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Changes in newborn lamb proteomic profile upon colostrum ingestion: an iTRAQ proteomics study

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Introduction Colostrum contains a complex mixture of proteins that participate in the protection of the neonate, mainly immunoglobulins. Due to characteristics of ruminant placenta, the transfer of immunoglobulins from the dam to the foetus is not enough to ensure the survival of the newborn. As a consequence, colostrum intake and protein absorption plays an essential role in Passive Immune Transfer, and ultimately in the newborn survival rate. The aim of this study was to determine which proteins present in the colostrum are absorbed by newborn lambs during the first 14 hours after birth using the iTRAQ (isobaric tag for relative and absolute quantitation) method.

Material and methods Two groups of lambs (of 4 each) were fed colostrum at two different time points after birth. The first group (C group) was fed with colostrum, at 2, 14 and 26 hours after birth, while the other group (DC group) received colostrum at 14 and 26 hours after birth. At the end of the colostrum feeding period (26 hours after birth), each animal (from both groups) received the same amount of fresh colostrum from a pool with 64.74 mg of IgG/mL. Blood samples were collected before feeding at 2 and 14 hours after birth, and the obtained plasma was frozen at -80°C until further analyses. Samples were homogenized using TES buffer (10mM Tris-HCl (pH 7.6), 1mM EDTA, 0.25M sucrose). After protein quantitation, 100 µg of protein from each sample were obtained after precipitation with 6 volumes of ice-cold acetone at 15.000 x g for 10 minutes at 4°C. Samples were treated according to Danielsen *et al.* (2011) and then they were labelled according to the manufacturer (Applied Biosystems Inc., Foster City, CA). Finally, samples were combined to create 4-plexed samples. An Agilent 1100 Series capillary HPLC equipped with a Zorbax Bio-SCX Series II was used to fractionate protein mixtures generated from the digestion of 50 µg of protein and an increasing NaCl solution was used for elution. Fractions were collected every minute for 65 minutes and then combined according to their peptide loads into 9-10 pooled samples. Pooled samples were then separated by a reverse phase liquid chromatography using an Agilent 1100 Series nano-flow HPLC system. LC-MS/MS analyses were performed on a QSTAR Elite mass spectrometer (AB Sciex). The collected files were used to interrogate an in-house assembled sheep and goat database consisting of sequences from TrEMBL, Swiss-Prot and NCBInr (Updated November 2012) using Mascot 2.3.02 (Matrix Science). Results were analysed using MS Data Miner v.1.1 (MDM; Dyrlund *et al.*, 2012) and a final report was generated, comparing all identified and quantified proteins from the 6 sets of iTRAQ data. Statistical analysis was performed using SAS, Version 9.00 (SAS Institute Inc., Cary, NC). The SAS PROC MIXED procedure for repeated measurements was used to evaluate the effect of colostrum intake (C group vs. DC group) at 2 and 14 hours after birth. A Bonferroni's test was used to evaluate differences between groups.

Results From this result, a total of 31 proteins were selected, as they followed a normal distribution in at least 3 of the 4 biological replicates in each of the studied groups. A statistical analysis was performed in the selected proteins, as described. A total of 8 proteins were found increased in the Colostrum group at 14 hours after birth, as shown in table 1.

Table 1. Identification and function of colostrum proteins overexpressed in the colostrum group (C).

Protein	Function
Apolipoprotein A-IV, B-100 and E	Fat absorption (Intestinal synthesis). Reduces gastric acid secretion. Immunomodulatory effect
Ceruloplasmin precursor	Iron metabolism and Copper transport
Fibrinogen Alpha Chain	Coagulation. Increase during acute-phase reaction. Promotion of adhesion, migration, chemotaxis and phagocytosis of monocytes and macrophages
Immunoglobulin M	Immune function
Tetranectin	Regulation of plasmin activation. Neutrophil migration to the infection
Trypsin inhibitor	Reduction of biologically active trypsin. Protein structure protection

Conclusions Early colostrum intake increased eight plasma proteins in the C group, demonstrating the importance of the non-immunoglobulin proteins that play a fundamental role in the activation and attraction of immune cells, the low gastric secretion, among others. These results can be used to decrease lamb mortality rates and increase the economic benefit of the sheep producers.

References
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