Comparative Proteomic Analysis Of Mammary Gland In Dairy Sheep Of Different Breeds

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Introduction

Milk component synthesis and secretion by the mammary gland varies dramatically across species and involves the expression of a large number of genes whose regulation remains poorly defined. Although much is known about the biochemistry of milk synthesis, the regulatory and cellular signaling systems of mammary gland are not well understood (Andrechek et al., 2008). Proteomics can be a new tool for the understanding of metabolic and biochemical pathways, to elucidate the genetic mechanisms underlying economic traits. The most important output of sheep farming in Italy is cheese production; Gentile di Puglia is an historical triple-purpose Merino-type breed, widely distributed throughout Southern Italy until the mid-1960's; then it was largely substituted by a specialized dairy breed, the Sarda. Milk production traits of the two breeds differ dramatically, both in daily yield, which is about 60% higher, and in lactation duration, which is about 60% longer, in the Sarda, compared to the Gentile di Puglia. The objective of this study was to evaluate temporal changes in mammary protein profiles, by comparing protein expression in mammary gland of the Sarda and the Gentile di Puglia, at different lactation stages.

Material and methods

Animals were reared in the same farm; one ewe for each of the two breeds was slaughtered at each of the following lactation stages: one week after lambing (1), at weaning of the lamb, i.e. about 40 days after lambing (2) and one week before the dry-off (3). Mammary gland tissue samples were immediately collected and kept frozen (-80 °C) until the processing. They were then homogenised in lysis buffer, containing 8M urea, 2M thiourea, 4% CHAPS, 1% DTT, and then sonicated in ice. The homogenates were kept shaking for 1 hour at 15°C, and centrifuged for 5 min at 14000 rpm. Total protein concentration was quantified by 2D Quant Kit (G.E.) and 250 μg were loaded on 17 cm strips (pH 3-10 NL). IEF was carried out for a total of 80000 Vhr. After equilibration, strips were applied onto 12% SDS PAGE and the second dimensional protein separation was performed till about 1100 V. Gels (triplicate) were stained with colloidal Coomassie and then analysed by PDQuest software.

Statistical analyses. The data were processed by analysis of variance (SAS, 1999) with breed, lactation and their interactions as factors; means values were compared by Fisher's LSD test. Only protein spots showing significant differences ($P<0.05$) for either breed or

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lactation stage were picked up and submitted to trypsin digestion, then directly identified by MALDI-MS or LC-MS/MS

Stepwise discriminant analysis was performed, to identify the proteins that could discriminate either the breed or the lactation stage, using Wilk's lambda as the statistical selection for the variables.

Results and discussion

The differentially expressed proteins, classified using the KEGG pathway, are presented in Figure 1. In addition to milk proteins, we identified proteins involved in processes linked to both lactation and mammary involution, as carbohydrate and lipid metabolism, apoptosis, cell cycle control, cell communication and cellular processes. Many of the proteins, differentially expressed during lactation or mammary involution, consist of metabolic enzymes. This is not surprising, because cellular metabolism, during lactation, is expected to be high, so to fulfill the enormous energy requirements of mammary cells during milk secretion and subsequent tissue remodeling during involution. Differentially expressed proteins along the lactation include proteins associated with cytoskeleton, such as gelsolin isoform B, lamin B1, lamin A/C, vimentin, B-actin. Further more, many proteins involved in protein turnover, signaling and transport were identified. These proteins contribute to essential cellular processes, such as vesicle trafficking, which is required for transporting the milk proteins during lactation, therefore are extremely important during mammary development.

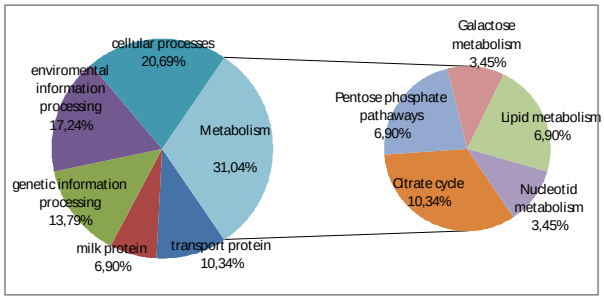

Figure 1: Classification of identified proteins based on KEGG biological function

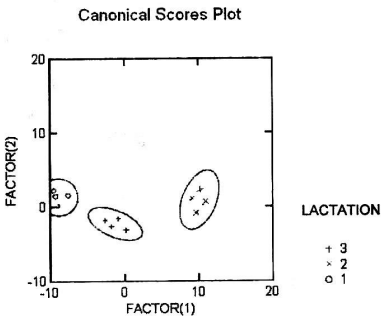

Figure 2: Discriminant analysis of differentially expressed proteins during lactation stages

The stepwise discriminant analysis (Figure 2) could discriminate the lactation stages, by 100%, by using only 5 proteins: phosphogluconate dehydrogenase, cytosolic NAP-isocitrate dehydrogenase, galactose mutarotase, staphylococcal nuclease and tudor domain containing 1, methylenetrathydrofolate dehydrogenase1. These are all proteins involved in energetic metabolism, protein synthesis and cellular signal during lactation.

It has been proposed that ruminant mammary tissue (Bauman et al., 1973) generate NADPH from acetate, via NADP-isocitrate dehydrogenase, and from glucose, via the pentose phosphate cycle (phosphogluconate dehydrogenase.) Rudolph et al., (2007) reported that mRNA levels of the enzymes involved in tricarboxyl acid (citrate) transporter increase three- to four fold at mid lactation. Consequently, through increasing protein levels, they would serve to focus mitochondrial activity on the generation and export of citrate to cytoplasm, where it would be converted to substrates for fatty acid and cholesterol synthesis as well as for generation of NADPH via the malate shuttle. In early biochemical studies (Mellenberger et al., 1973) the increased activity of the penthose phosphate shunt at the onset of lactation was found to be a major contributor to increased lipogenesis.

In the pathway for biosynthesis of the lactose and lipids of milk, our data highlight that the expression of cytosolic NADP-isocitrate dehydrogenase, phosphogluconate dehydrogenase1 and galactose mutarotase exhibits the same trend of milk production, with an up regulation at mid lactation followed by a down regulation at late lactation.

During lactation signal transducers and activators of transcription play a critical role in prolactin-induced transcription of several milk protein genes. Paukku et al. (2003) reported that the expression of a staphylococcal nuclease-like domains tudor was increased, in mammary epithelial cells, during lactation, in response to lactogenic hormones, and was correlated with the induction of β-casein gene expression.

Our results showed a differential expression of staphyloccocal nuclease and tudor domain containg 1 during lactation with an increase at peak of lactation.

Methylentetrahydrofolate dehydrogenase is required for several key reactions in metabolic processes: purine, pyrimidine and methionine synthesis, glycine and serine metabolism. Cell division and protein biosynthesis characterized the onset of lactation until peak lactation, and then the biosynthetic activity progressively decreases.

The stepwise discriminant analysis could discriminate the two breeds, by 75%, using only 2 proteins: transferrin and gelsolin isoform B. The gelsolin isoform B is a member of gelsolin protein family that exerts a powerful regulatory role on actin assembly and disassembly, and is believed to regulate cell motility through modulation of the actin network (Kusano et al., 2000). Furthemor, gelsolin mediates epidermal growth factors that control ductal outgrowth, elongation and branching (Desvrieres et al., 2003). The opposite trend on the expression of gelsolin isoform B was observed between two breed, and dowregulation of gelsolin isoform B expression was found in the Gentile di Puglia breed compared to the Sarda. These results suggest that gelsolin regulates cell motility through modulation of the actin network, and actin may form part of a contractile apparatus involved in lipid secretion.

Lee et al. (1987) documented, in mice, that small amounts of transferrin are locally produced by epithelial cells in the mammary gland and that its synthesis increases throughout gestation and lactation. Growing evidence suggests that transferrin synthesis is associated with growth and functional differentiation in non hepatic tissues. Ours results showed that expression of transferrin was dowregulated in the Gentile di Puglia breed compared to the Sarda.

Conclusion

In the present study, we identified proteins that are differentially expressed during lactation in both breeds: these proteins are related to specific biochemical and physiological functions of the mammary gland: enzymes involved in carbohydrate metabolism, cell communication and cellular processes. At mid lactation, the differential expression of the enzymes involved in metabolism was linked to the increase of milk production in both breed. In addition, the upregulation of ferritin and gelsolin detected in the Sarda breed at all stages, may be associated to the higher milk yield of this breed.

Acknowledgements

This study is part of the "GENZOOT" research programme, funded by the Italian Ministry of Agriculture

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