RESEARCH ARTICLE

Relative Gene Expression Study on Casein Protein and its Regulatory Genes in Mammary Epithelial Cells of Surti Goat

Mamta Janmeda[1]*, Gaurav Pandya[1], Umed Ramani[2], Balkrushna Brahmkshtri[3], Navin Patel[4], Vishnu Kharadi[1]

Abstract

This investigation was carried out to study the relative gene expression of casein proteins and its regulatory genes in mammary epithelial cells of Surti goats at 30 and 90 days interval postpartum. 10 healthy Surti goats were selected from Livestock Research Station, Navsari. The non-invasive method was used for the isolation of Mammary Epithelial Cells (MEC) from goat milk samples. The relative gene expression of *CSN1S1* (αs1-casein), *CSN1S2* (αs2-casein), *CSN2* (β-casein), *CSN3* (κ-casein) and its regulatory genes C/EBP and STAT5A genes and *KRT14* (epithelial cell marker) gene were studied in MEC. The relative gene expression of *CSN1S1, CSN1S2, CSN3* and *C/EBP* genes were significantly up-regulated with the advancement of lactation at 90 days postpartum. The relative gene expression of *CSN2* was highly significant and had a positive correlation with its regulatory genes *C/EBP* and *STAT5A* at 30 days and 90 days, respectively. The relative gene expression of CSN1S2, CSN3, and C/EBP genes was positively correlated with protein percent at 30 days and 90 days postpartum in Surti goats.

Keywords: Casein proteins, Gene expression, Mammary epithelial cells, Surti goat.

Ind J of Vet Sci and Biotech (2020): 10.21887/ijvsbt.16.1.12

Introduction

Goat is one of the most important livestock and the oldest domesticated species worldwide and has long been used for their milk, meat, hair and skins. Goats have a short gestation period, early maturation, smaller body size and relatively high production of milk (Nowak *et al*., 2011) and hence are one of the best model organisms for mammary gland bioreactor studies by targeting protein expression in their mammary glands (MacHugh and Bradley, 2001). Goat milk has been recognized as beneficial for human nutrition (Finocchiaro and van Kaam, 2004) and possesses medicinal properties (Bepost *et al*., 2006). The nutritional value of goat milk is mainly attributable to the fat and protein fractions secreted from mammary glands.

The milk protein genes encode proteins which are produced and secreted by the mammary glands epithelial cell. More than 95% of the proteins in the milk of goats are encoded by casein and whey protein genes (Martin *et al*., 2002). These genes are encoded by four tightly linked casein genes (CSN1S1, CSN1S2, CSN2, and CSN3) within a 250 kb of the gene cluster mapped on the chromosome 6 (CHI6) (Dagnachew *et al*., 2011). The caprine casein genes are highly polymorphic (Boutinaud *et al*., 2002; Shi *et al*., 2014). These genes significantly affect the physical, chemical, and nutritional quality of the goat's milk (Martin *et al*., 2002). High levels of *CSN1S1* have been found and associated with higher total protein and casein concentrations in goats (Clark and Sherbon, 2000). *CSN2* is the gene, which is an important fraction of casein, is the most extensively studied milk protein gene.

[1]Department of Animal Genetics and Breeding, Navsari Agricultural University, Navsari, Gujarat

[2]Department of Animal Biotechnology, Navsari Agricultural University, Navsari, Gujarat

[3]Department of Instructional Livestock Farm Complex, College of Veterinary Sciences & AH, Navsari Agricultural University, Navsari, Gujarat

[4]Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat

Corresponding Author: Mamta Janmeda, Department of Animal Genetics and Breeding, Navsari Agricultural University, Navsari, Gujarat, e-mail: mamtajanmeda@nau.in

How to cite this article: Janmeda, M., Pandya, G., Ramani, U., Brahmkshtri, B., Patel, N. & Kharadi, V. (2020). Relative Gene Expression Study on Casein Protein and its Regulatory Genes in Mammary Epithelial Cells of Surti Goat. Ind J Vet Sci and Biotech, 16(1):54-57.

Source of support: Nil

Conflict of interest: None.

Submitted: 22/08/2020 **Accepted:** 24/08/2020 **Published:** 04/09/2020

Milk protein gene expression is influenced by the complex interplay of both positive and negative transcription factors and the hormone-regulated signaling pathway (Rijnkels *et al*., 2003). The proximal promoter of β-casein has lactogenic response elements that harbor multiple or a single binding site(s) for transcription factors, mainly including signal transducer and transcription activator 5 (STAT5A), and CAAT/enhancer-binding protein β (C/EBPβ) (Qian and Zhao, 2014). Despite the nutritional and biological importance of goat

---

milk, transcriptome-wide information from mammary glands is still lacking. Considering the above facts, the present study was designed to estimate the relative gene expression of casein proteins (*CSN1S1, CSN1S2, CSN2,* and *CSN3*) and its regulatory genes (*STAT5A* and *C/EBPβ*) in mammary epithelial cells of Surti goats during the lactation cycle.

MATERIALS AND METHODS

Ten healthy Surti goats were selected from Livestock Research Station, NAU, Navsari (Gujarat), and were categorized into two groups for the ease of data analysis and comparisons, *viz.,* S30 (30th day postpartum) and S90 (90th day postpartum). Whole milk sample 800 ml from each selected animal was collected during milking into a sterile container, and milk yield and its composition were determined.

The non-invasive method was used for isolation of somatic cells and Mammary Epithelial Cells (MEC) from goat milk samples using antibody-mediated magnetic bead separation adapted from Sigl *et al*. (2012).

Total RNA was extracted from the purified MEC using miRNeasy MiniKit as per the manufacturer's (Qiagen) protocol. After quantity and quality analysis of the RNA, 500 ng of the samples were subjected to reverse transcription for cDNA synthesis using QuantiTect® Reverse Transcription kit. The real-time PCR of milk casein, regulatory, and keratin (*KRT14* epithelial cell marker) genes was carried out using previously published caprine specific primers (Faucon *et al*., 2009) and commercially synthesized ones (Eurofins Genomics, India).

Relative gene expression was quantified by real-time PCR (ABi, USA) and analyzed using Applied Biosystems 7500 software v2.0.5. The real-time PCR reaction master mix was prepared, and running protocol involved denaturation step (94°C, 15 sec), annealing combined with extension step (60°C, 30 sec), cycling program (45 times) followed by melt curve analysis. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPD*) was used as a reference index and used for normalization. The Quantitative cycle ΔCq values were calculated as ΔCq = Cq target gene transcript – Cq reference gene transcript. Additionally, the amount of target normalized to an endogenous reference gene and relative to a calibrator was obtained by the following equation:

$$\text{Fold increase/ decrease in target} = 2^{-\Delta\Delta Cq}$$

The data on milk yield, milk composition, and gene expression analysis were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS, Version 20.0) software. The ΔCq values were normalized individually in relation to the housekeeping gene index of *GAPD* prior to statistical analysis. Bivariate correlations were calculated using Pearson correlation coefficient.

Table 1: Mean milk yield and composition traits at day 30 and 90 postpartum in Surti goats

Traits / Groups	*S30 (n = 10)*	*S90 (n = 10)*	*'t' value*
Test day milk yield (kg)	2.14 ± 0.13	1.18 ± 0.09	5.73**
Cumulative milk yield (kg)	31.0 ± 3.66	128.0 ± 8.58	10.46**
Fat percent	3.58 ± 0.07	3.96 ± .06	3.8**
SNF percent	8.15 ± 0.03	8.55 ± 0.13	2.9*
Protein percent	3.15 ± 0.03	3.34 ± 0.06	2.5*
Lactose percent	4.12 ± 0.12	4.29 ± 0.05	1.22

*Significant at $p \leq 0.05$, **highly significant at $p \leq 0.01$, n = Number of observations.

RESULTS AND DISCUSSION

Milk yield and composition of goats are given in Table 1. All the parameters pertaining to milk yield and milk composition differed significantly at both the stages of lactation, except for lactose percent.

In agreement with present finding, butterfat, protein, lactose and energy values were significantly tended to rise with advancing lactation as reported in West African dwarf goats (Charnobai *et al*., 1999), whereas Zeng *et al*. (1997) reported high fat, protein, solids non-fat and total solids with the exception of lactose, in the first month after parturition, which declined slightly and then remained constant until drying-off in Alpine does. In accordance with the present study, daily milk production increased steadily for the first 4 weeks following parturition and then decreased gradually as reported by Zeng *et al*. (1997), however, Yakan *et al*. (2018) reported increased daily milk yield (g) from 1st month to 3rd month of lactation in Damascus goats.

In the present study, Mammary Epithelial Cells (MEC) were successfully recovered from of Surti goats' milk and further used for recovering RNA for down step quantification of major milk casein protein gene expression. Somatic Cell Counts (SCC), MEC, MEC recovery %, and RNA yield were significantly higher at day 90 postpartum in Surti goats (Table 2).

In goat milk, SCCs range from 50 to 1,000 $\times 10^3$/ml and are influenced by the stage of lactation (Rota *et al*., 1993). SCC shows an upward trend with the progression of the productive period resulting in a significant negative correlation between SCC and milk production (Gomes *et al*., 2006). Significantly higher SCC was reported by Zeng *et al*. (1997) in Alpine does during the first 2 weeks of lactation

Table 2: Mean Somatic cell counts, mammary epithelial cells, MEC recovery % and RNA yield at day 30 and 90 postpartum in Surti goats

Traits/Groups	*S30 (n = 10)*	*S90 (n = 10)*	*'t' value*
SCC (× 1000/ml)	240. 60± 9.84	384.60±18.60	6.84**
MEC (×1000/ml)	5.64 ± 0.08	6.34 ± 0.14	4.23*
MEC recovery %	2.38 ± 0.10	1.68 ± 0.09	4.92*
RNA yield (µg)	3.43 ± 0.28	4.42 ± 0.25	5.18*

*Significant at $p \leq 0.05$, **highly significant at $p \leq 0.01$, n = Number of observations.

(887 ± 400 X 10^3/ml) and Damascus goats from 1st month (488.04 ± 107.86) to 3rd month (730.08 ± 189.23 ×1000/ml) as reported by Yakan *et al*. (2018).

In the present study, the mean relative expression of *CSN1S1, CSN1S2, CSN3,* and *C/EBP* genes differ significantly up-regulated ($p \leq 0.05$) with the advancement of lactation in Surti goats with 7.79, 32.87, 21.41 and 24.68 fold increase, respectively (Table 3), whereas the mean relative expression of *CSN2* and *STAT5A* genes did not differ significantly ($p > 0.05$) (Table 4).

In accordance with the present study, Shi *et al*. (2014) studied caseins genes expression at non-pregnant, 100 and 310 days of lactation in Saanen dairy goats and reported more than 1000 fold change for *CSN1S1, CSN1S2*, and *LALBA* during lactation. Similarly studies revealed, *CSN1S1* significantly expressed only during lactation (Ollier *et al*., 2008; Faucon *et al*., 2009; Ogorevc and Dovc, 2015; Crisa *et al*., 2016). CSN2 gene shown significant upregulation with fold changes of 6.631 in the 3rd month of lactation compared to the 1st month in Damascus goats by Yakan *et al*. (2018).

The relative expression of *KRT14* gene was almost similar in both the groups in the present study (Table 3). A similar result was reported by Faucon *et al*. (2009). *KRT14* was equally expressed at every developmental stage, suggesting that their numbers change little during pregnancy and the transition to lactation in Saanen and Alpine goats.

In the present study, at 30 days**,** the relative gene expression of *CSN1S1* with *C/EBP*, *CSN1S2* with *CSN3,* and *C/EBP* with *STAT5A* was significantly and positively correlated. The relative gene expression of *CSN2* had a highly significant and positive correlation with its regulatory genes *C/EBP* and *STAT5A* at 30 days and 90 days, respectively, in Surti goats (Table 5).

The relative gene expression of *CSN2* with Test Day Milk Yield and *CSN3* with Cumulative Milk Yield were positively correlated at day 30 postpartum in Surti goats (Table 6). Also the relative gene expression of *CSN1S2, CSN3,* and *C/EBP* genes were positively correlated with protein percent at 30 days and 90 days postpartum in Surti goats (Table 6, 7).

Table 3: Folds increase/decrease in major milk casein, regulatory genes and keratin gene at day 30 and 90 postpartum in Surti goats

Groups	*S30*	*S90*
CSN1S1	1	7.79
CSN1S2	1	32.87
CSN2	1	2.78
CSN3	1	21.41
C/EBP	1	24.68
STAT5A	1	2.40
KRT14	1	0.72

Table 4: Mean relative expression of major milk casein, regulatory and keratin genes at day 30 and 90 postpartum in Surti goats

Groups	*S30 (n = 10)*	*S90 (n = 10)*	*'t' value*
CSN1S1	3.68 ± 0.12 (14.86)	3.98 ± 0.12 (17.82)	2.71*
CSN1S2	3.52 ± 0.10 (13.49)	4.04 ± 0.13(18.53)	3.02*
CSN2	3.30 ± 0.14 (11.86)	3.50 ± 0.14 (13.34)	0.98
CSN3	3.41 ± 0.12 (12.63)	3.91 ± 0.06(17.05)	3.56**
C/EBP	3.28 ± 0.10 (11.76)	3.84 ± 0.17 (16.39)	2.75*
STAT5A	3.06 ± 0.08 (10.38)	3.27 ± 0.11 (11.69)	1.4
KRT14	3.44 ± 0.08 (12.91)	3.38 ± 0.07 (12.45)	0.56

Values in parenthesis are mean (ΔCq) values.

Table 5: Correlation coefficients among relative expression of major milk casein and regulatory genes in Surti goats at day 30 (above diagonal) and day 90 (below diagonal) postpartum

Gene transcripts	*CSN1S1*	*CSN1S2*	*CSN2*	*CSN3*	*C/EBP*	*STAT5A*
CSN1S1	1	0.158	0.049	0.455	0.843**	0.514
CSN1S2	0.121	1	0.249	0.845**	0.370	0.380
CSN2	0.246	0.661	1	0.149	0.738*	0.817**
CSN3	0.027	0.554	0.534	1	0.015	0.226
C/EBP	0.302	0.251	0.741*	0.073	1	0.717*
STAT5A	0.382	0.043	0.702*	0.141	0.30	1

*significant at $p \leq 0.05$, **highly significant at $p \leq 0.01$

Table 6: Correlation coefficients among milk yield, composition traits and relative expression of milk casein and regulatory genes in Surti goats at day 30 postpartum

Traits/Gene transcripts	*CSN1S1*	*CSN1S2*	*CSN2*	*CSN3*	*C/EBP*	*STAT5A*
TDMY (kg)	0.092	0.219	0.813**	0.517	0.111	0.120
Fat percent	0.349	0.036	0.318	0.595	0.099	0.204
SNF percent	0.106	0.092	0.204	0.345	0.211	0.263
Protein percent	0.423	0.659**	0.470	0.668**	0.734**	0.444
Lactose percent	0.142	0.257	0.423	0.190	0.115	0.210
CMY 30 (kg)	0.563	0.228	0.396	0.705*	0.017	0.160

*significant at $p \leq 0.05$, **highly significant at $p \leq 0.01$

Table 7: Correlation coefficients among milk yield, composition traits and relative expression of milk casein and regulatory genes in Surti goats at day 90 postpartum

Traits/Gene transcripts	*CSN1S1*	*CSN1S2*	*CSN2*	*CSN3*	*C/EBP*	*STAT5A*
TDMY (kg)	0.012	0.255	0.065	0.457	0.065	0.325
Fat percent	0.279	0.277	0.170	0.116	0.421	0.128
SNF percent	0.162	0.207	0.125	0.456	0.510	0.431
Protein percent	0.143	0.634**	0.450	0.935**	0.765**	0.469
Lactose percent	0.273	0.253	0.269	0.103	0.129	0.265
CMY90 (kg)	0.017	0.170	0.001	0.545	0.060	0.447

*significant at $p \leq 0.05$, **highly significant at $p \leq 0.01$

CONCLUSIONS

In conclusion, thus, gene expression profiling as determined from milk epithelial cells, provide a convenient means to more dynamically characterize events within the mammary gland. The high expression of caseins protein genes seemed to partly agree with previous data demonstrating that casein encoding genes are highly expressed in goat mammary glands at every stage of lactation.

ACKNOWLEDGMENTS

Authors thank the Dean, College of Veterinary Science and Animal husbandry and the University authorities, Navsari Agricultural University, Navsari for the facilities provided for this research work.

REFERENCES

Bepost, F., Hosokawa, M., Tanaka, L., Kohno, H., Tanaka, T., & Miyashita, K. (2006). Potent inhibitory effect of trans9, trans11 isomer of conjugated linoleic acid on the growth of human colon cancer cells. *Journal of Nutrition and Biochemistry*, 17, 830–836.

Boutinaud, M., Rulquin, H., Keisler, H., Djiane, J., & Jammes, H. (2002). Use of somatic cells from goat milk for dynamic studies of gene expression in the mammary gland. *Journal of Animal Science,* 80, 1258-1269.

Charnobai, C.A., Damasceno, J.C., Vise, J.V., Souza, M.E., & Matsushita, M. (1999). Physical-chemical composition of Mathura goat milk from cross Saanen throughout lactation period. *Archivas Latina Americanos-de-Nutrician*, 49(3), 283-286.

Clark, S., & Sherbon, J.W. (2000). AlphaS1-casein, milk composition and coagulation properties of goat milk. *Small Ruminant Research*, 38, 123-134.

Crisa, A., Ferre, F., Chillemi, G., and Moioli, B. (2016). RNA-Sequencing for profiling goat milk transcriptome in colostrum and mature milk. *BMC Veterinary Research*, 12, 264.

Dagnachew, B.S., Thaller, G., Lien, S., & Adnoy, T. (2011). Casein SNP in Norwegian goats: additive and dominance effects on milk composition and quality. *Genet Selection and Evolution*, 43, 31.

Faucon, F.E., Rebours, C., Bevilacqua, J.C. Helbling, J.A., Makhzami, S., Dhorne, S.R., & Martin, P. (2009). Terminal differentiation of goat mammary tissue during pregnancy requires the expression of genes involved in immune functions. *Physiology of Genomics*, 40, 61–82.

Finocchiaro, R., & Vankaamj, T. (2004). Perspectives for goat milk in human nutrition. *Animal Science Paper Report*, 22, 375-380.

Gomes, V., Melville, A.M., Paiva, M., Madureira, K.M., and Araujo, W. P. (2006). Effect of the stage of lactation on somatic cell counts in healthy goats (Caprae hircus) breed in Brazil. *Small Ruminant Research*, 64(1-2), 30-34.

MacHugh, D.E., & Bradley, D.G. (2001). Livestock genetic origins: goat buck the trend. *Proceedings of National Academy of Science*, 98, 5382–5384.

Martin, P., Szymanowska, M., Zwierzchowski, L., & Leroux, C. (2002). The impact of genetic polymorphisms on the protein composition of ruminant milks. *Reproduction Nutritional and Development*, 42(5), 433-459.

Nowak, I.M., Kues, W., Carnwath, J.W., & Niemann, H. (2011). Pluripotent stem cells and reprogrammed cells in farm animals. *Microsc Microanalanlysis*, 17, 474-497.

Ogorevc, J., & Dovc, P. (2015). Relative quantification of beta-casein expression in primary goat mammary epithelial cell lines. *Genetics and Molecular Research,* 14(2), 3481-3490.

Ollier, S., Chauvet, S., Martin,P., Chilliard, Y., & Leroux, C. (2008). Goat's aS1-casein polymorphism affects gene expression profile of lactating mammary gland. *Animal,* 2(4), 566-573.

Qian, X., & Zhao, F. (2014). Current Major Advances in the Regulation of Milk Protein Gene Expression. *Critical Reviews™ in Eukaryotic Gene Expression*. 24(4), 357-378.

Rijnkels, M., Elnitski, L., Miller, W., & Rosen, J. M. (2003). Multispecies comparative analysis of a mammalian-specific genomic domain encoding secretory proteins. *Genomics*. 82(4), 417-432.

Rota, A.M., Gonzalo, C., Rodriguez, P.L., Rojas, A.I., Martin, L., & Tovar, J.J. (1993). Effects of stage of lactation and parity on somatic cell counts in milk of Verata Goats and algebraic models of their lactation curves. *Small Ruminant Research*, 12, 211-219.

Shi, H, Jiangjiang, Z.J., Jun, L J, Wenting, C.W., Huaiping, S.H, Dawei, Y.D, Jun, L.J., Sun, Y., & Loor, J.J. (2014). Genes Regulating Lipid and Protein Metabolism are Highly Expressed in Mammary Gland of Lactating Dairy Goats. *Functional Integrity of Genomics*., 15, 309-321.

Sigl, T., Meyer, H.H., & Wiedemann, S. (2012). Gene expression of six major milk proteins in primary bovine mammary epithelial cells isolated from milk during the first twenty weeks of lactation. *Czech Journal of Animal Science*, 57(10), 469-480.

Yakan, A., Ozkan, H., Eraslan, Aysel., Unal, N., & Ozbeyaz, C. (2018). Gene expression levels in some candidate genes for mastitis resistance, milk yield, and milk quality of goats reared under different feeding systems. *Turkey Journal of Veterinary and Animal Science,* 42, 18-28.

Zeng, S.S., Escobar, E.N., & Popham, T. (1997). Daily variations in somatic cell count, composition, and production of Alpine goat milk. *Small Ruminant Research,* 26, 253-260.