J. Dairy Sci. 106:3641–3649
https://doi.org/10.3168/jds.2022-22704

Effects of chronic low-dose aflatoxin B_1 exposure in lactating Florida dairy goats

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ABSTRACT

In the past few years there has been a growing trend in the prevalence of aflatoxins, attributable to climate change, in substances destined for animal feeding, together with an increase in dairy product consumption. These facts have triggered great concern in the scientific community over milk pollution by aflatoxin M_1. Therefore, our study aimed to determine the transfer of aflatoxin B_1 from the diet into milk as AFM_1 in goats exposed to different concentrations of AFB_1, and its possible effect on the production and serological parameters of this species. For this purpose, 18 goats in late lactation were divided into 3 groups (n = 6) and exposed to different daily doses of aflatoxin B_1 (T1 = 120 μg; T2 = 60 μg, and control = 0 μg), during 31 d. Pure aflatoxin B_1 was administered 6 h before each milking in an artificially contaminated pellet. The milk samples were taken individually in sequential samples. Milk yield and feed intake were recorded daily, and a blood sample was extracted on the last day of exposure. No aflatoxin M_1 was detected, either in the samples taken before the first administration, or in the control group ones. The aflatoxin M_1 concentration detected in the milk (T1 = 0.075 μg/kg; T2 = 0.035 μg/kg) increased significantly on a par with the amount of aflatoxin B_1 ingested. The amount of aflatoxin B_1 ingested did not have any influence on aflatoxin M_1 carryover (T1 = 0.066% and T2 = 0.060%), these being considerably lower than those described in dairy goats. Thus, we concluded that the concentration of aflatoxin M_1 in milk follows a linear relationship with respect to the aflatoxin B_1 ingested, and that the aflatoxin M_1 carryover was not affected by the administration of different aflatoxin B_1 doses. Similarly, no significant changes in the production parameters after chronic exposure to aflatoxin B_1 were observed, revealing a certain resistance of the goat to the possible effects of that aflatoxin.

Key words: aflatoxin B_1, aflatoxin M_1, carryover, mycotoxins

INTRODUCTION

Aflatoxins are considered the most important mycotoxins worldwide in animal and human feeding due to their carcinogenicity and hepatoxicity (Strosnider et al., 2006). In this respect, the International Agency for Research on Cancer (IARC) has concluded that there is sufficient evidence in humans of the carcinogenicity of the aflatoxins B_1, B_2, G_1, G_2, and M_1, which can cause liver cancer (hepatocellular carcinoma). That carcinogenicity is produced by a genotoxic action mechanism, involving the activation of an epoxide metabolite, DNA adduct formation, and the modification of the tumor suppressor gene *TP53*. Thus, aflatoxins are included in group 1 as cancerigenous substances for humans (IARC, 2012).

These mycotoxins are difuranocoumarins, mainly produced by 2 species of *Aspergillus*, *Aspergillus flavus* and *Aspergillus parasiticus*, which contaminate grain and cereals at several stages during harvesting, transport, or the storage of raw materials (Kumar et al., 2017). In addition, *A. flavus*, the principal aflatoxin-producing fungus, adapts itself very well to warm, dry, climate conditions. Therefore, the European Food Safety Authority's Unit for Emerging Risks (EFSA) has considered the effect of climate change to be a key factor in the greater risk of aflatoxin pollution in European crops (Battilani et al., 2012, 2016).

The most toxic aflatoxin is B_1 (**AFB_1**). This becomes biotransformed in the liver by means of enzymes belonging to the cytochrome P450 superfamily into various metabolites, among which is aflatoxin M_1 (**AFM_1**; Deng et al., 2018; Rushing and Selim, 2019). With respect to AFM_1, this is a hydroxylated metabolite that can be found in mammal milk which have ingested

Received August 27, 2022.
Accepted November 17, 2022.
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some AFB_1-contaminated feed (Creppy, 2002). In addition to milk, aflatoxins can be excreted in eggs, urine, semen, bile, and feces (Coppock et al., 2018).

Although all animal species are susceptible to aflatoxins, ruminants are regarded as being less sensitive than monogastric species due to ruminal detoxification processes (Gallo et al., 2015). Because of their lesser susceptibility, the chief repercussion of chronic exposure in these animals lies in the decline in their production parameters and, above all, in the presence of residues in their milk (Mostrom and Jacobsen, 2020). In this sense, AFM_1 is thermally stable and, once it is present in raw milk, it is highly unlikely that its concentration will be reduced, in spite of applying routine heat treatments such as pasteurization or sterilization (Becker-Algeri et al., 2016). Another key factor in small ruminant milk is that there is no physical method capable of preventing the binding of AFM_1 to whey proteins, which implies a higher AFM_1 concentration in cheese (Barbiroli et al., 2007). This is especially relevant in some continents such as Europe, Oceania, and America because cheese production from goat milk is a significant industry (Silanikove et al., 2010).

According to the corporative statics database of the Organization for Agriculture and Food of the United Nations (FAOSTAT), in 2019, the world milk production reached 864,854 thousand tons, and the mean consumption per capita was established at 70.76 kg/yr. The production of raw goat milk contributed 23.9% of all milk production in that year, being Asia and Africa the main producers (FAOSTAT, 2022). In view of the importance to humans of consuming milk, AFM_1 limits in this food have been established in different legislations. In the European Union, the maximum AFM_1 levels in milk and processed dairy products must not exceed 0.05 μg/kg, reducing this limit to 0.025 μg/kg in infants and follow-on food preparations (European Commision, 2010). In addition, the maximum level of AFB_1 for compound feed for dairy cattle and calves, dairy ewes and lambs, and dairy goats and kids has been limited to 5 μg/kg (European Commision, 2011). In the United States, the Food and Drug Administration (FDA) has fixed an action level of 0.5 μg/kg in whole, low-fat, skim milk (US FDA, 2005).

The presence of AFM_1 in milk samples has been described in many studies in the past few years, thus evidencing a high incidence rate of this pollutant. Although concentrations in European countries are generally found below the maximum level permitted, the high concentrations and the degree of effect in developing countries demonstrate the need to study, monitor and control AFM_1 contamination (Mollayusefian et al., 2021; Saha Turna and Wu, 2021; Sharafi et al., 2022). However, international trade and the climate change have made AFM_1 also become a serious problem in developed countries (Frazzoli et al., 2017).

With the growing concern for AFM_1 in the dairy industry, in addition to the limited scientific studies on goats, we have considered that investigating the AFM_1 excretion dynamics for the milk of this species would be of great importance. Additionally, the repercussions of climate change on the concentration of aflatoxin in feed materials destined for animal feeding mark the target for studying the possible influence of chronic exposure to AFB_1 on production parameters in goats.

MATERIALS AND METHODS

Housing and Ethical Considerations

The experimental phase of the study was carried out in the Small Ruminant Experimental Unit at Córdoba University (Spain), a center registered as an establishment for the employment of animals for experimentation and other scientific purposes. All the applicable national, international, or institutional guidelines for the care and use of those animals were followed. All procedures complied with the instructions of the Animal Experimentation Committee at Córdoba University, following the indications of Directive 2010/63/EU (European Parliament, 2010).

Animals, Feeding, and Milking

Eighteen Florida breed goats in late lactation (>120 d in lactation) were used. They had a mean weight of 64.83 ± 3.12 kg, and were stabled individually so that one animal only could access to its food and drink troughs. They were fed with a ration of concentrate (protein: 17%; fat: 4.5%; fiber: 9.5%, and ash: 8.4%) established at 1,350 g/animal per d, and they had access to hay and water ad libitum. The absence of aflatoxins in concentrate and hay was determined by ELISA (Bio-Shield Aflatoxin Total ES, Prognosis Biotech). Regarding milking, the goats were mechanically milked individually using a portable milking machine at 1200 h. Concentrate intake was measured daily by weighing the amount left over from the previous day. Hay intake was not accounted. Milk yield also was measured daily by weighting the milk amount produced for every goat.

Experimental Design

The animals were randomly allocated to 3 groups (n = 6) exposed to different concentrations of AFB_1 [T1 = 120 μg/AFB_1 per d; T2 = 60 μg/AFB_1 per d; and control (CON) = 0 μg/AFB_1 per d]. These concentrations were chosen because they are environmentally relevant.

For example, Tarazona et al. (2020) determined a range of AFB_1 in maize kernel in Spain at 0.87 to 124.1 μg/kg. Pure AFB_1 (Sigma-Aldrich, A6636) was dissolved in methanol and administered by means of a an artificially contaminated pellet, 6 h before each milking session. According to Battacone et al. (2012) the average AFM_1 concentration in goat milk is higher at 3 and 6 h after the AFB_1 administration in a single dose with respect to others times. The contaminated pellet was placed directly into the oral cavity of each goat. Previously, the remaining amount of feed from the previous day had been removed and a new ration had been added. The experimental phase lasted 34 d, with an exposure time of 31 d. Previously, the animals spent 14 d in the facilities to acclimatize themselves. The health status of animals was monitored constantly during both periods.

Milk Composition Samples and Analysis

Once milking was finished and the amount of milk produced by each goat was weighed, 100-mL homogenized sample from each animal was taken to determine milk's composition (% protein, % fat, and % lactose). These samples were taken on the day before the first AFB_1 administration; on d 3 of exposure; and every 7 d successively up to the exposure end (d 31). The samples were stored at −18°C until their analysis.

The milk composition were ascertained with the MilkoScan FT120 (Foss Electric) milk analyzer in the Milk Control Laboratory, Faculty of Veterinary Medicine, at Córdoba University. Before their analysis, frozen milk samples were tempered in a 33°C water bath while carefully stirring to homogenize the fat.

AFM_1 in Milk and Analysis

To determine the AFM_1 excretion curve in milk, following the same mechanic, other 100-mL milk samples from each animal were taken the same days as for analyzing the milk composition. However, in this case, the first 2 and the last 3 d of the experimental phase were also sampled. These samples was frozen too until their posterior analysis when the study ended.

The AFM_1 in milk was determined in the Mass Spectrometry and Chromatography Unit of the Central Research Support Service at Córdoba University, by liquid chromatography coupled to a Tandem Mass Spectrometry detector (LC-MS/MS), following the protocol described by Pallarés et al. (2021). For this purpose, an Agilent 1200 chromatograph (Agilent Technologies), equipped with a 3200 QTRAP mass spectrometer (Applied Biosystems, AB Sciex), was deployed. The components of the samples obtained in the liquid chromatography were moved to the mass spectrometer by electrospray ionization.

Biochemical Parameters

The blood samples were extracted by jugular venipuncture into heparinized tubes on the last day of exposure and analyzed to find out their biochemical profile. Those samples were centrifuged at 1,400 × *g* for 10 min at 25°C to obtain the blood plasma. The plasma samples were analyzed with commercial colorimetric kits (BioSystems S.A.) by spectrophotometry. The biochemical parameters determined were glucose, cholesterol, urea, creatinine, total proteins, globulins, bilirubin, alkaline phosphatase, aminotransferase aspartate, and gamma-glutamyl transferase (**GGT**).

Data Analysis

The AFM_1 carryover was calculated individually for each animal, following the formula used by Aazami et al. (2019):

$$AFM_1 \text{ carryover} = [AFM_1 \text{ concentration } (\mu g/kg) \times \text{milk production } (kg/d)]/[AFB_1 \text{ ingested } (\mu g/d)] \times 100.$$

As in Battacone et al. (2009), the carryover of AFM_1 in milk was calculated when the toxin output in milk reached a steady state (from d 3 to 31 of the exposure period).

The statistical analysis of the data were carried out with SAS/STAT Software (version 15.2; SAS Institute Inc.). The normality of the data was verified by the Kolmogorov-Smirnov test, and they were analyzed following the mixed linear model reported by Battacone et al. (2003):

$$y_{ijk} = \mu + T_i + P_j + (T \times P)_{ij} + E_k + \varepsilon_{ijk},$$

where y = dependent variable (AFM_1 concentration, carryover, milk yield, milk composition and feed intake); μ = general mean; T_i = fixed effect of the dose of AFB_1 (i = 0; 60; 120 μg); P_j = fixed effect of the exposure time (j = day); E_k = random effect of each animal; and ε_{ijk} = residual error. The Tukey test was used as a post hoc method. For milk yield, milk composition and feed intake only data in exposure phase (d 1 to 31) were used. Regarding AFM_1 concentration, only steady-state (d 3 to 31) data were used.

Due to the non-normality in most of the data obtained on the biochemical parameters, the nonpara-

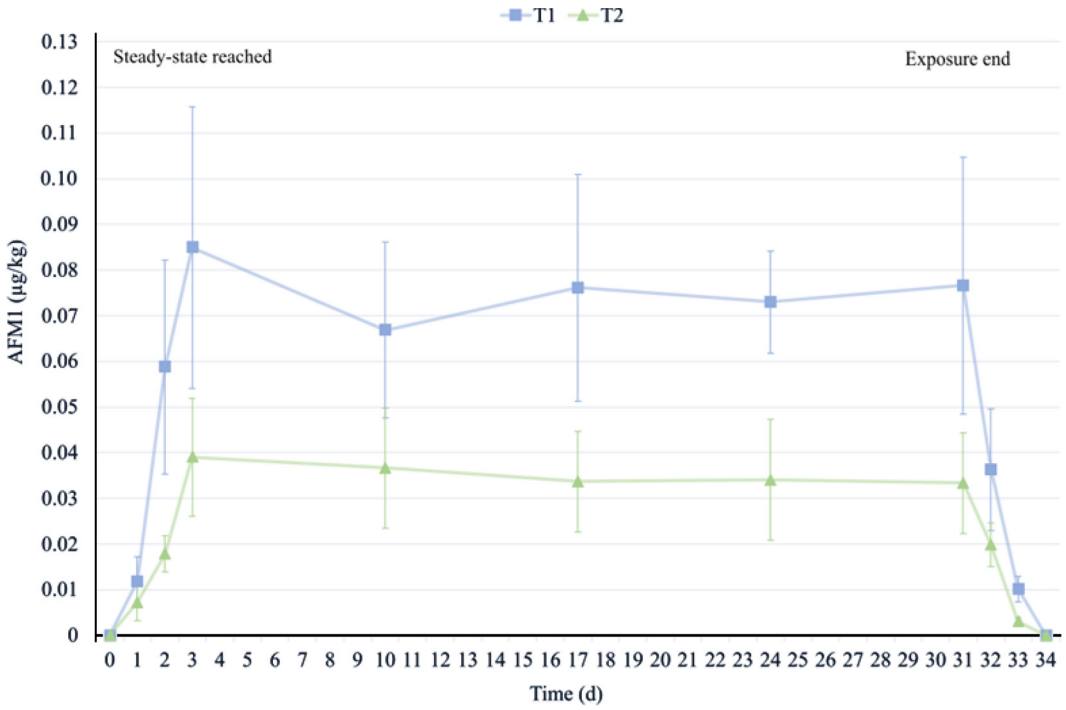

Figure 1. Aflatoxin M_1 (AFM_1) concentration in the milk of groups T1 (120 µg/d of aflatoxin B_1) and T2 (60 µg/d of aflatoxin B_1) throughout the study.

metric Kruskal-Wallis test was employed to establish the differences between the different groups. In this case, the data were presented as the median (25th–75th percentile). In all the cases, statistical significance was established for a value of $P < 0.05$. The data that support the findings of this study are available on request from the corresponding author.

RESULTS AND DISCUSSION

AFM_1 in Milk and Carryover

In the samples before exposure, no AFM_1 traces were detected in any case. Nor was AFM_1 found in the milk of goats belonging to the CON group during the whole experimental time. In groups T1 and T2, AFM_1 was detected from d 1 to 33. In these groups, at d 34 the milk was free of AFM_1. The AFM_1 concentration pattern in the milk of groups T1 and T2 is shown in Figure 1.

The presence of AFM_1 in the milk collected after AFB_1 exposure agreed with the results obtained in previous studies made by other authors, both in goats and in ewes exposed to a single dose of AFB_1 mixed with their feed (Battacone et al., 2003, 2012). In fact, its early appearance in the milk was due to the rapid absorption of the aflatoxins in the intestinal tract because it is possible to detect AFM_1 in blood plasma at scarcely 5 min after exposure to AFB_1, as was demonstrated by Gallo et al. (2008) in their study on cows. Although, it is possible that bovine mammary epithelial cells can metabolize AFB_1 into AFM_1 in a tiny percentage (Caruso et al., 2009), most of AFM_1 is derived from hepatocytes (Deng et al., 2018; Rushing and Selim, 2019). When AFM_1 reaches the mammary gland from blood circulation, it is able to excrete into milk via passive diffusion. However, the active transport mediated by effux transporter of the ABC-family in the epithelial cells of mammary gland could probably be more important than passive diffusion (Min et al., 2021).

In Figure 1, it can be observed how AFM_1 reaches its maximum peak on d 3 and the excretion is stable until d 31, when the exposure ends. After exposure, no traces of AFM_1 are found at 78 h. Battacone et al. (2012) reported that AFM_1 can be found in goat milk 1 h after the AFB_1 administration. In their study, the average AFM_1 concentration in goat milk was higher at 3 and 6 h after a single AFB_1 dose. In our case, in the first 6 h the AFM_1 concentration was low, it could be explained because they used much higher doses than us. Although it is possible to detect AFM_1 before, it is generally detectable at 12 h after the ingestion of aflatoxins and no traces of it are found 72 h after the removal of aflatoxins from the diet (Mollayusefian et

Table 1. Effect of the intake of different doses of aflatoxin B_1 [AFB_1; T1 = 120 μg/d, T2 = 60 μg/d, and CON (control) = 0 μg/d] on the concentration of aflatoxin M_1 (AFM_1) in the milk, and its carryover in Florida goats[1]

	Group				*P*-value[2]		
Parameter	CON	T1	T2	SEM	Treatment	Time	Interaction
AFB_1 (μg/d)	0	120	60	—	—	—	—
AFM_1 (μg/kg)	0[a]	0.075[b]	0.035[c]	0.0036	<0.001	0.871	0.703
Carryover[3] (%)	0[a]	0.066[b]	0.060[b]	0.0361	<0.001	0.696	0.596

[a–c]Means within a row with different superscripts significantly differ ($P < 0.05$).

[1]Data shown belong to steady-state period (d 3 to 31).

[2]Treatment = AFB_1 doses; Time = exposure time; Interaction = treatment × time interaction.

[3]Carryover = percentage of AFB_1 which turns into AFM_1 and is excreted in the milk.

al., 2021). Frobish et al. (1986), in dairy cows, reported that, at 24 h after exposure to AFB_1, the mean AFM_1 concentrations were already near to being stationary. Also in cows, Diaz et al. (2004) observed similar dynamics given that the AFM_1 concentration was in a steady state after approximately 48 h. Both studies reached steady state before us. However, Masoero et al. (2007), who also observed that AFM_1 appeared quickly in milk, determined that the steady state was reached later, between d 7 and 12 after AFB_1 intake.

In the steady state, the mean concentrations of AFM_1 determined for groups T1 and T2 were of 0.075 ± 0.023 μg/kg and 0.035 ± 0.012 μg/kg, respectively (Table 1). Also, the minimum and maximum values were of 0.042–0.129 for group T1 and 0.018–0.062 for group T2. The results of the statistical analysis made with the mixed linear model showed the significant influence of the effect of the AFB_1 dose ingested ($P < 0.01$) on the AFM_1 concentration in the milk, whereas no significant differences were found for the exposure time and the interaction between effects (Table 1). The post hoc analysis indicated that group T1, exposed to a higher dose of AFB_1, excreted significantly more AFM_1 than group T2, that was exposed to a lower dose ($P < 0.01$). Nevertheless, it is possible that an animal exposed to a lower AFB_1 concentration may excrete more AFM_1 than another one exposed to a higher concentration, in specific situations because the maximum AFM_1 concentration determined for group T2 established at 0.063 μg/kg was higher than the minimum concentration for T1, at 0.042 μg/kg.

Furthermore, whereas AFM_1 excretion is stable, we determined a positive linear relationship between the AFB_1 ingested and the AFM_1 concentration in milk. This type of relationship has already been described previously by other authors (Price et al., 1985; Battacone et al., 2003, 2005). This relationship can be expressed by the following equation:

$$AFM_1\ (\mu g/kg) = 0.00063 \times AFB_1\ (\mu g/d);$$

$$SE = 0.0148;\ R^2 = 0.81;\ P < 0.01.$$

For this equation, no significant values were found for the intersection ($P = 0.75$).

The mean AFM_1 carryovers are shown in Table 1. These values were 0.066 ± 0.020% and 0.060 ± 0.021% for groups T1 and T2, respectively. It is also important to highlight that there was a certain variability between the animals in the carryover regardless of the group because the minimum percentage detected was established at 0.032% and the maximum was as high as 0.129%.

The same as happened with the AFM_1 concentration in the milk, the statistical analysis demonstrated that the AFB_1 dose had a significant effect, although there were no significant differences in the exposure time or in the interaction between effects. However, the post hoc analysis did not show any significant differences ($P = 0.37$) in the carryover between groups T1 and T2 as it happened with AFM_1 concentration. In this case, both groups presented significant differences ($P < 0.01$) with respect to the CON group. The dynamics of the carryover and the AFM_1 concentration throughout the study in groups T1 and T2 is shown in Figure 2.

The same as us, Veldman et al. (1992), in dairy cows, and Battacone et al. (2003) in ewes, determined that this variable is not significantly affected by the dose of AFB_1 ingested. However, it also has been described that the carryover of AFB_1 into AFM_1 decreased significantly as the AFB_1 intake increased (Battacone et al., 2009). Frobish et al. (1986) exposed high-producing cows to 492, 1,144, and 2,491 μg/d of AFB_1 and determined that the carryover decreased when the AFB_1 dose increased (2.33, 2.13, and 1.94%, respectively). Thus, we could suggest that at least in range of intake from 60 to 120 μg/d of AFB_1 in goats, the carryover is not affected by these doses. In our study, the exposure time was also not significant such as Battacone et al. (2003) reported. Nevertheless, the results of other studies are contradictory in this regard. Aazami et al.

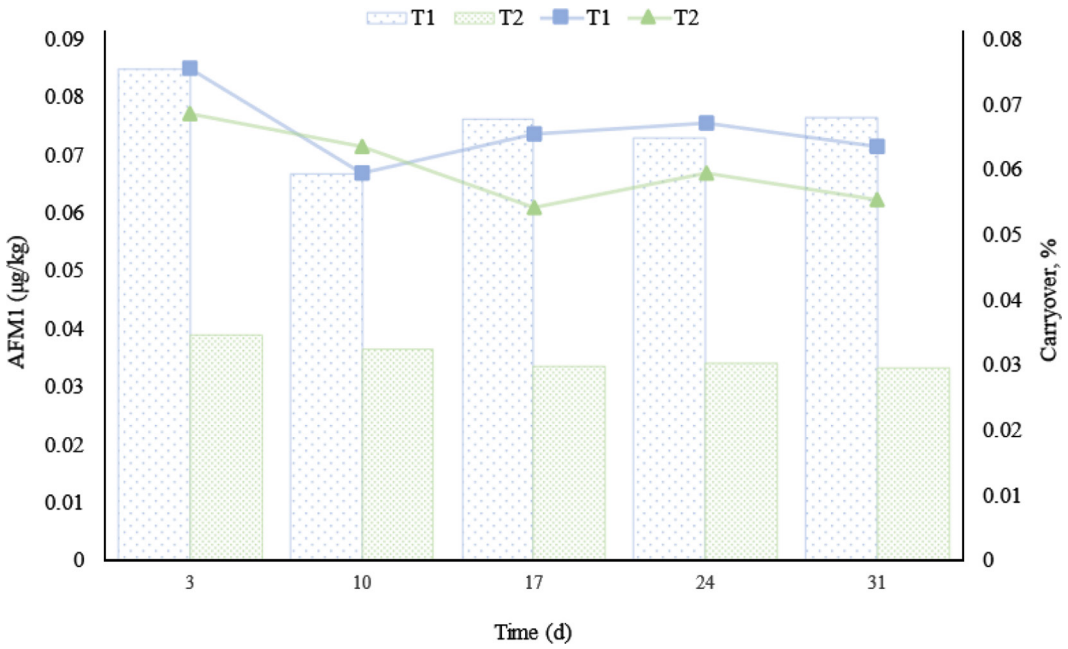

Figure 2. Aflatoxin M_1 (AFM_1) concentration in milk (bars) and carryover (lines) in groups T1 (120 µg/d of aflatoxin B_1) and T2 (60 µg/d of aflatoxin B_1) during the steady state.

(2019), who exposed Saanen breed goats daily to 25 µg of AFB_1/kg of feed, detected a decline in the AFM_1 carryover as their study progressed (from 0.29 to 0.22). On the contrary, Rao y Chopra (2001), who exposed goats to a dose of 100 µg of AFB_1 daily, notified a rising tendency in the AFM_1 carryover (from 0.14 to 0.39). Thereby, the carryover dynamics through the time does not seem to be clarify.

The carryovers obtained in this study were lower than those described by other authors in milking goats: 0.18 to 0.38% (Helferich et al., 1986), 0.22 to 0.29% (Aazami et al., 2019), 0.26% (Mazzette et al., 2009), and 0.17% (Battacone et al., 2012). These studies used differents conditions, such as different breeds or lactating periods, so we suggest that, perhaps, these factors could explain this lower carryover. In cows, the carryover is much higher, which is placed at between 2% and 6.2% (Fink-Gremmels, 2008). In this regard, the presence of AFB1 in the cows' urine and feces is also higher compared with that of small ruminants, which suggests that the latter had a higher ruminal microbiota detoxification activity (Loh et al., 2020). Indeed, Upadhaya et al. (2009) demonstrated in vitro that the ruminal fluid of native Korean goats was capable of degrading approximately 11% more AFB1 than that of young Holstein steers. Due to such a low carryover, it can be seen how the administration of 120 µg of AFB_1 (i.e., a concentration that is 24 times the maximum limit permitted in complete feed for dairy animals by the European Union, 5 µg/kg) triggers AFM_1 concentrations in milk that slightly exceed the maximum limit for this residue, established at 0.05 µg/kg.

Feed Intake, Milk Yield, and Composition

The data referring to feed intake, milk yield, and milk composition during the exposure phase are shown in Table 2. The statistical analysis of the data did not reveal any significant differences between the various groups. Therefore, these parameters were not seen to be affected by the ingestion of these AFB_1 doses during the exposure period.

Regarding feed intake and milk yield, Sulzberger et al. (2017) reported in cows that 100 µg of AFB_1/kg DMI did not affect these parameters. Similarly, Rodrigues et al. (2019) showed that feeding 105.5 µg/kg DMI of a aflatoxins mixture (B_1, B_2, G_1, and G_2) did also not affect milk yield and feed intake. Kutz et al. (2009) did not detected changes in DMI and milk yield when 112 µg of AFB_1/kg DMI was fed to dairy cows. In fact, cow and ewe milk production does not seem to be affected by AFB_1 consumption (Frobish et al., 1986; Battacone et al., 2005). Though chronic aflatoxicosis is associated with a reduction in performance parameters (Mostrom and Jacobsen, 2020), it is suggested that the dose range and the exposure time used were not enough to decrease these parameters.

Table 2. Effect of the ingestion of different doses of aflatoxin B_1 [AFB_1; T1 = 120 μg/d, T2 = 60 μg/d, and CON (control) = 0 μg/d] on feed intake, milk yield, and composition of the milk of Florida goats[1]

	Group				*P*-value[2]		
Parameter	CON	T1	T2	SEM	Treatment	Time	Interaction
Feed intake (g)	1,118.933	1,102.900	1,115.167	11.604	0.374	0.071	0.068
Milk yield (mL)	1,032.183	1,056.617	1,031.527	14.664	0.160	0.194	0.126
Protein (%)	3.886	4.019	3.906	0.045	0.289	0.802	0.667
Fat (%)	4.867	4.928	4.771	0.053	0.532	0.278	0.260
Lactase (%)	4.813	4.825	4.875	0.028	0.638	0.421	0.532

[1]Data shown belong to exposure phase (d 1 to 31).
[2]Treatment = AFB_1 dose; Time = exposure time; Interaction = treatment × time interaction.

With respect to milk composition, Kourousekos et al. (2012) determined a negative relationship between the content in fat and the AFM_1 concentration. In their study on dairy goats, the animals receiving 100 μg/d AFB1 presented the highest concentration of AFM_1 and the lowest fat content in their milk, in comparison with the group that received 50 μg/d AFB_1. In our work, with a dose of AFB_1 and a very similar duration, we did not observe that negative relationship. In the same context, neither did Kourousekos et al. (2012) or Battacone et al. (2003) identify any significant differences attributable to AFB_1 intake in protein and lactose percentages.

Biochemical Parameters

Table 3 compiles the medians and the percentiles (25th to 75th) of the serum parameters analyzed. The statistical analysis revealed the influence of the intake of AFB_1 on the enzyme GGT ($P < 0.01$). In this respect, the activity of that enzyme was significantly higher in groups T1 and T2 than in group CON. In the rest of the parameters analyzed, no significant differences were found between groups.

The increase in the concentration and activity of the serum enzymes is a sign of liver damage. The liver enzymes most consistently described in cases of

Table 3. Effect of the ingestion of different aflatoxin B_1 (AFB_1) doses [T1 = 120 μg/d, T2 = 60 μg/d, and CON (control) = 0 μg/d] on the serum parameters of Florida breed goats[1]

	Group			
Parameter[2]	CON	T1	T2	*P*-value
Glucose (mg/dL)	58.00 (51.25–62.50)	51.00 (50.25–53.25)	57.50 (54.00–58.00)	0.20
Cholesterol (mg/dL)	102.50 (96.00–115.75)	97.00 (91.25–104.25)	89.50 (82.75–94.75)	0.10
Urea (mg/dL)	35.00 (31.33–39.95)	34.10 (29.95–38.85)	33.85 (30.20–37.05)	0.85
Creatinine (mg/dL)	0.59 (0.54–0.71)	0.58 (0.55–0.64)	0.60 (0.56–0.65)	0.93
Albumin (g/dL)	3.61 (3.32–3.87)	3.39 (0.21–3.65)	3.65 (3.42–3.85)	0.82
Total proteins (g/dL)	7.30 (7.00–7.38)	7.25 (6.80–7.55)	6.65 (6.53–7.00)	0.32
Globulins (g/dL)	3.62 (3.05–4.03)	3.57 (3.23–3.90)	3.12 (2.85–3.47)	0.45
Bilirubin (mg/dL)	0.26 (0.20–0.27)	0.24 (0.21–0.26)	0.22 (0.20–0.26)	0.93
ALP (U/L)	129.50 (116.00–144.50)	101.00 (93.00–114.25)	132.00 (110.25–149.25)	0.39
AST (U/L)	53.00 (49.13–60.03)	47.75 (43.05–52.45)	48.25 (47.23–56.40)	0.48
GGT (U/L)	36.00 (32.00–40.75)	58.00 (54.50–60.75)	50.00 (43.75–52.50)	<0.01
Cholinesterase (U/L)	118.50 (95.25–148.50)	85.50 (78.50–102.25)	94.50 (85.50–116.25)	0.33

[1]Mean values and 25th to 75th percentiles in parentheses.
[2]ALP = alkaline phosphatase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase.

aflatoxicosis are: GGT, aminotransferase aspartate, alkaline phosphatase (in nonruminants), and succinate dehydrogenase (Coppock et al., 2018). In our study the enzyme GGT increased significantly in groups T1 and T2, with respect to the CON group. However, this increase barely exceeds the reference values for this enzyme in goats (20–56 U/L; Kaneko et al., 2008).

CONCLUSIONS

The AFM_1 concentration found in Florida goats' milk followed a linear relationship with the AFB_1 ingested; however, the AFM_1 carryover was not affected by the administration of different AFB_1 doses, that carryover being lower than the one described previously by other authors in this species. Therefore, to exceed the maximum limit of AFM_1 in milk established by the EU, the goats would have to ingest much higher concentrations of AFB_1 than the maximum limit permitted, in complete feed for dairy animals. In the same context, the daily administration of AFB_1 during 31 d at concentrations of 60 and 120 μg, did not significantly affect either the feed intake, or the milk yield, or the milk composition or biochemical parameters of the animals exposed, which would indicate a certain resistance on the part of the goat to the possible effects of that aflatoxin.

ACKNOWLEDGMENTS

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The authors have not stated any conflicts of interest.

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