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PROTEOMIC CHARACTERIZATION OF THE PORCINE NEUTROPHIL RESPONSE TO LPS FROM *SALMONELLA TYPHIMURIUM* BY 2D-GEL ELECTROPHORESIS AND MASS SPECTROMETRY

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Neutrophils are the first line of defense against pathogens and participate in a wide range of normal and pathological responses of the organism to diverse stimuli. The activated neutrophils attack microorganisms by phagocytosis or by releasing a combination of reactive oxygen species (ROS), enzymes and antimicrobial peptides. Lipopolysaccharide (LPS) is a component of the outer wall of Gram-negative bacteria that evokes a variety of functional responses in neutrophils. Interaction of bacterial LPS with the swine PMN represents a model system for studying the innate immune response during infection and inflammation. The objective of this study were to identify proteins involved in the response of swine neutrophils to LPS, using 2D gel electrophoresis and mass spectrometry technology. Blood samples were collected at the slaughterhouse from five healthy pigs and neutrophils were isolated with Dextran sedimentation and centrifugation through Ficoll-Paque. For LPS stimulation, the neutrophils were incubated for 18 hours in the presence or absence of 100ng/ml LPS. Proteins were solubilized, the extracts were pooled and six replicate 2-DE gels for condition (untreated cells and treated with LPS) were analysed by 2-DE. The LPS-induced changes in proteins was subjected to statistical analysis with a Student's *t* test after checking normality by the Wilks-Shapiro test and those spots with $p< 0.05$ were analyzed by MALDI-TOF/TOF. The number of protein differentially expressed in the cells after LPS treatment was 73 and 32 of this proteins were identificated (19 were down-regulates, 11 up-regulated and 2 proteins were presented in control samples only). We used the Ingenuity Pathway Analysis software to analyze our data sets. The association of the proteins afected by LPS treatment with canonical pathways highlighted two major pathways: acute phase response signalling and regulation of actin-based motility by Rho protein. Networks whose activities are most likely affected were cell-to-cell signaling and interaction and cell cycle.