Journal of Animal and Veterinary Advances 12 (18): 1454-1460, 2013
ISSN: 1680-5593


# Differential Protein Composition of Goat Whey Before and After Intramammary Plasmid Infusion

Jinfeng Miao, Qiaoxiu Wang, Jian Lin, Qiang Zhang and Qian Yang
College of Veterinary Medicine, Nanjing Agricultural University, 210095 Nanjing, China

**Abstract:** The aim of this study was to determine if intramammary infusion of recombinant expression vectors and subsequent analyses of whey proteins can be used to evaluate plasmid function and to elucidate their mechanisms of improving milk production. On the day of the experiment, 6 goats were infused with 100 μg $g^{-1}$ and of recombinant vectors pcGH-20 or pIN in both right and left glands. At intervals of 0 (control group), 1, 3 and 7 days Post Infusion (PI), milk was collected. Whey from each time point was compared to controls by 2-Dimensional gel Electrophoresis (2-DE) with colloidal coomassie staining and matrix-assisted desorption/ionization mass spectrometry. In the pIN infusion group, 19 protein spots with a 2 fold or greater change from the controls were obtained. In the pcGH-20 infusion group 14 protein spots were produced. The proteins were sorted into 5 functional classes based on Conserved Domain Database (CDD) comparison. Some spots corresponded to the major milk proteins. Some are involved in host defense/immune functioning, others in metabolism and still others are hormone and hormone-like factor. The remaining proteins serve other functions. The upregulation of some important metabolism related proteins (including hormone and hormone-like factors) indicates that mammary epithelial cells respond to recombinant vector infusion. The data also show that different plasmids induce different whey profiles. Further, development of this model could be used to evaluate the function of recombinant vectors and to elucidate their potential mechanism of action prior to the production of transgenic animals.

**Key words:** Recombinant vectors, whey proteins, two dimensional gel electrophoresis, cells, action

## INTRODUCTION

Milk is a nutritious, cost-efficient source of protein, minerals and vitamins for human beings. Improving milk production and composition has been ongoing for centuries. Increasing milk production is especially important in China because of its large population and fewer dairy resources per capita than in developed countries. Genetic selection, nutrition and disease control have contributed to the success of the milk industry. Transgenic engineering offers additional possibilities for improving milk production, optimizing milk composition, protecting the mammary gland against infectious diseases and producing pharmaceuticals (Houdebine, 2000; Hunter *et al*., 2005; Bosze *et al*., 2008).

Transgenic engineering provides the possibility of expressing foreign genes in the mammary gland. The first successful targeted expression of transgenes in the mammary gland was produced more than a decade ago (Gordon *et al*., 1987). However, the development of transgenic ruminants is limited by the difficulty of adding foreign genes to farm animals (Samiec and Skrzyszowska, 2011). Transgene expression is often ill-controlled and a number of technical problems remain to be solved. For example, expression vectors may not be constructed in an appropriate manner the foreign genes may be integrated in the wrong position (such as centromeres or telomeres where DNA is inactive); the promoter may not work the expression rate may vary among different gene constructs and gene constructs which are quite active in cultured cells may be silent in transgenic animals (Petitclerc *et al*., 1995; Dobie *et al*., 1996; Clark, 1998; Manhes *et al*., 2006). Accounting for the difficulty and cost of generating transgenic farm animals, it would be useful to determine the function of foreign genes including the efficiency of vectors, *in vivo*. Foreign genes expressed in mammary tissue after infusion with genetically engineered vectors can persist for at least 7-11 days (Patton *et al*., 1984; Fan *et al*., 2004). This indicated that the mammary gland might be an appropriate tool to evaluate the function of recombinant vectors before transgene.

Complex physiological processes are controlled by multiple genes and their protein products. The mammary gland is a complex organ consisting of a number of cell

**Corresponding Author:** Qian Yang, College of Veterinary Medicine, Nanjing Agricultural University, 1 Weigang, Jiangsu Province, 210095 Nanjing, China

types within an extracellular matrix. During mammary development and lactation, there is interaction of a great variety of regulatory factors. Hormones, including steroid and polypeptide mammogens, may be important factors (Knight *et al*., 1998). However, they need to express receptors and other mediator proteins to be effective (Wolf *et al*., 1997). In ruminant galactopoiesis, Growth Hormone (GH) function dominates that of prolactin. A positive relationship has been observed between plasma GH concentrations and milk yield in dairy cows. Exogenous GH increases milk production in cattle by 6-30% (Davis *et al*., 1989; Hadsell *et al*., 2008). GH acts indirectly in the mammary gland and increases milk yield likely via the rapamycin (mTOR) pathway and Insulin-like Growth Factor I (IGF-I) synthesis (Rius *et al*., 2010). IGF-I is the primary mediator of the actions of GH (Ruan *et al*., 1992). IGF-I is synthesized and secreted by many tissues but principally by the liver. In mammary tissue, IGFs are synthesized by stromal cells (fibroblasts and adipocytes). The actions of IGFs can be modulated by interaction with a family of insulin-like, Growth-Factor Binding Proteins (IGFBPs) which regulate the bioavailability of IGFs in the circulation (Ruan *et al*., 1992; Hadsell *et al*., 2008). However, their functions at the cellular level are not fully understood.

Whey is the fluid left in milk following removal of casein (the most abundant milk protein). Whey contains low levels of various proteins including signal mediator proteins such as those of the IGF family, complement proteins and others that are synthesized and secreted by mammary tissues or derived from serum. Detailed proteomic analysis of whey proteins may help gain a better understanding of the mechanisms of local regulation of lactation (Hogarth *et al*., 2004; Smolenski *et al*., 2007).

In this study, researchers use goats as a model to evaluate the effects of recombinant GH and IGF-I plasmid infusion on the mammary gland. The goal is to determine if this model can be used to evaluate the function of recombinant vectors prior to the production of transgenic animals and to explore the potential mechanisms of GH and IGF-I in improving milk production.

## MATERIALS AND METHODS

**Reagents:** Isoelectric pH Gradient (IPG) strips (pH 3.0-10.0 NL, 17 cm), urea, Pharmalyte pH 3-10, glycerol (87% w/w), Tris (electrophoresis grade), TEMED (electrophoresis purity reagent), acrylamide (40% solution, acrylamide to bisacrylamide ratio 37.5:1), CHAPS (electrophoresis grade), thiourea (ACS grade), dithiothreitol (DTT, electrophoresis grade), iodoacetamide (electrophoresis grade), mineral oil and low melting-point agarose were all obtained from Bio-Rad (Richmond, CA, USA). Coomassie Brilliant Blue G-250 was purchased from Amresco (Solon, OH, USA). High-purity water was prepared with a Milli-Q gradient water purification system (Millipore, Bedford, MA) and was used for all protocols in this study.

**Plasmids:** The vectors pcGH-20 and pIN which express caprine GH and IGF-I, respectively were constructed by Dr. Zhang Qiang and Lin Jian. The backbone vector pBC1 contained the goat β-casein 5' arm and the β-casein 3' arm which regulate genes expressed in mammary tissue.

### Animals and treatment

**Ethics statement:** All animal care and procedures were in accordance with national and institutional policies for animal health and well-being. All goat milk samples collection and field study were approved by the Nanjing Nannong high-tech Co., Ltd. The license number was SYXK (SU) 2006 to 0004. Fresh water was always available through automatic dispensers. All efforts were made to minimize the number of animals used and their suffering.

**Goats:** Twelve, healthy, Suining white goats at early stages of their second lactation and free of intramammary inflammation were used for this study. To allow adaptation to their new environment, the animals were transferred to the experimental stable 7 days prior to plasmid infusion. Milk samples from each half of the mammary gland were collected daily for 7 days before inoculation for bacteriological examination and determination of milk Somatic Cell Counts (SCC). Only glands with a SCC <150,000 cells $mL^{-1}$ and milk samples that cultured negative for mastitis pathogens were included in the study. To ensure maintenance of the SCC level until the start of the experiment, the halves were carefully milked out twice daily.

**Treatment:** On the day of the experiment, after morning milking, the goats were intramammary infusion with recombinant vector pcGH-20 or pIN in both right and left glands (100 μg $g^{-1}$ land, N = 6). At intervals of 0, 1, 3 and 7 days Post Infusion (PI) milk was collected from each gland. The milk samples collected before vector infusion constituted the control group. Whole milk was centrifuged at 4000 g for 20 min at 4°C and the lipid-rich supernatant (cream) and cell debris pellets were discarded. The resultant skim milk was stored at -80°C until analyzed. After all mammary infusions and sample collections, teats were sterilized with 75% alcohol and iodine to prevent infection.

**Protein sample preparation and two dimensional gel electrophoresis**

**Protein sample preparation:** Samples from the each experimental group were pooled. Caseins which comprise approximately 80% of the total protein content in milk were precipitated by the addition of saturated, ice-cold $(NH_4)_2SO_4$ to the milk samples in an ice bath to a final concentration of 35% v/v of $(NH_4)_2SO_4$. After centrifugation at 4000 g for 20 min at 4°C, the supernatant (milk whey) was removed and dialyzed overnight against deionized water 4°C. The protein concentration for each of the final supernatants was measured by the Bradford assay using Bovine Serum Albumin (BSA) as the standard.

**Two dimensional gel electrophoresis:** The 800 μg of each protein extract were separated by Iso-Electrophoresis (IEF) using IPG strips (pH 3.0-10.0 NL, 17 cm) in the protean system (Bio-Rad) at 20°C. Focusing was conducted through 1 h at 250 V, 1 h at 500 V, 1 h at 2,000 V, 2 h at 8,000 V and then at 8,000 V until a total of at least 60,000 V h was reached. After the IEF run was complete, strips were removed and equilibrated with gentle shaking in two subsequent steps for 15 min each in 5 mL equilibration buffer (0.05 M Tris-HCl pH 8.8, 6M urea, 30% glycerol, 2% SDS) containing 0.1 mg DTT in the first step and 0.125 mg iodoacetamide in the second step. The second dimension was run on a 12.5% polyacrylamide SDS gel using the Multiphor system (Amersham Biosciences). The 2-DE for each sample was repeated three times. Neuhoff's colloidal Coomassie blue G-250 staining was carried out according to the method described by Candiano *et al.* (2004). Stained gels were scanned and analyzed using PDQuest V 710 Software (Bio-Rad). After alignment, gel spots were automatically matched. The matched spots were then examined manually to ensure accuracy. Only those spots with a quality of over 50 were chosen for further analysis. Spot quantity normalization was conducted in the 'total quantity of valid spots' mode.

**MALDI-TOF-MS analysis and database queries:** In gel trypsin digestion of protein spots and MALDI-TOFMS (Reflex III, Bruker-Daltonics, Germany) analyses were based on procedures described by Wang *et al.* (2005). MS fingerprinting data searches were performed by the search engines of MS-fit (http://prospector.ucsf.edu) against the NCBInr database in the taxa of *Gallus gallus* with the parameter sets of trypsin digestion, two missed cleavages, complete modification of iodoacetamide (Cys), partial modification of methionine oxidation, protein mass = ±20% of the observed protein mass, p = ±1 of observed PI and a mass tolerance for monoisotopic data of 100 ppm. Protein identification was assigned when there were at least four matching peptides and >20% sequence coverage.

**Functional annotation of identified proteins:** The functions of proteins identified through MS fingerprinting data were annotated by querying against the protein function database Pfam (http://www.sanger.ac.uk/Software/Pfam (Finn *et al*., 2010)) or Inter-Pro (http://www.ebi.ac.uk/interpro (Apweiler *et al*., 2001)).

## RESULTS AND DISCUSSION

Good separation of whey protein spots was apparent in the 2D gel electrophoresis (Fig. 1 and 2). After auto-matching and a manual quality check, 391 valid spots were identified. To determine the repeatability among gels of the same sample, spot to spot correlation coefficients were calculated. They varied from 0.81-0.92 with all being significant at $p \geq 0.0001$. In each sample, at least one of the three correlation coefficients was over 0.85 while the difference in spot volume among replicates was not significant, based on ANOVA.

In general, the protein profiles from four gels of each group were similar. However, certain spots displayed obvious differences in volume or abundance. In this study, only the spots with a fold change >2 were considered for further identification. In the pIN infusion group, 7 spots (1, 2, 3, 4, 5, 6, 7) in whey PI day 1 had differences in volume as compared to control whey, 7 spots (1, 8, 9, 10, 11, 12, 13) at PI day 3 and 8 spots (11, 12, 14, 15, 16, 17, 18, 19) at 7 days PI. Of 19 spots, 6 (1, 2, 3, 7, 8, 18) were down regulated after gene infusion. The remainder of the 19 spots was up-regulated. Spot 1 was down-regulated at 1 and 3 days PI; spots 12 and 13 were up-regulated on days 3 and 7 PI. After pcGH-20 infusion, a single spot (22) was down regulated at day 1; 5 spots (20, 21, 22, 23, 24) had differences in volume on day 3 and 10 spots (7, 13, 17, 23, 25, 26, 27, 28, 20, 30) on day 7 PI. Five spots (21, 22, 24, 28, 29) were down regulated after pcGH-20 infusion.

Proteins from all 30 spots were subjected to in gel digestion and analyzed by MALDI-TOF-MS. Protein identification using the MS fingerprint data was conducted by querying the NCBInr protein database. Positive identifications were obtained for all 30 spots (Table 1 and 2).

The 30 identified proteins were sorted into five functional classes based on the Conserved Domain Database (CDD) comparison. Some spots (9, 17, 18, 22, 23, 25, 26, 27, 29) corresponded to the major milk proteins.

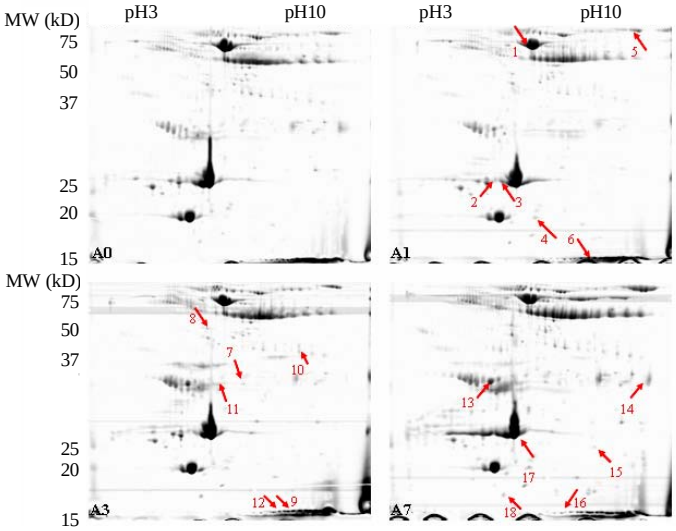


Fig. 1: Protein profiles of goat whey before and after mammary gland infusion with the recombinant vector pIN. A0: whey protein profiles prior to intramammary infusion with pIN; A1, A3, A7: whey proteins profiles 1, 3 and 7 days after intramammary infusion with pIN. Arrows indicate proteins with at least a 2.0 fold change compared to controls

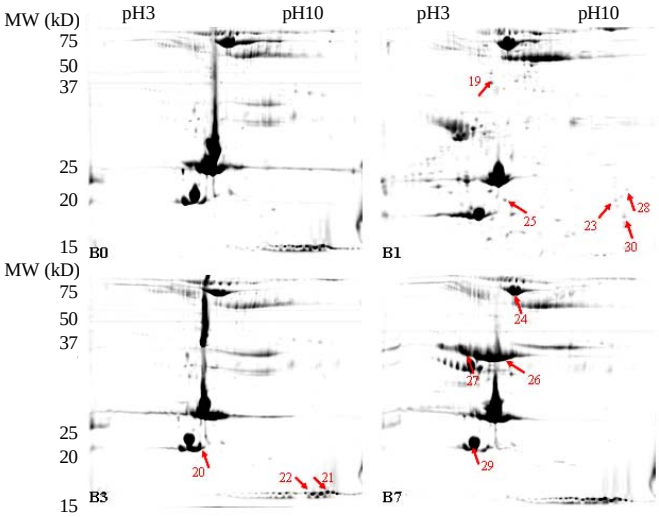


Fig. 2: Proteins profiles of goat whey before and after intramammary infusion with recombinant vector pcGH-20. B0: whey protein profiles before intramammary infusion with pcGH-20 (control); B1, B3, B7: whey protein profiles 1, 3 and 7 days after intramammary infusion with pcGH-20. Arrows indicate proteins with at least a 2.0 fold change compared to controls

Other spots (4, 5, 6, 7, 8, 11, 12, 19, 20, 30) are involved in host defense/immune mechanisms, metabolism (1, 10, 14, 15, 24) and hormone and hormone-like factor upregulation (3, 16, 21, 28). The remaining proteins were related to other functions.

It is well known that GH and IGF-I stimulate lactation-related metabolic processes in mammary epithelial cells and have a positive influence on milk secretion in ruminants (Davis *et al*., 1989). However, no reports discuss the direct effect of recombinant vectors (*GH* or *IGF-I* genes) on the mammary gland. In this study, researchers found that intramammary infusion of pcGH-20 or pIN improved the production of milk, respectively. During the period of experiment, the mammary glands viewed filling apparently after recombinant plasmids challenged. Unfortunately, researchers could not give the accurate comparison of milk product for the limited of experimental condition. This indicates that the vectors

Table 1: Differentially expressed proteins of goat whey before and after intramammary infusion with recombinant vector pIN identified by PMF query

| Spot No. | Up or down | Protien names | Score | Sequnce coverage (%) | NCBI accession No. | MW (kDa) | PI |
|---|---|---|---|---|---|---|---|
| 1 | Down | Albumin precursor | 111 | 25 | gi\|193085052 | 68.27 | 5.58 |
| 2 | Down | Cellular FLICE-like inhibitory protien short-form | 203 | 83 | gi\|148283490 | 24.34 | 5.35 |
| 3 | Down | Preproenkephalin | 291 | 68 | gi\|269979221 | 26.99 | 5.65 |
| 4 | Up | Immunoglobulin γ2 heavy chain constant region | 436 | 97 | gi\|147744654 | 21.48 | 6.33 |
| 5 | Up | Lactoferrin | 308 | 46 | gi\|556807 | 79.35 | 8.55 |
| 6 | Up | T-cell receptor β-chain | 422 | 100 | gi\|2618640 | 16.16 | 6.81 |
| 7 | Down | Interleukin 12 p35 subnit precursor | 261 | 58 | gi\|50261482 | 23.06 | 6.71 |
| 8 | Down | Immunoglobulin mu heavy chain constant region | 113 | 38 | gi\|162424563 | 52.88 | 5.15 |
| 9 | Up | κ-casein | 303 | 97 | gi\|117958741 | 15.96 | 7.89 |
| 10 | Up | Ornithine transcarbamylase | 295 | 66 | gi\|284157311 | 39.72 | 8.20 |
| 11 | Up | Interleukin-1α | 185 | 84 | gi\|6016355 | 30.84 | 5.04 |
| 12 | Up | Interleukin-2 | 458 | 88 | gi\|1200120 | 15.55 | 6.19 |
| 13 | Up | Stem cell fact | 239 | 59 | gi\|1881771 | 29.23 | 5.24 |
| 14 | Up | Putative pheromone receptor gV1R1 | 337 | 70 | gi\|18148936 | 35.35 | 9.72 |
| 15 | Up | Glyceraldehyde-3-phosphate dehydrogenase | 357 | 97 | gi\|27525391 | 20.30 | 8.51 |
| 16 | Up | Glyceraldehyde-dependent cell adhesion molecule 1 | 103 | 34 | gi\|22096365 | 17.05 | 5.27 |
| 17 | Up | β-lactoblobulin | 110 | 70 | gi\|165839 | 20.33 | 5.29 |
| 18 | Down | Chain A, recombinant goat α-lactalbumin T29v | 99 | 61 | gi\|13399946 | 14.77 | 4.92 |
| 19 | Up | Immunoglobulin mu heavy chain constant region | 172 | 40 | gi\|162424563 | 52.20 | 5.15 |

Table 2: Differentially expressed proteins of goat whey before and after intramammary infusion with recombinant vector pcGH-20 identified by PMF query

| Spot No. | Up or down | Protien names | Score | Sequnce coverage (%) | NCBI accession No. | MW (kDa) | PI |
|---|---|---|---|---|---|---|---|
| 7 | Up | Interleukin 12 p35 subnit precursor | 261 | 58 | gi\|50261482 | 23.06 | 6.71 |
| 13 | Up | Stem cell factor | 239 | 59 | gi\|1881771 | 29.23 | 5.24 |
| 17 | Up | β-lactoblobulin | 110 | 70 | gi\|165839 | 20.33 | 5.29 |
| 20 | Up | Interferon-tau 5 | 352 | 70 | gi\|34014819 | 22.35 | 5.44 |
| 21 | Down | Myostatin | 465 | 95 | gi\|21886638 | 14.40 | 8.11 |
| 22 | Down | Casien-κ | 208 | 42 | gi\|40795921 | 18.36 | 6.08 |
| 23 | Up | α-S2-casien | 283 | 81 | gi\|416751 | 26.37 | 8.24 |
| 24 | Down | Albumin precursor | 179 | 34 | gi\|193085052 | 68.27 | 5.58 |
| 25 | Up | β-lactoblobulin | 93 | 64 | gi\|165839 | 20.33 | 5.29 |
| 26 | Up | β-casien | 247 | 60 | gi\|4495057 | 24.85 | 5.26 |
| 27 | Up | α-S1-casien | 221 | 75 | gi\|311943 | 24.16 | 5.19 |
| 28 | Down | α-melanocyte-stimulating hormone | 172 | 73 | gi\|158323608 | 23.15 | 9.30 |
| 29 | Down | Chain A, recombinant goat α-lactalbumin T29v | 147 | 61 | gi\|13399946 | 14.77 | 4.92 |
| 30 | Up | Interferon-α | 311 | 56 | gi\|238837048 | 21.27 | 8.84 |

pcGH-20 and pIN which contain the goat β-casein 5' arm and the β-casein 3' arm, express in caprine mammary tissue for at least 7 days.

Using 2-DE and MALDI-TOF-MS analysis, 19 whey proteins exhibit differential changes in profile after the recombinant vector pIN is infused into mammary glands. The 8 (42%) of these proteins are involved in host defense/immune function. This seems reasonable for an exogenous molecule inducing changes in the immune response of tissues which have active defense capabilities such as mammary gland. Some whey proteins are involved in important aspects of metabolism including hormone and hormone like factor upregulation.

Ornithine Transcarbamylase (OTC, EC 2.1.3.3), also called ornithine carbamoyltransferase, is an enzyme that catalyzes the reaction between carbamoyl phosphate and ornithine to form citrulline and phosphate. In mammals, OTC is localized in the mitochondrial matrix, mainly in the liver but also in other epithelial cells (Mori *et al*., 1982). Its function is mainly involved in the urea cycle and the metabolic transformation of arginine and proline as well as in other systems (Diaz-Munoz and Hernandez-Munoz, 2010). Recent evidence suggests that OTC may function as a marker enzyme of cell proliferation in swine during liver regeneration (Diaz-Munoz and Hernandez-Munoz, 2010). The upregulation of OTC in the current study may indicate that it also enhances transamination and protein metabolism in mammary tissue. This is consistent with the increase in milk yield observed.

Protein synthesis requires energy which is provided by carbohydrate metabolism. Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH, EC 1.2.1.12) catalyzes the sixth step of glycolysis or glucose aerobic oxidation and is an important enzyme in the carbohydrate metabolism pathway (De La Monte *et al*., 2008). GAPDH has recently also been implicated in several non-metabolic processes including transcription activation, initiation of apoptosis and in endoplasmic reticulum to Golgi vesicle shuttling. Zheng *et al*. (2003) discovered that GAPDH could move between the cytosol and the nucleus and was part of a transcriptional coactivator complex and linked with histone gene expression and DNA replication

(Zheng *et al*., 2003). The upregulation of GAPDH in the current study indicates that mammary epithelial cells become active after pIN infusion into mammary glands.

It is reported that GH may regulate lactation in ruminants via mediation of IGF-I (Hadsell *et al*., 2008; Rius *et al*., 2010). Indirect evidence supporting this observation from the current study is that only 1 spot (22) changed 1 day after pcGH-20 infusion whereas 7 changed after pIN challenge. Of the 14 identified proteins, 7 correspond to major milk proteins (including α-S1-casein, α-S2-casein, β-casein, κ-casein, β-lactoglobulin, etc.). These data are consistent with a previous study showing pretreatment of milk by ammonium sulphate precipitation only partially removes caseins masking the identification of some low abundance whey proteins (Hogarth *et al*., 2004; Smolenski *et al*., 2007). Thus, contrary to the expectations, only 3 proteins linked to metabolism (including hormone and hormone-like factors) were identified. This suggests a future study using staining methods such as silver or sypro-ruby that are more sensitive than colloidal coomassie to examine low abundance proteins without interference from the major milk proteins.

## CONCLUSION

The utilizing 2-DE and MALDI-TOFMS analyses, researchers documented changes in low abundance proteins in goat whey after recombinant expression vectors were directly infused into mammary glands. The data show that the different plasmids induce different protein profiles. Increasing the sensitivity of this model could facilitate evaluation of the functions of recombinant vectors prior to the engineering of transgenic ruminants.

## ACKNOWLEDGEMENTS

This project was supported by the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions, the Natural Science Foundation of Jiangsu Province, China (No. BK2010444), the Youth Sci-Tech. Innovation Fund, NJAU (No. Y200917) and the authors wish to express their thanks to Dr. Howard Gelberg (Oregon State University) for manuscript editing.

## REFERENCES

Apweiler, R., T.K. Attwood, A. Bairoch, A. Bateman and E. Birney *et al*., 2001. The InterPro database, an integrated documentation resource for protein families, domains and functional sites. Nucleic Acids Res., 29: 37-40.

Bosze, Z., M. Baranyi, C. Bruce and A. Whitelaw, 2008. Producing recombinant human milk proteins in the milk of livestock species. Adv. Exp. Med. Biol., 606: 357-393.

Candiano, G., M. Bruschi, L. Musante, L. Santucci and G.M. Ghiggeri *et al*., 2004. Blue silver: A very sensitive colloidal Coomassie G-250 staining for proteome analysis. Electrophoresis, 25: 1327-1333.

Clark, A.J., 1998. Gene expression in the mammary glands of transgenic animals. Biochem. Soc. Symp., 63: 133-140.

Davis, S., P. Gluckman, S. Hodgkinson, V. Farr, B. Breier and B. Burleigh, 1989. Comparison of the effects of administration of recombinant bovine growth hormone or n-met insulin-like growth factor-i to lactating goats. J. Endocrinol., 123: 33-39.

De La Monte, S.M., J.E. Yeon, M. Tong, L. Longato and R. Chaudhry *et al*., 2008. Insulin resistance in experimental alcohol-induced liver disease. J. Gastroenterol. Hepatol., 23: e447-e487.

Diaz-Munoz, M. and R. Hernandez-Munoz, 2010. Molecular and biochemical features of the mitochondrial enzyme ornithine transcarbamylase: A possible new role as a signaling factor. Curr. Med. Chem., 17: 2253-2260.

Dobie, K.W., M. Lee, J.A. Fantes, E. Graham and A.J. Clark*et al*., 1996. Variegated transgene expression in mouse mammary gland is determined by the transgene integration locus. Proc. Nat. Acad. Sci., 93: 6659-6664.

Fan, W., K. Plaut, A.J. Bramley, J.W. Barlow, S.A. Mischler and D.E. Kerr, 2004. Persistency of adenoviral-mediated lysostaphin expression in goat mammary glands. J. Dairy Sci., 87: 602-608.

Finn, R.D., J. Mistry, J. Tate, P. Coggill and A. Heger *et al*., 2010. The pfam protein families database. Nucleic Acids Res., 31: D211-D222.

Gordon, K., E. Lee, J.A. Vitale, A.E. Smith, H. Westphal and L. Hennighausen, 1987. Production of human tissue plasminogen activator in transgenic mouse milk. Nat. Biotechnol., 5: 1183-1187.

Hadsell, D.L., A.F. Parlow, D. Torres, J. George and W. Olea, 2008. Enhancement of maternal lactation performance during prolonged lactation in the mouse by mouse gh and long-r3-igf-i is linked to changes in mammary signaling and gene expression. J. Endocrinol., 198: 61-70.

Hogarth, C.J., J.L. Fitzpatrick, A.M. Nolan, F.J. Young, A. Pitt and P.D. Eckersall, 2004. Differential protein composition of bovine whey: A comparison of whey from healthy animals and from those with clinical mastitis. Proteomics, 4: 2094-2100.

Houdebine, L.M., 2000. Transgenic animal bioreactors. Transgenic Res., 9: 305-320.

Hunter, C.V., L.S. Tiley and H.M. Sang, 2005. Developments in transgenic technology: Applications for medicine. Trends mol. Med., 11: 293-298.

Knight, C.H., M. Peaker and C.J. Wilde, 1998. Local control of mammary development and function. Rev. Reprod., 3: 104-112.

Manhes, C., C. Kayser, P. Bertheau, B. Kelder and J.J. Kopchick *et al*., 2006. Local over-expression of prolactin in differentiating mouse mammary gland induces functional defects and benign lesions, but no carcinoma. J. Endocrinol., 190: 271-285.

Mori, M., S. Miura, T. Morita, M. Takiguchi and M. Tatibana, 1982. Ornithine transcarbamylase in liver mitochondria. Mol. Cell. Biochem., 49: 97-111.

Patton, S., U. Welsch and S. Singh, 1984. Intramammary infusion technique for genetic engineering of the mammary gland. J. Dairy Sci., 76: 1323-1326.

Petitclerc, D., J. Altai, M.C. Thrron, M. Bearzotti and P. Bolifraud *et al*., 1995. The effect of various introns and transcription terminators on the efficiency of expression vectors in various cultured cell lines and in the mammary gland of transgenic mice. J. Biotechnol., 40: 169-178.

Rius, A.G., J.A.D.R.N. Appuhamy, J. Cyriac, D. Kirovski and O. Becvar *et al*., 2010. Regulation of protein synthesis in mammary glands of lactating dairy cows by starch and amino acids. J. Dairy Sci., 93: 3114-3127.

Ruan, W., C.B. Newman and D.L. Kleinberg, 1992. Intact and amino-terminally shortened forms of insulin-like growth factor I induce mammary gland differentiation and development. Proc. Nat. Acad. Sci., 89: 10872-10876.

Samiec, M. and M. Skrzyszowska, 2011. Transgenic mammalian species, generated by somatic cell cloning, in biomedicine, biopharmaceutical industry and human nutrition/dietetics-recent achievements. Pol. J. Vet. Sci., 14: 317-328.

Smolenski, G., S. Haines, F.Y. Kwan, J. Bond and V. Farr *et al*., 2007. Characterization of host defence proteins in milk using a proteomic approach. J. Proteome Res., 6: 207-215.

Wang, Y., L. Yang, H. Xu, Q. Li, Z. Ma and C. Chu, 2005. Differential proteomic analysis of proteins in wheat spikes induced by fusarium graminearum. Proteomics, 5: 4496-4503.

Wolf, E., P.M. Jehle, M.M. Weber, H. Sauerwein and A. Daxenberger *et al*., 1997. Human insulin-like growth factor I (IGF-I) produced in the mammary glands of transgenic rabbits: Yield, receptor binding, mitogenic activity and effects on IGF-binding proteins. Endocrinology, 138: 307-313.

Zheng, L., R.G. Roeder and Y. Luo, 2003. S phase activation of the histone h2b promoter by oca-s, a coactivator complex that contains gapdh as a key component. Cell, 114: 255-266.