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Stage Specific Expression Profile of Lipogenic Genes in Mammary Epithelial Cells of Surti and Jaffarabadi Buffaloes

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Abstract

The present study was conducted to study the relative gene expression of lipogenic genes at 15- and 60-day post-partum (pp) in primary bovine mammary epithelial cells (pBMEC) of Surti and Jaffarabadi buffaloes. Milk sample was collected from 10 healthy buffaloes of each breed selected at random from Livestock Research Station, Navsari and Cattle Breeding Farm, Junagadh, Gujarat. Non-invasive approach of obtaining pBMEC from buffalo milk samples was used to study relative gene expression of major milk lipogenic genes. The relative expression of the eight major milk lipogenic genes i.e. ABCG2, FASN, BTN1A1, SCD, LPL, GPAM, ACACA and LPIN were studied. Non-significant changes were observed in all the genes in both the lactation stages in both Surti and Jaffarabadi buffaloes except FASN which showed significant difference in Jaffarabadi buffaloes from 15 to 60 days pp in expression pattern suggesting steady expression of mammary lipid metabolism upto 60 days pp.

Keywords: Buffalo, Jaffarabadi, Gene Expression, Lipogenic Genes, Surti

Introduction

The role of buffalo as a major milk producing species is well recognized in Indian subcontinent, especially in India and Pakistan. Important chemical composition determining nutritive properties has long been valued in buffalo milk. Fat is the major energy source in milk, and lipid synthesis by the mammary glands. Milk lipid synthesis as well as droplet formation and secretion received particular interest due to their influence on the manufacturing properties and organoleptic quality of milk and dairy products (Keenan and Mather, 2006).

Gene expression analysis is becoming more prevalent in livestock species since it promotes our understanding of complex biological processes such as lactation physiology (Wang *et al.,* 2009). Studies in different species have also highlighted the importance of milk purified mammary epithelial cells (pMEC) over mammary tissue to study expression patterns of genes related to milk synthesis (Yadav *et al.,* 2014), as expression pattern in MEC would give a real picture of the molecular events involved in milk synthesis during lactation. The majority of genes in the fat metabolism pathway had high expression in transition and peak lactation milk (Wickramasinghe *et al.,* 2012). On the basis of previous studies in bovine, some genes were shortlisted for assessment, which were involved in different pathways of lipid synthesis and transcription regulation of lipogenic genes presented in Table 1.

Table 1: Genes, symbols and processes involved for lipogenic genes under study by Bionaz and Loor (2008)

Gene	Symbol	Process involved
ATP-binding cassette, sub-family G (WHITE), member 2	*ABCG2*	It is present in the milk fat globule membrane (MFGM), probably in the external bilayer originating from plasma membrane and plays essential role in secretion of some important milk constituent.
Fatty acid synthase	*FASN*	de novo Fatty acid synthesis, TAG synthesis and secretion
Butyrophilin sub family 1 member A	*BTN1A1*	Regulates the assembly, transport, or secretion of milk fat droplets
Steroyl-CoA desaturase	*SCD*	Key enzyme for converting the saturated fatty acid stearic acid to monounsaturated fatty acid oleic acid.
Lipoprotein lipase	*LPL*	fatty acid uptake from blood
Glycerol-3-phosphate O-acyl transferase mitochondrial	*GPAM*	Esterification enzymes in mono, di and triacylglycerol synthesis
Acetyl-coA carboxylase alpha	*ACACA*	Catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, a rate-limiting step in fatty acid synthesis
Lipin	*LPIN*	Acts as enzyme, which catalyzes the conversion of phosphatidic acid to diacylglycerol, Triacylglycerol synthesis

Genetic variability remained the prime focus of breeders to explore reasons for differential production potential among different breeds. Considering these facts, the present study has been designed to analyze the relative gene expression pattern of lipogenic genes at 15- and 60-day post-partum (pp) in lactating Surti and Jaffarabadi buffalo breeds of Gujarat as these two are the divergent breeds of buffalo in relation to body size, fat % and feed conversion efficiency.

Materials and Methods

Samples were collected from 10 healthy Surti and Jaffarabadi buffaloes of each breed selected at random from Livestock Research Station, Navsari and Cattle Breeding Farm, Junagadh, Gujarat respectively. Fifteen hundred milliliter of the whole milk from 10 selected buffaloes of each breed group was collected during milking at 15- and 60-day post-partum into a sterile bucket and was subjected for cell isolation. The method for isolation of somatic cells and pBMEC from buffalo milk samples were adapted from Sigl *et al.* (2012) using primary mouse monoclonal antibody against cytokeratin 8 antibody (clone C-43). miRNeasy MiniKit used for total RNA extraction from the purified pBMEC as per the manufacturer's (Qiagen) guidelines. 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. Relative expression of milk lipogenic and keratin genes was quantified by Real Time PCR (ABi, U.S.A) and analyzed using Applied Biosystems 7500 software v2.0.5.The primers were selected from published references in HF cows by Sigl *et al.* (2014) and in Murrah buffaloes by Yadav *et al.* (2015) and commercially synthesized from Eurofins Genomics, India. For normalization and as reference index, the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPD*) was used in study. Quantitative cycle (Cq) values were calculated by Applied Biosystems 7500 software v2.0.5. The ΔCq values were calculated as ΔCq = Cq target gene transcript – Cq reference gene transcript (Pfaffl, 2001). Additionally, the amount of target normalized to an endogenous reference gene and relative to a calibrator was obtained by following equation:

$$\text{Fold increase/ decrease in target} = \quad 2^{-\Delta\Delta Cq} \text{ (Livak and Schmittgen, 2001)}$$

The data 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 as per Hinkle *et al.* (2003).

Results and Discussion

The detailed statistics of somatic cells count (SCC), primary bovine mammary epithelial cells (pBMEC) obtained from total somatic cells and RNA yield from pBMEC in milk of Surti and Jaffarabadi buffaloes are presented in Table 2.

Table 2: Somatic Cell Count, pBMEC and RNA yield in different groups of buffaloes

Traits/Groups	**S15**	**S60**	**J15**	**J60**	**F**
N	**10**	**10**	**10**	**10**	**Values**
SCC (× 1000/ml)	241.45^{d}±6.75	196.68^{b}±4.74	218.37^{c}±6.40	174.38^{a}±5.51	22.93**
pBMEC (×1000/ml)	5.10±0.41	4.90±0.52	5.73±0.41	5.59±0.45	0.63
pBMEC recovery %	2.13^{a}±0.17	2.58^{ab}±0.27	2.62^{ab}±0.16	3.17^{b}±0.28	3.71*
RNA yield (µg)	4.64^{a}±1.02	7.52^{b}±0.81	3.89^{a}±0.90	6.35^{ab}±1.09	2.94*

**Significant at p≤0.05, **highly significant at p≤0.01, N= Number of observations. Means bearing different superscript between groups differed significantly. S15 (Surti buffaloes 15th day pp), S60 (Surti buffaloes 60th day pp), J15 (Jaffarabadi buffaloes 15th day pp) and J60 (Jaffarabadi buffaloes 60th day pp)*

In the present study, higher SCC was observed in Surti buffaloes as compared to Jaffarabadi buffaloes. A decrease in the mean SCC in milk from day 15 pp to day 60 pp was also been reported by Tyagi (2016) which is in accordance with the present finding. Boutinaud *et al.* (2012) reported average SCC of $1.99 \pm 0.03 \times 10^5$ cells/ml with the range of 1.86×10^5 - 2.12×10^5 cells/ml of buffalo milk that is comparable with the SCC reported in the present study. In the present study, the mean pBMEC recovered (×1000/ml milk) from total somatic cells were comparable in all groups. Pandya *et al.* (2018) reported, mean pBMEC values (×1000/ml) and pBMEC recovery percent obtained from total somatic cells were found to be similar in S15 and S60 groups in Surti buffalo. Although lower pBMEC that ranged from ($1.10 \pm 0.06 \times 10^3$ to $1.40 \pm 0.03 \times 10^3$ cells/ml milk) at different stage of lactation had also been reported in cows by Sigl *et al.* (2012). The percentages of pBMEC in relation to total milk cells among different groups in the present study were steady with non-significant at 60 pp. However, lower 1.54×10^3 pBMEC/ml in Holstein-Friesian cow's milk around day 162 pp which comprised 2 percent of total milk cells was reported by Boutinaud *et al.* (2015). An optical density 260/280 ratio (OD 260/280) ~ (1.8 to 2.0) was observed in samples of the present study indicating purity of RNA. Significant difference in RNA yield was observed with advancement of lactation in Surti buffalo. However, no significant variation was observed in Jafarabadi buffaloes. Similar results were obtained in cows by Sigl *et al.* (2012), where extracted quantity of pBMEC mRNA did not vary during experimental timeframe.

Relative Gene Expression of Lipogenic and Keratin Genes

The mean relative expression values of milk lipogenic genes among different groups have been presented in Table 3 and table 4 presented the relative folds increase / decrease in groups for various gene transcripts in terms of unit folds expression.

Table 3: Relative expression of milk lipogenic genes among different groups

Groups	S15	S60	J15	J60	F value
N	10	10	10	10	
ABCG2	3.75 ±0.17(6.53)	3.66 ±0.22(5.94)	3.68 ±0.05(2.25)	3.78 ±0.03(1.95)	1
FASN	3.68^{a}±0.12(2.20)	3.63^{a}±0.14(4.64)	3.89^{ab}±0.17(5.53)	3.18^{b} ±0.22(6.94)	6.13*
BTN1A1	3.98 ±0.03(-0.72)	3.96 ±0.10(-0.57)	4.00 ±0.04(-0.96)	3.98 ±0.13(-0.73)	0.03
SCD	3.60±0.70(2.16)	3.60 ±0.11(2.83)	3.60 ±0.06(2.90)	3.53 ±0.15(3.47)	0.36
LPL	3.82 ±0.07(0.91)	3.68 ±0.12(2.20)	3.60 ±0.07(2.85)	3.63 ±0.14(2.64)	0.84
GPAM	3.74 ±0.09(1.68)	3.50 ±0.12(3.68)	3.72 ±0.06(1.85)	3.58 ±0.13(3.05)	1.13
ACACA	3.39 ±0.09(4.52)	3.08 ±0.17(6.53)	3.40 ±0.12(4.42)	3.18 ±0.22(5.94)	1.03
LPIN	3.53 ±0.09(3.44)	3.33 ±0.12(4.94)	3.54 ±0.13(3.34)	3.49 ±0.18(3.73)	0.54
KRT8	3.63 ±0.04(2.59)	3.62 ±0.06(2.73)	3.67 ±0.05(2.25)	3.71 ±0.03(1.95)	0.75

Values in parenthesis are mean (ΔCq) values.

Table 4: Folds increase/decrease in milk lipogenic genes among different groups

Groups	A				B				C				D			
	S15	S60	J15	J60	S15	S60	J15	J60	S15	S60	J15	J60	S15	S60	J15	J60
ABCG2	1	0.26	0.86	0.45	3.91	1	3.35	1.75	1.17	0.3	1	0.52	1	0.52	0.67	1
FASN	1	0.4	1.96	0.05	4.02	1	8.06	1.11	0.51	0.2	1	0.52	6.42	6.01	2.04	1
BTN1A1	1	0.9	1.18	1.01	1.11	1	1.31	1.12	0.85	0.76	1	0.85	0.99	0.9	1.18	1
SCD	1	0.63	0.6	0.4	1.6	1	0.95	0.65	1.68	1.05	1	0.68	2.49	1.56	1.49	1
LPL	1	0.41	0.26	0.3	2.46	1	0.64	0.74	3.86	1.57	1	1.16	3.32	1.36	0.86	1
GPAM	1	0.25	0.89	0.39	4.03	1	3.59	1.56	1.12	0.28	1	0.43	2.59	0.66	2.3	1
ACACA	1	0.25	1.07	0.37	4.05	1	4.32	1.52	0.94	0.23	1	0.35	2.67	0.66	2.86	1
LPIN	1	0.35	1.07	0.82	2.84	1	3.05	2.33	0.93	0.33	1	0.76	1.22	0.43	1.31	1
KRT8	1	0.91	1.27	1.5	1.1	1	1.4	1.72	0.79	0.72	1	1.23	0.64	0.58	0.81	1

A, B, C and D represents relative folds increase / decrease in groups for various gene transcripts against unit folds expression of one rotationally chosen group.

The relative expression of the major milk lipogenic genes *ABCG2, BTN1A1*, *SCD*, *LPL*, *GPAM*, *ACACA* and *LPIN* were studied, out of which all the genes did not show changes in expression patterns at 15 and 60 pp of lactation in both Surti and Jaffarabadi buffaloes.

In contrast to the present study, Bionaz and Loor (2008) observed, a 30-fold increase was in *ABCG2* transcript, >40-fold up-regulation of *SCD* at peak lactation (60-day pp) with subsequent decline in late lactation and ~ 20-fold up-regulation of *LPIN* mRNA during lactation in Holstein Friesian cattle. Yadav *et al.* (2015) reported contrasting results in Murrah buffaloes in which *LPL* mRNA level showed up-regulation during early lactation (45-day pp) up until peak lactation and then remained unchanged throughout lactation. *ACACA* was slight decrease in mRNA level during 30–45-day pp which further increased at peak lactation, followed by sharp decline at 90 day (Yadav *et al.*, 2015). Bionaz and Loor (2008) observed *ACACA* and *FASN* both of which are key enzymes regulating de novo Fatty acid synthesis, showed peaked expression at 60 days in HF cattle. As per Anonymous (2014), the expression level of *GPAM* in milk somatic cells of Murrah buffaloes and Sahiwal cows were significantly higher in early (0-20 days) compared to mid (90-130 days) and late (>240 days) lactation stages. Wickramasinghe *et al.* (2012) reported higher expression in transition lactation milk somatic cells and a significant decrease in the expression levels in *BTN1A1*.

In present study, relative expression of *FASN* gene was significantly lowered with advancement of lactation in Jaffarabadi buffaloes however it remained steady in Surti buffaloes at day 15 and 60 pp. Bionaz and Loor (2008) observed, *ACACA* mRNA abundance accounted for <1% whereas *FASN* accounted for 7% of total genes measured. However, *ACACA* mRNA had greater up-regulation during lactation compared with *FASN*. The relative expression of *KRT8* was almost similar in all the groups in accordance with other studies in buffaloes (Tyagi, 2016; Pandya *et al.*, 2018). Taylor-Papadimitriou *et al.* (1989) stated that *KRT8* was almost constantly expressed irrespective of breed and stage of lactation.

Table 5: Correlation coefficients of milk lipogenic genes in Surti buffaloes at day 15 (above diagonal) and day 60 post-partum (below diagonal)

Gene transcripts	***BTN1A1***	***SCD***	***LPL***	***GPAM***	***ACACA***	***LPIN***	***ABCG2***	***FASN***
BTN1A1	--	0.45	0.52	0.1	0.49	0.46	0.56	0.53
SCD	0.65*	--	0.84**	0.58	0.86**	0.80**	0.90*	0.80*
LPL	0.82**	0.88**	--	0.80**	0.80**	0.77**	0.89*	0.56
GPAM	0.91**	0.86**	0.91**	--	0.62	0.53	0.78*	0.67
ACACA	0.66*	0.84**	0.83**	0.88**	--	0.91**	0.67	0.56
LPIN	0.78**	0.85**	0.84**	0.94**	0.96**	--	0.68	0.67
ABCG2	0.58	0.65	0.87	0.54	0.56	0.59	--	0.89
FASN	0.68	0.85*	0.54	0.44	0.56	0.56	0.67	--

**significant at p≤0.05, **highly significant at p≤0.01*

Table 6: Correlation coefficients of milk lipogenic genes in Jafarabadi buffaloes at day 15 (above diagonal) and day 60 post-partum (below diagonal)

Gene transcripts	***BTN1A1***	***SCD***	***LPL***	***GPAM***	***ACACA***	***LPIN***	***ABCG2***	***FASN***
BTN1A1	--	0.29	0.19	0.65*	0.21	0.19	0.56	0.53
SCD	0.64*	--	0.74*	0.29	0.38	0.42	0.90*	0.80*
LPL	0.89**	0.6	--	0.41	0.58	0.64*	0.89*	0.56
GPAM	0.81**	0.6	0.81*	--	0.34	0.53	0.78*	0.67
ACACA	0.87*	0.71*	0.84**	0.69*	--	0.95**	0.67	0.56
LPIN	0.87**	0.66*	0.79**	0.63*	0.98*	--	0.68	0.67
ABCG2	0.58	0.53	0.56	0.53	0.56	0.53	--	0.53
FASN	0.90*	0.80*	0.90*	0.80*	0.90*	0.80*	0.8	--

**significant at p≤0.05, **highly significant at p≤0.01*

In the present study, significant to highly significant correlations were observed among almost all lipogenic genes. Significant positive correlations between relative expressions of *SCD* and *FASN* were observed in all the stages of buffalo breeds as presented in Table 5 and 6. In accordance with our study, Yadav *et al.* (2015) reported similar findings that *LPIN* has strong positive correlation with *SCD* (0.80) and *LPL* (0.38) but negative correlation with *ACACA* (-0.57) in Murrah buffaloes.

Conclusion

Lactating ruminants have been of special value as a model to investigate milk fat synthesis as it decides about ranking of buffaloes as fat per cent and total fat content. In present study overall relative expression of the milk lipogenic genes *ABCG2*, *BTN1A1*, *SCD*, *LPL*, *GPAM*, *ACACA* and *LPIN* did not show changes in expression patterns at 15- and 60-days pp of lactation in both Surti and Jaffarabadi buffaloes suggesting genes expression in mammary lipid metabolism remains steady and peaked at 60 days post-partum and exactly follows the lactation curve. The relative expression of *FASN* gene was downregulated significantly with advancement of lactation in Jaffarabadi buffaloes however it remained steady in Surti buffaloes at day 15 and 60 pp. *FASN* expression in Jaffarabadi buffaloes is not following the pattern of expression of other genes which suggests that fatty acid uptake from blood predominated during the first month of lactation and after 1st month post-partum *de novo* fatty acid synthesis predominated and continued upto 10 to 12 weeks as mammary cells utilize fatty acids derived both from *de novo* synthesis and fatty acids absorbed from blood circulation for milk lipid synthesis. Hence, conclusion is difficult for both breeds. Further in-depth study of key genes regulating milk fat synthesis is required to provide clear picture.

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Conflict of Interests

There is no conflict of interest.

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References

1. Anonymous. (2014). Analysis of Mammary Gland Transcriptome and Proteome during Lactation and Involution in Indigenous Cattle and Buffalo for Identification of Probable Mammary Biomarkers. *Final Report: NAIP – ICAR National Dairy Research Institute Karnal*, 71.
2. Bionaz, M. and Loor, J. J. (2008). Gene networks driving bovine milk fat synthesis during the lactation cycle. *BMC Genomics, 9*, 366-374.
3. Boutinaud, M., Herve, L. and Lollivier, V. (2015). Mammary epithelial cells isolated from milk are a valuable, non-invasive source of mammary transcripts. *Frontiers in Genetics, 6*, 323-335.
4. Boutinaud, M., Lollivier, V., Finot, L., Bruckmaier, R. M. and Lacasse, P. (2012). Mammary cell activity and turnover in dairy cows treated with the prolactin- release inhibit or quinagolide and milked once daily. *Journal of Dairy Science, 95*, 177–187.
5. Hinkle, D. E., Wiersma, W. and Jurs, S. G. (2003). Applied Statistics for the Behavioral Sciences 5th ed. Boston: Houghton Mifflin.
6. Keenan, T.W. and Mather, I.H. (2006). Intracellular origin of milk fat globules and the nature of the milk fat globule membrane. In: Fox, P.F., McSweeney, P.L.H. (Eds.), Advanced Dairy Chemistry: Lipids. Springer, New York, 137–171.
7. Livak, K.J. and Schmittgen, T.D. (2001). Analysis of relative gene expression data using RealTime Quantitative PCR and the Method. *Methods, 25*, 402–408.
8. Pandya, G., Ramani, U., Janmeda, M., Kharadi, V., Brahmkshtri, B. and Tyagi. K. (2018). Relative Gene Expression Analysis of beta-Casein Milk Protein and its Transcription Regulatory Genes in Surti Buffalo. *International Journal of Livestock Research, 8*(4), 121-127.
9. Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research, 29*(9), e45.
10. Sigl, T., Meyer, H.H.D. and 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.
11. Sigl, T., Meyer, H.H.D. and Wiedemann, S. (2014). Gene expression analysis of protein synthesis pathways in bovine mammary epithelial cells purified from milk during lactation and short-term restricted feeding. *Journal of Animal Physiology and Animal Nutrition, 98*, 84-95.
12. Taylor-Papadimitriou, J., Stampfer, M., Bartek, J., Lewis, A., Boshell, M., Lane, E.B. and Leigh, I.M. (1989). Keratin expression in human mammary epithelial cells cultured from normal and malignant tissue: relation to in vivo phenotypes and influence of medium. *Journal of Cell Science, 94*(3), 403-413.
13. Tyagi, K. K. (2016). Relative gene expression analysis of milk protein genes in primary milk epithelial cells of Surti and Mehsani buffaloes (Published doctoral dissertation). Navsari Agricultural University, Navsari, India.
14. Wang, Y. H., Bower, N. I., Reverter, A., Tan, S. H., Jager, N. D., Wang, W., McWilliam, S. M., Cafe , L. M., Greenwood, P. L., Lehnert, S. A. (2009). Gene expression patterns during intramuscular fat development in cattle. *Journal of Animal Science, 87*, 119–130.
15. Wickramasinghe, S., Rincon, G., Islas-Trejo, A. and Medrano, J. F. (2012). Transcriptional profiling of bovine milk using RNA sequencing. *BMC Genomics, 13*, 45-59.
16. Yadav, P., Kumar, P., Mukesh, M., Kataria, R.S., Yadav, A., Mohanty, A.K. and Mishra, B.P. (2015). Kinetics

of lipogenic genes expression in milk purified mammary epithelial cells (MEC) across lactation and their correlation with milk and fat yield in buffalo. *Research in Veterinary Science*, *99*,129-136.

17. Yadav, P., Singh, D. D., Mukesh, M., Kataria, R. S., Yadav, A. and Mohanty, A. K. (2014). Expression profiling of glucose transporter 1 (GLUT1) and apoptotic genes (BAX and BCL2) in milk enriched mammary epithelial cells (MEC) in riverine buffalo during lactation. *Animal Biotechnology*, *25*, 151–159.

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