.548 | 0.016 | 0.115 | 0.131 |
| | Appetizers | 10 | 0.017 | 0.340 | 0.357 | 0.002 | 0.313 | 0.315 | 0.015 | 0.027 | 0.042 |
| | Pasta | 10 | 0.032 | 0.222 | 0.253 | 0.021 | 0.213 | 0.234 | 0.010 | 0.009 | 0.019 |
| | Soup | 10 | 0.011 | 0.247 | 0.258 | 0.006 | 0.242 | 0.248 | 0.006 | 0.005 | 0.011 |
| | Salad | 10 | 3.594 | 0.255 | 3.849 | 3.585 | 0.215 | 3.800 | 0.009 | 0.040 | 0.049 |
| | Main course | 10 | 2.323 | 0.924 | 3.247 | 2.319 | 0.904 | 3.223 | 0.004 | 0.020 | 0.024 |
| Panelist characterization | Age | 3 | 0.193 | 0.046 | 0.239 | 0.192 | 0.046 | 0.239 | 0.000 | 0.000 | 0.000 |
| | Sex | 2 | 0.183 | 0.012 | 0.195 | 0.183 | 0.012 | 0.195 | 0.000 | 0.000 | 0.000 |
| | Academic level | 3 | 0.015 | 0.475 | 0.490 | 0.008 | 0.475 | 0.483 | 0.007 | 0.000 | 0.007 |

In total, 39 variables with either a nominal, numeric or ordinal scaling level are included in the analysis (Supplementary Table S3). Variables can be classified into two or more sets and scaled as multiple nominal, single nominal, ordinal, or numerical and the interpretation of their direction is obtained from the position of projected centroids. Most of the variables considered in the present study are ordinal. This implies that the order of the categories within each variable must be preserved. Then, if actual and projected centroids are not separated, ordinal variables should have been considered as nominal [28]. As suggested by van der Burg and Dijksterhuis [29], sensory scores were reorganized into fewer new categories to minimize the existence of empty categories, we decided to adopt this organization system, thus minimizing the occurrence of unique marginal frequencies.

## 3. Results

Kruskal–Wallis H reported significant differences ($p < 0.05$) for all egg sensory attributes across egg type categories except for white color ($p > 0.05$). Dunn's tests reported egg categories were significantly different, with Utrerana egg reporting the highest median (5), followed by ecologic (3), and commercial (2). The Utrerana egg scored one median-unit higher than commercial lineage, which also presented a significantly lower score for flavor, overall and on plate broken egg visual value when compared to ecologic eggs (1 point higher) and Utrerana eggs (2 points higher), with no significant difference between Utrerana and ecologic eggs. Texture was only significantly different between commercial and ecologic eggs ($p < 0.05$), with the latter reporting a one-point-higher median than commercial or Utrerana eggs. Commercial eggs' whole egg visual value was significantly different to that of ecologic and Utrerana eggs.

Single ICC, determining how a single observation taken at random may correlate to another single observation, was 0.105, 0.120 and 0.125, for commercial, Utrerana and ecologic eggs, respectively. This could be expected, given that we were considering panelists' personal appreciation of certain products, and no correlation should be expected beforehand as they may be strongly conditioned by subjective factors. However, average ICC and Cronbach's alpha, that is, how consistent the whole panel of panelists is on average, were 0.800, 0.826 and 0.829, for commercial, Utrerana and ecologic eggs, respectively, reporting an excellent repeatability. This suggested the survey and scales used were sound and the panel was properly instructed and reliable.

Eigenvalues were high (0.673 and 0.645 for dimensions 1 and 2, respectively). Hence, the actual fit value was 1.318. A bi-dimensional solution was chosen, so 1.318/2 = 65.9% of the variation was calculated in the analysis, with 0.673/1.318 51.1% of the actual fit calculated by the first dimension and 0.645/1.318 48.9% by the second dimension.

Table 2 shows a summary of loss functions for each dimension and set. Average loss was 2 − 1.318 = 0.682 in our study and not necessarily high. The number of dimensions was equal to 2 (0.682 + 1.318). The single and multiple fit of variables is presented in Table 3. Component loadings are presented in Table S6. The visual maps depicted in Figures 1–6 are defined by all variables listed in Tables S1 and S2. Those variables, which in sum showed a multiple fit of more than 0.1 (Table 3), may play a more important role in the explanation of variance. Variable values, which are not displayed, were mainly spread around the axis of coordinates. By neglecting this proportion of data none of the influential values are lost, but the readability of figures is improved. Component loadings (interpanelist agreement) are shown for each variable separately in Table S5. Dimension 1 shows that the panelists agree very much on the attribute whole egg visual, fish consumption, drug prohibition, commercial egg price, free range egg price, ecological egg price, ecological product status relevance, and salad applicability. The second dimension shows panelist agreement is dominated by environmental aspects, egg applicability in desserts, and academic level.

Apparently, when analyzing each set separately, yolk color, flavor and overall score were the attributes on which the panelists agreed less. These lowest agreement values are also reported for vegetable and meat consumption, hen welfare and genetically modified organism (GMO) banning, Utrerana knowledge, seasonal product conception or egg applicability in soup.

Two very distinct professional profiles are identified regarding attitudes towards eggs (Figures 1–6), Professional Profile A—non-affine profile (PPA, in red in plots), and Professional Profile B—affine profile (PPB, in green in plots). The egg sensory attributes set is assessed in Figure 1. PPA scored yolk color from 2 to 4. The most of the observers from PPB scored yolk color higher than 5 out of 8 levels in the scale. PPA normally scored white color 1 or 2, while PPB scored white yolk from 3 to 6. PPA scored odor from 1 to 4, while PPB scored odor from 4 to 8. PPA scored flavor from 2 to 4, while PPB scored it from 5 to 8. PPA scored texture with the values of 2, from 4 to 5 and from 7 to 8, while PPB provided constantly increasing values from 1 to 7. For the overall score, PPA provided scores of 2 or 8 while PPB scores increasingly ranged from 4 to 7. For whole egg, PPA provided 4 or 8 scores, while PPB provided values from 2 to 6 (excluding 4). The on-plate broken egg value was irregularly scored by PPA and PPB.

PPA does not usually consume eggs, while PPB consumes more than four eggs a week more than two days per week (Figure 2). PPA either does not consume vegetables or consumes them from six to seven days a week, while PPB consumes vegetables from one to five days a week. PPA consumes fruit six to seven days a week while PPB or does not eat fruit or eat it less than five days a week. PPA eats meat one day per week while PPB eats meat more than two days per week. Fish and dairy consumption habits were reported by PPA in six to seven days, while PPB used to consume fish and dairy products less than five days a week or did not consume them at all. PPA did not consume ecological products while PPB did consume ecological products.

PPA provided a low importance to hen welfare, while PPB provided it with a higher importance (Figure 3). Contrastingly, PPA provided the highest importance for hens being kept in free range conditions in the scale, while PPB scored differently from 1 to 7 and from 8 to 9. PPA scored drug prohibition importance with 1 to 2 values and 4, while PPB scored its importance from 4 to 6 and from 8 to 9. A more irregular trend, supported by the low component loadings (Supplementary Table S6), was described in both profiles for environmental respect and GMO banning.

PPA scored product closeness from 1 to 3, while PPB scored it from 4 to 8. PPB was acquainted with the Utrerana breed while PPA was not (Figure 4). Contrary to PPB, PPA progressively misattributed the highest prices to commercial, free range and ecological eggs, respectively, and provided the lowest importance to ecological products or to the product being linked to an Andalusian autochthonous or endangered breed. The importance conferred to the seasonal attribute of the product described an irregular scoring pattern for both PPA and PPB.

PPA presented a lower trend to use eggs in desserts, as appetizers, in pasta, soup or salad than PPB (Figure 5). PPA mostly comprise women under 20 years old with no studies, while PPB comprised men from 21 to 40 years old and with secondary studies (Figure 6).

## 4. Discussion

The eigenvalues of the two dimensions that result from the nonlinear canonical correlation analysis are quite high, with 0.673 for the first dimension and 0.645 for the second dimension. Our total fitness value of 1.318 can be considered appropriate for this type of treatment [29], as it has been reported by several authors and food products. Other two-dimensional solutions reported in the literature have produced total fit indexes of 1.644 in apples, 1.763 in luncheon meat, 1.192 in water and 1.856 in cheese [24,29]. This makes the conclusions driven from the present study valid and reliable.

European consumers prefer darker yolks, given the psychological healthier egg qualities misattribution. Observers scored Utrerana yolk color and odor significantly higher, which may be based on Utrerana's acknowledged darker yolk color when compared to laying lineages' yolk color [4]. The higher pigmentation found in some strains may be due to different genetic capabilities to absorb and deposit pigments in yolk [30]. Different egg yolk color preferences have been reported between northern and southern European countries [31], with a taste towards intensely colored (golden-orange) yolks in southern countries, contrasting with what occurs in the majority of consumers worldwide, where consumers show a greater affinity towards brighter yolks. Similarly, consumers of ecologic/organic eggs generally accept paler yellow yolks, as reported by Grashorn [32].

Yolk color has been reported to depend directly on the carotenoid level and on the proportion between yellow and red carotenoids in the feed provided to laying hens. The content of yellow carotenoids (lutein, zeaxanthin, cryptoxanthin, violaxanthin, ethyl ester of β-apo-8′-carotenoic acid, β-apo-8′-carotenal) stabilize the yellow color in the yolk, but do not intensify it. Contrastingly, for a rather intense, golden-orange color, red carotenoids, such as capsanthin/capsorubin, canthaxanthin, and citranaxanthin have to be added to the feed. Red carotenoids cover yellow carotenoids, and if their content is further increased then the yolk color presents a pinkish or red tone [32].

A stronger egg odor has been attributed to dual-purpose hens when compared to laying hens [33]. Additionally, native breeds' eggs have reported similar or superior values for aroma than improved breeds eggs [34]. These results contrast with those of Olugbemi et al. [35], who did not find significant differences between commercial laying hens and local breeds. Highly significant differences have been reported for egg composition across hen breeds and avian species, particularly regarding egg volatiles, fatty acids content, and albumen proteome composition [36]. Supportive findings by Rizzi and Marangon [34] indicated that dual-purpose hens present a stronger flavor. Haunshi et al. [34] also found flavor acceptability was significantly higher in local breeds than in improved breeds. No significant differences were found in either the texture across egg types, or in the literature for the texture of processed scrambled eggs belonging to hens fed on alternative products to dietary molt [37].

The Utrerana eggs' overall score was significantly higher than commercial eggs' score, agreeing with earlier reports [34]. The on-plate broken egg visual values were significantly lower in commercial eggs than ecologic and Utrerana eggs. This suggests that the Utrerana egg's higher proportion of yolk and a darker yolk [4] would have a greater market acceptability. Panelists agreed very much on the whole egg visual value, as previously reported [4]. Furthermore, external appearance and eggshell color has been suggested to hold some positive correlations with egg quality parameters [38].

Panelists' agreement was higher for fish consumption; drug prohibition; commercial, free range and ecological egg price; ecological product status relevance and egg in salad. Contextually, total drug banning led to the promotion of the consciousness of drug use, which develops a popular sense towards the effects of antibiotics and growth promoters on food safety and health in farms in Europe, which may be the basis for the panelists' high agreement on the subject [39]. Furthermore, it can be inferred that when prices are higher, environmentally friendly food production and ecological products are regarded as secondary priorities [40]. Still, a tendency for consumers to pay higher prices for these environmentally-friendly products has been described in the literature [41].

Panelists agreed with respect to the environment, use of eggs in dessert and academic level (second dimension). Food consumption and production trends and patterns are one of the main causes of environmental pressure. Consumers are aware of this; hence, their consumption choices guide the search for more sustainable productive systems [42]. Not only did the PPB group consume more eggs, but also scored sensory attributes higher than the PPA group (non-usual eggs consumers, <4 eggs/week) and were more conscious about ecological production. Contrastingly, PPA reported a higher fruit, fish and dairy consumption, contrasting with the more frequent PPB meat and ecological products consumption habits. A lower frequency of consumption of eggs or meat in habitual fish consumers has been observed [43]. This could be explained by the division of panelists depending on their consumption livestock derived products. European diets are characterized by a high intake of livestock products (meat, dairy and eggs) [44].

The PPA group scored product closeness lower, was not acquainted with the Utrerana breed, misattributed a higher price to Utrerana eggs than to other egg types, scored ecological product status relevance lower and provided a relatively low importance to the product being linked to an Andalusian autochthonous and endangered breed. Contrastingly, the PPB group was acquainted with the Utrerana breed and scored the product and local endangered breeds-based production systems higher. Links with local breeds and their products starting from childhood improve their marketing strategies [45]. Hence, alternative markets may help conserving endangered native breeds and valuable animal genetic resources, as consumer demand for specialty livestock products and the willingness of

consumers to pay for them largely depends on their lack of availability and their knowledge on the breed involved [46].

The functions of eggs, like coagulating, foaming, emulsifying and contributing nutrients, make them a useful ingredient in a lot of gastronomic preparations [47]. PPB provided a higher cuisine applicability to Utrerana eggs (desserts, appetisers, pasta, soup, salad and main courses) than PPA. Professionals acquainted with the product and its qualities, find a greater applicability than those who are not familiar. Polesel et al. [48] suggested egg consumption as an indicator of a diet rich in foods such as desserts and meat.

Women under 20 years old with no studies fit the PPA, while PPB would be characterized by men from 21 to 40 years old with secondary studies. These results agree with those of a previous study reporting that men usually consume more eggs than women, and individuals of 20–30 years old had the lowest odds of consuming eggs [49]. Stefanikova et al. [50] suggested men eat more meat, eggs and milk, while women eat more fruit and vegetables. Another study conducted in college students and nutrition educators suggested men consumed more meat, poultry, fish and eggs, while women consumed more vegetables and fruit [51]. Bejaei et al. [52] showed that free-run, free range, and organic eggs consumers have higher education levels compared with consumers of other egg types; this is supported by the fact that PPB–which valued more highly ecological and local hens' eggs, than commercial ones–also presented higher education levels.

The differentiated quality of a product can be protected in markets offering a wider scope of valuable products, adapted to the consumer's special needs [53]. Breed choice is mainly prescribed by the regulations of the producers, while the high quality of the products is appreciated by a small group of consumers, which indirectly promotes a local breed's preservation [54]. Customer profile analysis adds value to the formulation of investment projects, providing information on consumers' reactions to alternative products, generated through innovation or trend. In this context, the preferences of the target market among similar products, their purchase incentives, needs, among others, must be established. In this way, market research and the analysis of data collected enables the issuing of a diagnosis on the viability of the product in question, in turn translating into the sustainability of the breed that it originated from.

## 5. Conclusions

Involving autochthonous breeds, such as Utrerana, in common production systems and commercial chains seeking the characterization of differentiated products could be the key to improving profitability in future sustainable poultry productions. Defining different attitudes of costumers towards eggs may help outlining potential strategies for the design and implementation of marketing campaigns, indirectly identifying those sectors to which a greater effort should be made in an attempt to revalue a native breed's egg products. These profiles may also suggest strategies on how to successfully achieve the aim of covering the currently increasing demand for non-conventional quality products linked to particular breeds and production systems from markets that are different from those normally established for classical highly productive systems.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2076-2615/9/11/920/s1: Table S1. Recipe and chemical composition of the compound feed used for feeding the hen sets in the study; Table S2. Descriptive statistics for Utrerana native hen egg sensory attributes (yellow), Panelist diet habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and consumer characterization (grey) as perceived by cuisine instructed panelists (n = 192); Table S3. Scales for Utrerana native hen variables included in the sets of egg sensory attributes, Panelist diet habits, production context awareness, product consciousness, cuisine applicability and panelist characterization as perceived by cuisine instructed panelists (clustering set in bold); Table S4. Testing for normality using Shapiro-Francia W' in Utrerana native hen egg sensory attributes (yellow), Panelist diet habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and Panelist characterization (grey) as perceived by cuisine instructed panelists (n = 192); Table S5. Kruskal Wallis H Ranks, Dunn's test and Bonferroni's significance correction and Median sorted by egg type for Utrerana native hen egg sensory attributes; Table S6. Components loadings for nonlinear canonical correlation analysis for Utrerana native hen egg sensory attributes (yellow), Panelist diet

habits (red), production context awareness (purple), product consciousness (green), cuisine applicability (blue) and Panelist characterization (grey) as perceived by cuisine instructed panelists (n = 192).

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**Conflicts of Interest:** The authors declare no conflict of interest.

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# Discriminant Canonical Analysis as a Validation Tool for Multivariety Native Breed Egg Commercial Quality Classification

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**Abstract:** This study aimed to develop a tool to validate multivariety breed egg quality classification depending on quality-related internal and external traits using a discriminant canonical analysis approach. A flock of 60 Utrerana hens (Franciscan, White, Black, and Partridge) and a control group of 10 Leghorn hens were placed in individual cages to follow the traceability of the eggs and perform an individual internal and external quality assessment. Egg groups were determined depending on their commercial size (S, M, L, and XL), laying hen breed, and variety. Egg weight, major diameter, minor diameter, shell b*, albumen height, and the presence or absence of visual defects in yolk and/or albumen showed multicollinearity problems (variance inflation factor (VIF) > 5) and were discarded. Albumen weight, eggshell weight, and yolk weight were the most responsible traits for the differences among egg quality categories (Wilks' lambda: 0.335, 0.539, and 0.566 for albumen weight, eggshell weight, and yolk weight, respectively). The combination of traits in the first two dimensions explained 55.02% and 20.62% variability among groups, respectively. Shared properties between Partridge and Franciscan varieties may stem from their eggs presenting heavier yolks and slightly lower weights, while White Utrerana and Leghorn hens' similarities may be ascribed to hybridization reminiscences.

**Keywords:** egg quality; external quality traits; internal quality traits; DSM color; fan color coordinate decomposition; mechanical eggshell strength; pH-related traits



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## 1. Introduction

In 2019, the world's hen egg production exceeded 1.6 billion eggs, 28.7% higher production than a decade before [1]. Such a remarkable increase brought about a parallel increase in the concerns for animal welfare and environment in the European Union. Contextually, more than 50% of hens were reared in cage-free systems, while 18% of hens were reared in alternative production systems (free-range and organic) in Europe in 2019 [2].

This increasing interest in products obtained under non-industrial production systems allows the development of sustainable farming practices [3]. These sustainable farming practices may involve the use of native breeds adapted to the local environment, with great rusticity and resistance to meteorological situations and diseases, as well as great ability to search for food in the wild [4,5]. Consequently, it is through the conservation of animal genetic resources, that economic sustainability in the rural areas is promoted [6,7].

The Utrerana avian breed is one example of rustic Spanish hen, located in Andalusia (Southern Spain), which is officially considered to be endangered as stated in the Royal Decree—Law 45/2019 from the 8 February 2019. Its four varieties, namely, White, Franciscan, Black, and Partridge, are classified depending on the color of its feathers and tarsi [8].

It was initially oriented toward egg production, however, the introduction of rather productive commercial hybrid genotypes in Europe caused the displacement of the Utrerana hen breed to a secondary position [9]. As a result, the breed census reached a critical situation, which was parallel to a decrease in its productive indices derived from the patent lack of productive selection [10]. Although the number of individuals has multiplied in the last years, only 1548 animals were registered in the studbook of the breed during 2019 [11].

The enhancement of local products can be a strategy for the conservation of autochthonous genotypes, by avoiding the loss of connection between products, the local breeds from which they derive, and the area in which these were produced, as has been described for industrial products [12]. In line with this situation, the definition of the breed's productive role became compulsory to maximize the breed's potential to satisfy current commercial demands. The characterization of Utrerana's egg as the main product of the breed was configured in the context of a set of strategies that sought the obtention of competitive sustainable products in the framework of the recent emerging diseases and climate change [13].

The acceptability of the eggs by consumers is mainly affected by the characteristics that describe their quality. Egg quality depends on several parameters, which are related to the eggshell, the albumen, and the yolk. Quality traits can be classified into external and internal quality, depending on whether the egg has to be broken to be scored (internal quality) or not (external quality) [14,15]. Egg weight, eggshell strength, albumen quality, and yolk color intensity are among the most important egg traits of commercial interest [14,16–18]. Eggs are commercially classified into four classes depending on their total egg weight: S (<53 g), M (53–63 g), L (63–73 g), and XL (>73 g).

Egg quality traits have been reported to be multifactorially dependent mainly on the laying hen's age and nutritional factors [16,19–21]. However, there are some relevant pieces of evidence for the influence of the genotype on some of these egg quality traits on the relative proportion of yolk and albumen, albumen quality, or chemical composition [14,22–26].

Utrerana's egg not only constitutes a differentiated product in terms of internal and external quality–related traits [14] and chemical composition [23]. Additionally, its sensory characteristics have been reported to differ from the eggs of commercial lines [27].

Consequently, the present study aimed to determine the contributions of external and internal quality parameters to the eggs produced by each of the four varieties of Utrerana hens and a control flock of a commercial laying lineage. Canonical discriminant analysis was used to design a statistical tool that permits determining whether specific eggs may correctly fit the features of the different commercial size categories (S, M, L, and XL), which may support the standardization of the Utrerana varieties' eggs as products and may address and support their suitability to cover particular sections of the market for egg consumption.

## 2. Material and Methods

### *2.1. Institutional Animal Care and Use Committee Statement*

Avian-specific codes for good practices and the national guidelines for the care and use of laboratory and farm animals were followed in agreement with the standards found under the scope of the European Union legislation (2010/63/EU, from the 22 September 2010) and its transposed Spanish law document (Royal Decree Law 53/2013). As recommended by Royal Decree Law 53/2013 and its credited entity, the Ethics Committee of Animal Experimentation from the University of Córdoba, no additional permission was required as stated in the 5th section of the 2nd article of the aforementioned document given the zootechnical credited utilization of the animals participating in the present study.

### *2.2. Layer Flock and Environmental Conditions*

The public farm in which the study took place is located at the Agropecuary Provincial Center of Diputación of Córdoba, south Spain (Plus code: W77Q + MF El Levigar/37°54′50.9″ N 4°42′40.4″ W). The eggs used in the experiment were obtained from a layer flock com-

prising 60 Utrerana hens and 10 Leghorn Lohmann LSL-Classic lineage hens (hereinafter referred to as "Leghorn hens"), distributed depending on their breed or variety as described in Table 1. The laying flock was housed in individual cages (50 × 62 × 41 cm), to ensure that the traceability of each egg daily was feasible and following the Council Directive 1999/74/EC of 19 July 1999, which states minimum standards for the protection of laying hens. This whole year study ran from February 2019 to February 2020. All the birds were fed on the same commercial feed (15.20% crude protein, 4.60% crude fat and oils, 3.20% crude fiber, 14.00% crude ashes, 4.10% calcium, 0.66% phosphorus, 0.19% sodium, 0.31% methionine, 0.72% lysine). Feed and water were available *ad libitum*.

**Table 1.** Flock management information. All cages were chosen according to Council Directive 1999/74/EC of 19 July 1999, laying down minimum standards for the protection of laying hens.

| Flock Management Parameter | | Utrerana Varieties | | | | Leghorn (Control) |
|---|---|---|---|---|---|---|
| | | White | Franciscan | Black | Partridge | |
| Laying hens | | 15 | 15 | 15 | 15 | 10 |
| Subgroups | Hens (70 weeks old) | 8 | 8 | 8 | 8 | 0 |
| | Pullets (28 weeks old) | 7 | 7 | 7 | 7 | 10 |
| Stocking density [1] | | 4 animals per $m^2$ | | | | |
| Nest box density [1] | | 29 animals per $m^2$ | | | | |
| Waterer allotment/space | | Circle waterers of 5 cm in diameter per animal | | | | |
| Feeder allotment/space | | 41 cm per animal | | | | |
| Floor substrate | | Wood shavings covering the cage floor at a depth of approximately 1 cm | | | | |
| Nest box substrate | | Plastic turf mats covering the floor at a depth of approximately 1 cm | | | | |

[1] Stocking density and nest box density were determined after computing the whole cage's surface considering each cage's dimensions were 50 × 62 × 41 cm and its surface area was 0.25 $m^2$.

### 2.3. Work Sample

All statistical tests were performed on an egg sample comprising 541 eggs, laid during a complete laying cycle. The eggs were classified depending on their breed and variety and commercial size as shown in Table 2. The protocols are described below in Sections 2.4 and 2.5 were performed on each egg individually.

**Table 2.** Number of observations (eggs) classified per breed/variety and commercial size.

| | S (<53 g) | M (53–63 g) | L (63–73 g) | XL (>73 g) | Total |
|---|---|---|---|---|---|
| White | 2 | 46 | 45 | 3 | 96 |
| Franciscan | 7 | 71 | 25 | 2 | 105 |
| Black | 8 | 43 | 34 | 10 | 95 |
| Partridge | 8 | 32 | 32 | 3 | 75 |
| Leghorn | 12 | 83 | 60 | 15 | 170 |
| Total | 37 | 275 | 196 | 33 | 541 |

### 2.4. External and Internal Quality-Related Traits Description

External and internal quality-related traits were measured separately. For the external quality of the egg, noninvasive methods were used, and measurements were taken without breaking the eggshell. The external quality traits that were measured were as follows: egg weight; major and minor diameters of the egg; eggshell color lightness, redness, and yellowness coordinates (shell L*, shell a*, and shell b*). Shape index (SI) was computed through the following formula [28]:

$$SI = (ØM/Øm) * 100 \quad (1)$$

where ØM is the major diameter and Øm is the minor diameter.

Eggs were classified depending on their shape index as follows: sharp egg (SI < 72), standard egg (SI = 72–76), or round egg (SI > 76) [29].

Internal egg quality–related traits were evaluated after breaking the egg. Internal egg quality traits measures were as follows: eggshell resistance (eggshell strength and area under the force–displacement curve); albumen height; yolk color; yolk lightness, redness, and yellowness variables (yolk L*, yolk a*, and yolk b*); yolk diameter; eggshell weight; yolk weight, albumen weight; yolk pH; albumen pH; eggshell thickness; and the presence or absence of visual defects in yolk and/or albumen. Haugh units (HU) were calculated as a measure of the albumen quality, from the variables albumen height and egg weight via the following formula [30]:

$$HU = 100 * \log\left(h - 1.7w^{0.37} + 7.6\right) \quad (2)$$

where h is albumen height (mm) and w is egg weight (g).

### 2.5. Measurements on Eggs

The egg quality measurements were registered fortnightly for the whole duration of the study. Egg quality was assessed at 22 ± 1 °C. The traceability of the egg and the external and internal characterization of each individual egg were performed and registered within 24 h after oviposition. Eggs were weighed individually using an electronic scale (Cobos, CSB-600C, Barcelona, Spain). Eggshell color was assessed using a portable spectrophotometer (CM 700d, Konica Minolta Holdings Inc., Tokyo, Japan). Eggshell color results were expressed using the International Commission on Illumination (CIE) L*a*b* system color profile. Major and minor diameters were measured using a Vernier scale (Electro DH M 60.205, Barcelona, Spain).

Mechanical eggshell strength measurement was performed using a texturometer TA.XT2 Texture Analyzer (TA.XT2; Texture Technologies Corp., Scarsdale, NY, USA). Eggshells were punctured at the bottom (large end) of the eggshell with a polyoxymethylene (POM) probe with a 5 mm diameter. Eggshell strength and the area under the curve were determined from the graphical curve obtained by the texturometer. The approach followed started when each individual eggshell was broken, and the yolk and albumen were deposited on a glass surface to take measurements of the internal quality–related variables described above. Albumen height was computed as the arithmetic mean of three measurements performed using a Haugh digital micrometer (Baxlo, Barcelona, Spain). The intensity of the yellow–orange color of the yolk was measured both with the portable spectrophotometer (L*, a*, b*) and using a Roche color fan (yolk color) (DSM, DSM® YolkFan™, Heerlen, The Netherlands). The yolk diameter was measured on a Vernier scale. The eggshell, albumen, and yolk were separated and weighed using a precision balance. The pH was measured using a pH meter (Crison®, PH-25, Barcelona, Spain). Eggshell thickness was measured averaging three measurements around the blow-hole near the equator of the egg upon a Vernier scale. All the eggs were visually evaluated to detect blood or meat spots in the albumen, yolk, or both.

### 2.6. Canonical Discriminant Analysis

A canonical discriminant analysis was performed using egg weight, major diameter, minor diameter, shell L*, shell a*, shell b*, shape index, eggshell strength, area under the force–displacement curve, albumen height, Haugh units, yolk color, yolk L*, yolk a*, yolk b*, yolk diameter, eggshell weight, yolk weight, albumen weight, yolk pH, albumen pH, eggshell thickness, presence or absence of visual defects in yolk and/or albumen, and Haugh units per egg as explanatory variables. The commercial classification and the hen breed/variety were used as the labeling classification criteria to measure the variability in quality-related traits between and within classification groups, to establish, identify, and outline clusters [31,32].

The present discriminant tool permits to sort eggs across hen genotype and quality categories and to determine the clustering patterns described by the egg sample through a linear combination of quality-related traits. Canonical discriminant analysis was also

used to plot pairs of canonical variables building a territorial map to graphically interpret group differences. Variable selection was performed using regularized forward stepwise multinomial logistic regression algorithms as suggested by Marín Navas et al. [32]. Priors were regularized based on group sizes computed from the prior probability option in SPSS version 26.0 software rather than considering them to be equal, to prevent group with different sample sizes from affecting the quality of classification [33]. As the previous authors suggested, the statistical analysis used in the present research has been reported to be robust when sample sizes between groups are highly unequal. To palliate potential distortion effects, the smallest sample size should be at least 20 for every 4 or 5 predictors, and the maximum number of independent variables should be n − 2, where n is the sample size [34]. However, the fact of having 4 or 5 times more observations and dependent variables than previously described makes the discriminant approaches efficient [32]. This requirement is far surpassed in the present study, so the distorting effects mentioned are avoided.

Multicollinearity is a statistical phenomenon in which two or more variables are reciprocally dependent upon other variables in a way such that one can be linearly predicted from the rest with a high degree of accuracy. Multicollinearity analysis was performed before discriminant analysis to ensure that the regressors used were independent, so the variables chosen by the forward or backward stepwise selection methods were the same. Then, the forward stepwise selection method was chosen, as it is less time-demanding than the backward selection method.

Canonical discriminant analysis was performed by the use of the Discriminant routine of the Classify package of the SPSS version 26.0 software and the Discriminant Analysis routine of the Analyzing Data package of XLSTAT Pearson Edition.

#### 2.6.1. Multicollinearity Preliminary Testing

The multicollinearity assumption was tested to discard redundancies in the variables considered so that this phenomenon does not condition the structure of the matrices or overinflate the explanatory potential of variance, before performing a discriminant canonical analysis [32]. The variance inflation factor (VIF) was computed and used as an indicator of multicollinearity, following the formula:

$$\mathrm{VIF} = 1/\left(1 - \mathrm{R}^2\right) \quad (3)$$

where $R^2$ is the coefficient of determination of the regression equation. A VIF value of 5 was accepted in the present research, as reported by other authors [35]. The amount of variability in a certain independent variable that is not explained by the rest is called the tolerance and is calculated as $1 - R^2$ [36]. If tolerance has values lower than 0 and, simultaneously, the value of VIF is $\geq$10, multicollinearity can be considered a problem. For this, the Linear routine of the Regression package of the SPSS, version 26.0 software was used.

#### 2.6.2. Canonical Correlation Dimension Determination

The maximum number of canonical correlations (interpreted as Pearson's ρ) between two sets of variables is the number of variables in the smaller set. Although the first canonical correlation may often explain most of the relationship between sets, all canonical correlations must be considered [37]. Canonical correlation values of $\geq$0.30 may be indicative of a statistically significant dimension.

#### 2.6.3. Canonical Discriminant Analysis Efficiency

Variables that may significantly contribute to the discriminant function are evaluated by Wilks' lambda test. As Wilks' lambda approximates to 0, the contribution of the variable to the discriminant function increases. Functions can be used to explain group ascription if the significance (tested using $\chi^2$) is below 0.05 [38].

#### 2.6.4. Canonical Discriminant Analysis Model Reliability

The assumption of equal covariance matrices was evaluated through Pillai's trace criterion, which is the only acceptable test to be used in cases of unequal sample sizes [32,39]. Pillai's trace criterion was computed using the Multivariate routine of General Linear Model package of the software SPSS, version 26.0 software. Statistical differences in the dependent variables across the levels of independent variables are considered when significance is below 0.05.

#### 2.6.5. Variable Dimensionality Reduction

The overall variables were minimized to a few significant variables that contributed most to the different variations in the different types of eggs using a preliminary principal component analysis (PCA).

#### 2.6.6. Canonical Coefficients and Loading Interpretation and Spatial Representation

The percentage of allocation of an egg within its group (defined by its commercial size and the genotype of the hen that laid it) was determined using a discriminant function analysis. The variables that presented a discriminant loading of $\geq |0.40|$, were considered to be substantially discriminant. Non-significant variables were excluded from the function using stepwise procedures. The larger the absolute coefficients for each particular variable within a set, the better the discriminating ability [32]. Data were standardized following the premises described by Manly and Alberto [40]. Afterward, squared Mahalanobis distances were calculated. Squared Mahalanobis distances between groups were obtained using the following formula:

$$D_{ij}^2 = (\bar{Y}_i - \bar{Y}_j)\, COV^{-1} (\bar{Y}_i - \bar{Y}_j) \quad (4)$$

where $D_{ij}^2$ is the distance between population i and j; $\bar{Y}_i$ and $\bar{Y}_j$ are the means of variable x in the ith and jth populations, respectively; $COV^{-1}$ is the inverse of the covariance matrix of measured variable x.

The squared Mahalanobis distance was used to graphically depict the clustering patterns defined by the differences in the values for quality-related traits across the potential egg classifications considered in the present research. To this aim, a dendrogram representing the possible categories within egg quality classification was constructed using the underweighted pair-group method arithmetic averages (UPGMA) from the Universität Rovira i Virgili (URV), Tarragona, Spain, and the Phylogeny procedure of MEGA X 10.0.5 (Institute of Molecular Evolutionary Genetics, The Pennsylvania State University, State College, PA, USA).

#### 2.6.7. Discriminant Function Cross-Validation

The hit ratio can be defined as the percentage of correctly classified observations [41]. The leave-one-out cross-validation option was used to validate the discriminant functions used. The classification rate must be at least 25% higher than obtained by chance to be considered accurately enough [32].

Press' Q significance test was used to compare the discriminating power of the cross-validated function by using the following formula:

$$Press'Q = [N - (nK)]^2 / [N(K - 1)] \quad (5)$$

where N is the number of observations in the sample; n is the number of observations correctly classified; and K is the number of groups. Subsequently, the value of Press' Q statistic was compared with the critical value of 6.63 for $\chi^2$ with one degree of freedom in a significance of 0.01. If Press' Q exceeds the critical value of $\chi^2$ = 6.63, the cross-validated classification can be considered significantly better than chance.

## 3. Results

### 3.1. Canonical Discriminant Analysis Model Reliability

Egg weight, major diameter, minor diameter, shell b*, albumen height, and presence or absence of visual defects in yolk and/or albumen showed VIF values over 5 and were discarded from further analyses. A summary of the value of tolerance and VIF for each variable is shown in Table 3.

**Table 3.** Multicollinearity analysis of quality-related traits of eggs to discard for redundant variables.

| Statistics/Parameters | Tolerance ($1 - R^2$) | VIF |
|---|---|---|
| Shell L* | 0.2042 | 4.8980 |
| Shell a* | 0.2657 | 3.7642 |
| Yolk weight | 0.4428 | 2.2585 |
| Yolk diameter | 0.4504 | 2.2204 |
| Eggshell strength | 0.5250 | 1.9047 |
| Eggshell weight | 0.5526 | 1.8096 |
| Area under the force–displacement curve | 0.5733 | 1.7441 |
| Yolk color | 0.5877 | 1.7016 |
| Yolk a* | 0.6125 | 1.6326 |
| Yolk b* | 0.6184 | 1.6171 |
| Albumen weight | 0.6744 | 1.4829 |
| Eggshell thickness | 0.7037 | 1.4212 |
| Yolk L* | 0.7044 | 1.4196 |
| Haugh units | 0.7541 | 1.3261 |
| Albumen pH | 0.8314 | 1.2027 |
| Yolk pH | 0.8445 | 1.1841 |
| Shape index | 0.8735 | 1.1448 |

Interpretation thumb rule: variance inflation factor (VIF) = 1 (not correlated); 1 < VIF < 5 (moderately correlated); VIF $\geq$ 5 (highly correlated). VIFs > 5 are not presented in the table.

Pillai's trace criterion reported a significant difference across the different egg quality classification groups considered in the study ($p < 0.05$; Table 4).

**Table 4.** Summary of the results of Pillai's trace of equality of covariance matrices of canonical discriminant functions to determine the idoneity of data for discriminant canonical analyses to be performed.

| Parameter | Value |
|---|---|
| Pillai's Trace Criterion | 2.5016 |
| F (Observed value) | 4.7313 |
| F (Critical value) | 1.1357 |
| df1 | 323 |
| df2 | 8857 |
| Significance | <0.0001 |
| alpha | 0.05 |

F, Snedecor's F; df1, numerator degrees of freedom for the F-approximation; df2, denominator degrees of freedom for the F-approximation.

### 3.2. Canonical Coefficients, Loading Interpretation, and Spatial Representation

Six discriminating canonical functions were identified in the discriminating canonical analysis (Table 5). Table 6 reports the outcomes of discriminating ability testing. Higher eigenvalues were indicative of higher discriminatory power. Functions F1 and F2 with eigenvalues greater than 1 explain 75.63% of the total variance, while the rest contribute to the explanation of the variance with a low percentage of the information to the analysis.

**Table 5.** Canonical variable functions and percentages of self-explained and cumulative variance.

| Function | Eigenvalue | Variance, % | Canonical Correlation | Cumulative Variance, % |
|---|---|---|---|---|
| F1 | 3.2788 | 55.0163 | 0.8754 | 55.0163 |
| F2 | 1.2287 | 20.6172 | 0.7425 | 75.6335 |
| F3 | 0.4021 | 6.7477 | 0.5355 | 82.3812 |
| F4 | 0.3055 | 5.1253 | 0.4837 | 87.5064 |
| F5 | 0.2160 | 3.6241 | 0.4215 | 91.1306 |
| F6 | 0.1430 | 2.4002 | 0.3538 | 93.5307 |

**Table 6.** Canonical Discriminant analysis efficiency parameters to determine the significance of each canonical discriminant function.

| Test of Functions | Wilks' Lambda | Chi Square | df | Significance |
|---|---|---|---|---|
| 1 through 17 | 0.011 | 1320.792 | 323 | <0.001 |
| 2 through 17 | 0.063 | 802.254 | 288 | <0.001 |
| 3 through 17 | 0.155 | 541.386 | 255 | <0.001 |
| 4 through 17 | 0.254 | 398.607 | 224 | <0.001 |
| 5 through 17 | 0.358 | 298.246 | 195 | <0.001 |
| 6 through 17 | 0.474 | 217.171 | 168 | 0.010 |

df: degrees of freedom.

After discarding redundant variables, the test of equality of group means across egg quality classification groups was used to rank variables depending on their discriminating properties (Table 7).

**Table 7.** Results for the tests of equality of group means to test for difference in the means across egg groups once redundant variables have been removed.

| Variables | Rank | Wilks' Lambda | F | df1 | df2 | Significance |
|---|---|---|---|---|---|---|
| Albumen weight | 1 | 0.335 | 54.380 | 19 | 521 | <0.0001 |
| Eggshell weight | 2 | 0.539 | 23.440 | 19 | 521 | <0.0001 |
| Yolk weight | 3 | 0.566 | 21.010 | 19 | 521 | <0.0001 |
| Yolk diameter | 4 | 0.651 | 14.680 | 19 | 521 | <0.0001 |
| Haugh units | 5 | 0.700 | 11.760 | 19 | 521 | <0.0001 |
| Yolk b* | 6 | 0.764 | 8.470 | 19 | 521 | <0.0001 |
| Shape index | 7 | 0.800 | 6.860 | 19 | 521 | <0.0001 |
| Yolk color | 8 | 0.812 | 6.360 | 19 | 521 | <0.0001 |
| Area under the force–displacement curve | 9 | 0.832 | 5.550 | 19 | 521 | <0.0001 |
| Eggshell strength | 10 | 0.837 | 5.350 | 19 | 521 | <0.0001 |
| Shell L* | 11 | 0.844 | 5.080 | 19 | 521 | <0.0001 |
| Yolk a* | 12 | 0.845 | 5.050 | 19 | 521 | <0.0001 |
| Shell a* | 13 | 0.870 | 4.090 | 19 | 521 | <0.0001 |
| Eggshell thickness | 14 | 0.892 | 3.320 | 19 | 521 | <0.0001 |
| Yolk L* | 15 | 0.908 | 2.780 | 19 | 521 | <0.0001 |
| Yolk pH | 16 | 0.941 | 1.710 | 19 | 521 | 0.0300 |
| Albumen pH | 17 | 0.957 | 1.250 | 19 | 521 | 0.2200 |

F, Snedecor's F; df1, numerator degrees of freedom for the F-approximation (groups minus 1); df2, denominator degrees of freedom for the F-approximation (observations minus 1).

The greater the value of F and the lower the value of Wilks' lambda for a certain variable, the better its discriminating power was, and hence, the higher its position in the rank was as well.

As shown in Table 8, standardized discriminant coefficients were evaluated. This allowed us to determine the possibility of a reduction in the discriminant power of individual variables as a result of multicollinearity between pairs.

**Table 8.** Discriminant loadings for external and internal quality–related traits determining the relative weight of each trait on each canonical discriminant function.

| | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| Eggshell strength | −0.20 | −0.26 | −0.15 | 0.51 | 0.33 | −0.06 |
| Yolk L* | −0.08 | 0.02 | 0.07 | −0.10 | 0.51 | 0.18 |
| Yolk a* | −0.07 | −0.35 | −0.13 | −0.53 | −0.11 | −0.03 |
| Shape index | −0.04 | −0.04 | 0.69 | −0.13 | −0.05 | 0.27 |
| Shell a* | −0.04 | 0.05 | 0.09 | 0.64 | −0.22 | −0.62 |
| Yolk pH | −0.03 | 0.13 | −0.12 | −0.07 | 0.11 | 0.32 |
| Haugh units | −0.03 | −0.45 | 0.00 | 0.21 | 0.37 | −0.27 |
| Yolk b* | −0.02 | 0.44 | −0.01 | 0.15 | −0.47 | −0.48 |
| Area under the force–displacement curve | 0.01 | 0.28 | 0.43 | 0.02 | −0.06 | 0.04 |
| Albumen pH | 0.02 | 0.07 | 0.07 | 0.03 | −0.10 | −0.04 |
| Shell L* | 0.04 | −0.04 | 0.14 | 0.60 | 0.02 | −0.55 |
| Yolk color | 0.05 | 0.31 | 0.37 | −0.08 | −0.01 | 0.28 |
| Eggshell thickness | 0.06 | 0.06 | 0.23 | 0.29 | 0.09 | 0.05 |
| Yolk diameter | 0.08 | 0.35 | −0.12 | −0.08 | 0.29 | −0.36 |
| Yolk weight | 0.20 | 0.43 | 0.12 | 0.12 | 0.43 | 0.08 |
| Eggshell weight | 0.56 | −0.20 | 0.40 | −0.33 | −0.07 | −0.47 |
| Albumen weight | 0.83 | −0.07 | −0.21 | 0.30 | −0.27 | 0.27 |

The substitution of the values for quality-related traits in the first two discriminating functions was performed to obtain $x$ and $y$-axis coordinates, for the first and second dimensions, respectively. Once coordinates were obtained, each egg observation was sorted and classified across the different egg quality classification categories and laying hen genotype. Coordinates were used to depict eggs on a territorial map (Figure 1). Centroids represent the means of the discriminant function scores by egg quality classification group for each function calculated.

In this regard, Mahalanobis distances were used as they represent the probability that a case of an unknown background belongs to a particular egg quality classification group. It can be calculated through the relative distance of the problem egg to the centroid of its closest group. The probability of classification of observation into a group was calculated, following the premises in Hair et al. [42].

Consequently, the hit ratio, or successfully classified cases, was determined (Supplementary Table S1). Mahalanobis distances obtained after the evaluation of the discriminant analysis matrix were transformed into squared Euclidean distance and represented in Figure 2.

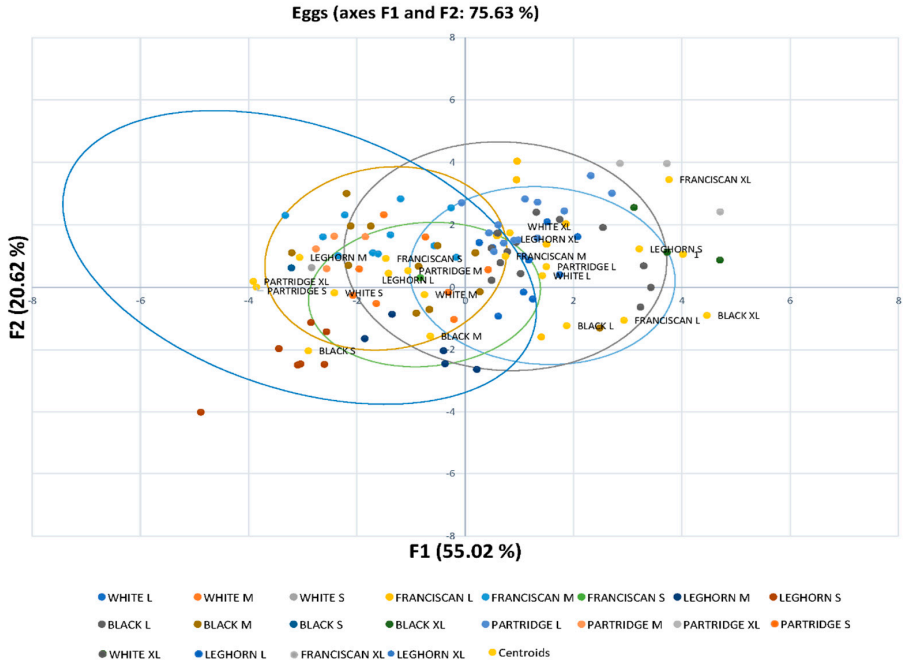


**Figure 1.** Territorial map depicting the eggs considered in the canonical discriminant analysis sorted across commercial quality categories (S, M, L, and XL) and laying hens genotypes (Leghorn and White, Black Franciscan, and Partridge Utrerana varieties). Centroids or canonical group means are the means for each group's canonical observation scores. The larger the difference between centroids, the better the predictive power of the canonical discriminant function in classifying observations.

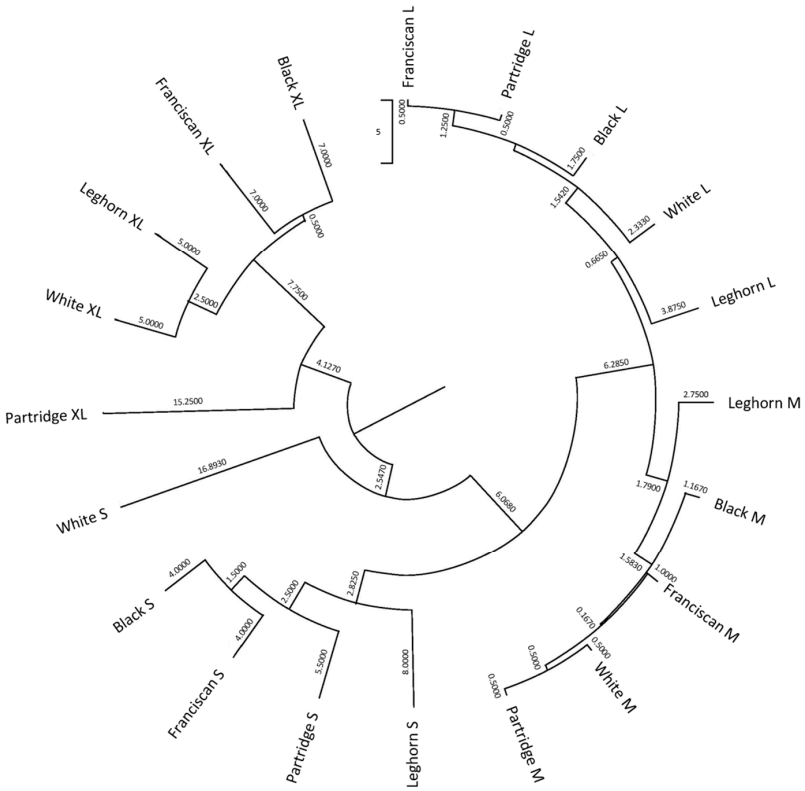


**Figure 2.** Cladogram constructed from Mahalanobis's distances between commercial quality classification categories and laying hen genotypes.

### 3.3. Discriminant Function Cross-Validation

Classification and leave-one-out cross-validation matrices were evaluated (Supplementary Tables S1 and S2). In all, 73.2% of original grouped cases were correctly classified in the different egg quality classification group, from which 57.1% of clustered observations were cross-validated. A Press' Q value of 5297.17 (N: 541; n: 396; K: 20) was obtained; hence, predictions were considered to be significantly better than those that would be obtained by chance at 95% [43].

## 4. Discussion

Involving autochthonous breeds in animal production systems may promote the evolution of sustainable ways of producing. Native breeds can be used in search of productive improvement by taking advantage of genotypes adapted to the climatology and orography, as well as to the technical, productive, and cultural conditions of the area. On the other hand, commercial chains are increasingly requesting more products derived from non-industrial processes. This context makes it necessary to characterize the eggs of the Utrerana avian breed according to their commercial size while defining how the different quality-related parameters affect the differentiation between eggs across the different varieties and breeds studied. The results obtained in the present study may suggest how to approach the different strategies to make an endangered breed profitable, thus ensuring its conservation by establishing production models to which it is adapted.

The selection of the individuals in the sample was performed considering that the hybrid commercial cycle and both genotypes reach 50% of laying (egg production during a laying cycle). Contextually, the typical production cycle in commercial layers (Leghorn hens among others) lasts about 72 weeks [44]. However, this cycle may extend until 156 weeks in around a third of the Utrerana population [45]. Additionally, according to Kuo et al. [46], the age at sexual maturity is estimated by age in weeks when 50% egg production is reached. In this regard, the same authors suggested the age when 50% egg production in White Leghorn is reached to be around 21 weeks. By contrast, the information reported by Orozco Piñán [45] suggested the average age of Utrerana hens at the moment of the first laying was 25 weeks. Furthermore, the breeding criterion of both breeds may differ, as while White Leghorn hens breeders have traditionally selected animals for precocity [47], Utrerana breeders have not sought this trait as a priority rather benefiting from the natural lay cycle of the breed [45]. Zita et al. [48] suggested that egg quality characteristics are affected by the interaction of genotype (breed and strain) and hen's age, rather than exerting their effects independently.

Multicollinearity analyses revealed high correlations between major diameter and minor diameter and egg shape index, since both measurements comprise the formula for its calculation. The same happens with the formula of Haugh units, which includes the variables of egg weight and albumen height, which consequently were eliminated due to multicollinearity problems. Moreover, egg weight can be calculated by separately summing albumen weight, yolk weight, and eggshell weight variables, which may be the logical source for the redundancies detected.

Degree of lightness (L*) and chromaticity coordinates (a* and b*) comprise the L*a*b* color space [49]. In this context, coordinates of shell a* and shell b* are difficult to interpret and can be correlated in white-shelled eggs, such as those of the Utrerana avian breed [20]. As suggested by other authors [50], a* and b* parameters measure chromaticity. More specifically, redness–greenness and yellowness–blueness, respectively. Positive values of a* are linked to increased amounts of redness in eggshell color, whereas negative values of a* relate to increased amounts of greenness in the eggshell color.

Similarly, the representativity of yellow and blue components in eggs of any color are represented by positive and negative values of b*, respectively. In this context, Odabaşi et al. [50], suggested that the lighter the shell color (higher L*), the lesser the redness of the color of the eggshell is as well. This was in line with the results reported by Aygun [51],

who reported shell L* could be considered as a discriminative color criterion as the lesser the amount of shell L*, the darker the eggshell color turns to be.

The visual defects in yolk and albumen are produced by meat and blood spots. The presence of these visual defects is regarded as an undesirable feature in eggs that causes rejection by consumers [52].

These undesirable findings may stem from the synthesis of the different parts of the egg during ovulation due to the rupture of an ovarian follicle at a different position from the stigma [53]. In these situations, variations in the chromaticity coordinates of the yolk color could appear, thus, may be one of the sources of multicollinearity problems between the presence of visual defects and yolk a* and yolk b*.

Albumen weight, eggshell weight, and yolk weight variables reported the best discriminating properties (Table 7). These three quality-related traits compose the egg weight, which is the main criterion on which the commercial classification of eggs relies. At the same time, albumen represents about 55–65% of the egg weight [54,55]. This explains the fact that albumen weight was ranked first at the test of equality of group means.

Hen strain has been reported to significantly affect albumen ratio [53,56]. Albumen is critical for the survival of the chicken embryo and the variations in the content of albumen in hen eggs can generate differences in skeletal muscle or liver metabolism during embryonic development [57]. In laying hens, albumen has great commercial importance, provided its unique functional properties and its use as an ingredient in a large number of culinary international preparations [58]. In previous studies, the Leghorn has been demonstrated to have a higher albumen weight than the Utrerana avian breed, due to the Leghorn's higher concentration of energy reserves [14]. Contextually, Peña-Villalobos et al. [57] suggested a significant reduction in metabolic rate occurs in the last fifth of embryonic life in albumen-removed eggs, which in turn derives into reduced catabolic activities in the skeletal muscle of chicks that eventually hatch.

Utrerana has been reported to present a lower eggshell weight and a higher yolk weight than Leghorns [14]. These results agree with the present research since these parameters have a high discriminating power when clusters differentiate. Modern commercial breeds showed clear differences in terms of eggshell weight when compared to native poultry, due to the high selection of all egg traits of eggs for its transport and commercial purposes [59,60].

Differences in the proportion of egg yolk have been reported between breeds and within highly productive laying hens strains such as the White Leghorn, which may be indicative of the presence of sufficient additive genetic variation [61]. Furthermore, selection based on additive genetic variation in yolk weight has been suggested as an option to promote seeking sustainability of local eggs [62], as native breeds could satisfy the growing demand for more energetically efficient eggs in the market [13,14,26].

Yolk diameter and Haugh units reported the best discriminating properties (fourth and fifth position in the rank) after weight-related traits (albumen, eggshell, and yolk weights). The relevance of these traits may be ascribed as suggested by Ukwu et al. [63], who reported significant differences in yolk weight and albumen height among light (less than 49.99 g), medium (50–55 g), and heavy eggs (more than 55 g) of Isa Brown egg layer chickens in Nigeria. This has also been reported by Alkan et al. [64], who addressed a parallel increase in yolk diameter as egg weight increases in partridge eggs. However, no differences between Utrerana and Leghorn breeds were detected in previous studies [14].

Haugh units are used as an indicator of internal egg quality [30]. Time of storage and storage conditions affect Haugh units values [65]. However, the strains or breed of the hen have been reported to quantitatively affect them. For instance, several authors have reported higher values for Haugh units in local breeds than in commercial hybrid strains [4,13,66]. In any case, albumen height is correlated with the percentage of albumen [67]. Hence, commercial strains could present a certain advanced position, provided a larger percentage of albumen is found in hybrid strains in comparison to that in native breeds.

Values for pH-related traits showed the lowest values of F and highest for Wilks' lambda. Egg pH allows the assessment of the egg's freshness [68,69]. The loss of $CO_2$ and $H_2O$ inside the egg produces an increase in albumen pH. The time of storage and high temperatures condition this loss of $CO_2$ and $H_2O$ and promote a decrease in albumen viscosity and flavor with detrimental effects for egg quality [19,70]. Albumen and yolk pH can be slightly influenced by the hen strain [13,14,71]. Nevertheless, in the present study, when all egg pH values were measured during the 24 h following oviposition, it was found that albumen pH and yolk pH have a low discriminating power between different groups of eggs, which may derive from the low variability in pH found. Such lack of variability may stem from the fact that the eggs considered in this study were fresh enough for those eggs presenting slightly lower values not to be detrimental on egg quality. Additionally, this finding may evidence a patent lack of importance provided to quality traits (such as the pH of the components of the egg) against quantitative traits among the current criteria that are considered for egg quality classification, as the quantity of the product may be better commercially valued than its quality. However, this commercial strategy may be erroneous given it may not match the current general trend of the customers preferring egg quality over quantity [72].

Figure 2 reports that egg quality classification clusters are mainly grouped depending on their commercial size. In addition, the Leghorn's egg groups differed from the rest of those from the Utrerana varieties, except for those of White Utrerana XL and Leghorn XL eggs, which reported a certain closeness. This may be indicative of the hybridization of the White Utrerana with the Leghorn breed, both with white plumage, which may have been historically performed by breeders as an attempt to decrease the consanguinity of the white variety, which is the Utrerana variety accounting with the smallest number of animals and the one which faces the highest endangerment risk.

Additionally, the present study may confirm the fact that product differentiation could be a feasible opportunity for the eggs of Utrerana varieties, which could constitute a favorable point when compared to eggs from other breeds that have traditionally been sold in the market [73].

The present discriminating tool allows to efficiently classify eggs based on quality-related traits as supported by the 73.2% of observations being correctly classified within their group. In this regard, weight traits play a pivotal role in the determination of the commercial quality of eggs.

All eggs belonging to the S category in White, Franciscan and Partridge, and L category of Franciscan and Partridge were correctly classified (Supplementary Table S1). However, 45.5% of M category Partridge eggs were classified as Franciscan M. Previous research suggested Partridge and Franciscan varieties present a significantly heavier yolk and slightly lower weight than the rest of the varieties or the Leghorn breed [14], hence similarities between egg quality-related traits of these two varieties could be expected. Moreover, 26.7% of XL category Leghorn eggs were classified as Leghorn L eggs, which may be explained as commercial genotypes have been selected to produce rather homogenous eggs, which may translate to a reduction in differences [74,75]. Furthermore, it may be worth mentioning that 23.8% of M category White Utrerana eggs were classified as M category Leghorn ones, with the likely hybridization between these two strains being the potential source for these similarities.

## 5. Conclusions

The present discriminating method has been proved and validated as an efficient tool to correctly classify eggs considering both external and internal traits. Additionally, this research confirms the fact that product differentiation could be a feasible opportunity for the eggs of Utrerana varieties, which could constitute a favorable point when compared to eggs from other breeds that have traditionally been sold in the market. Weight traits play a pivotal role in the determination of the commercial quality of eggs. This may evidence a patent lack of commercial attention provided to quality traits in favor of quantitative traits.

However, this commercial strategy may be erroneous given it may not match the current general trend of customers preferring egg quality over quantity. Partridge and Franciscan classification confusion may derive from the fact that these varieties present significantly heavier yolks and slightly lower weights. Similarities between the eggs of White Utrerana and Leghorn hens may evidence reminiscences of hybridization.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/2304-8158/10/3/632/s1, Table S1: Appropriately classified eggs (%) according to the commercial size and genotype of the laying hen; Table S2: Leave-one-out cross-validation (%) of eggs according to the commercial size and genotype of the laying hen.

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