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Kinetics of lipogenic genes expression in milk purified mammary epithelial cells (MEC) across lactation and their correlation with milk and fat yield in buffalo

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ABSTRACT

Expression patterns of lipogenic genes (LPL, ABCG2, ACSS2, ACACA, SCD, BDH, LIPIN1, SREBF1, PPARα and PPARγ) were studied in milk purified MEC across different stages of lactation (15, 30, 45, 60, 90, 120 and 240 days relative to parturition) in buffalo. PPARα was the most abundant gene while ABCG2 and ACSS2 had moderate level of expression; whereas expression of SREBF and PPARγ was very low. The expression patterns of some genes (BDH1, ACSS2, and LIPIN1) across lactation were positively correlated with milk yield while negatively correlated with fat yield. SCD also showed weak correlation with milk yield (p, 0.53) and fat yield (p, –0.47). On the other hand, expression pattern of ACACA was negatively correlated with milk yield (p, –0.88) and positively correlated with fat yield (p, 0.62). Strong correlation was observed between genes involved in de novo milk fat synthesis (BDH1, ACSS2, LIPIN2 and SCD) and milk yield.

1. Introduction

The mammary gland synthesizes and secretes a prodigious complement of products into milk including proteins, carbohydrates, membrane coated lipid droplets, water and ions. How secretion of such a diverse array of milk components is coordinated at the cellular level remains a major challenge for researchers working in area of lactation physiology. Over past 25 years, much progress has been made in unraveling potential mechanisms for the assembly and secretion of milk-lipid droplets. Understanding the mechanism of milk fat synthesis is important as milk lipids affect manufacturing properties and organoleptic quality of milk and dairy products. Progress in lactation biology of the bovine mammary gland advanced substantially during the 20th century (Bauman et al., 2006). Early studies in ruminants defined and quantified major metabolic aspects of mammary lipid metabolism, including *de novo* synthesis and fatty acid (FA) uptake from blood (Bauman and Davis, 1974). 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). Milk composition and in particular milk lipid synthesis are affected by the stage of lactation. Mammary cells utilize fatty acids derived both from de novo synthesis and fatty acids absorbed from blood circulation for milk lipid synthesis. Forty to sixty percent of total fatty acids come from blood, which are primarily derived from very low-density lipoproteins (VLDL) synthesized in liver. Short and medium chain fatty acids are synthesized in cytoplasm of mammary epithelial cells (MEC) by de novo synthesis (Fig. 1). Therefore, possible points of milk lipid synthesis regulation include either of these processes including intracellular fatty acid transport, desaturation, triglyceride synthesis, and fat secretion (Fig. 1). *Riverine buffalo* (*Bubalus bubalis*) is an economically important species in many Asian (especially India) and Mediterranean countries. It harbors several useful traits especially high milk fat yield and hence plays a major role in sustaining economy of Indian agriculture.

Genomic studies in other related species have shown that some metabolic adaptations are acquired in mammary gland during lactation, which includes expression pattern of lipogenic genes (Bionaz

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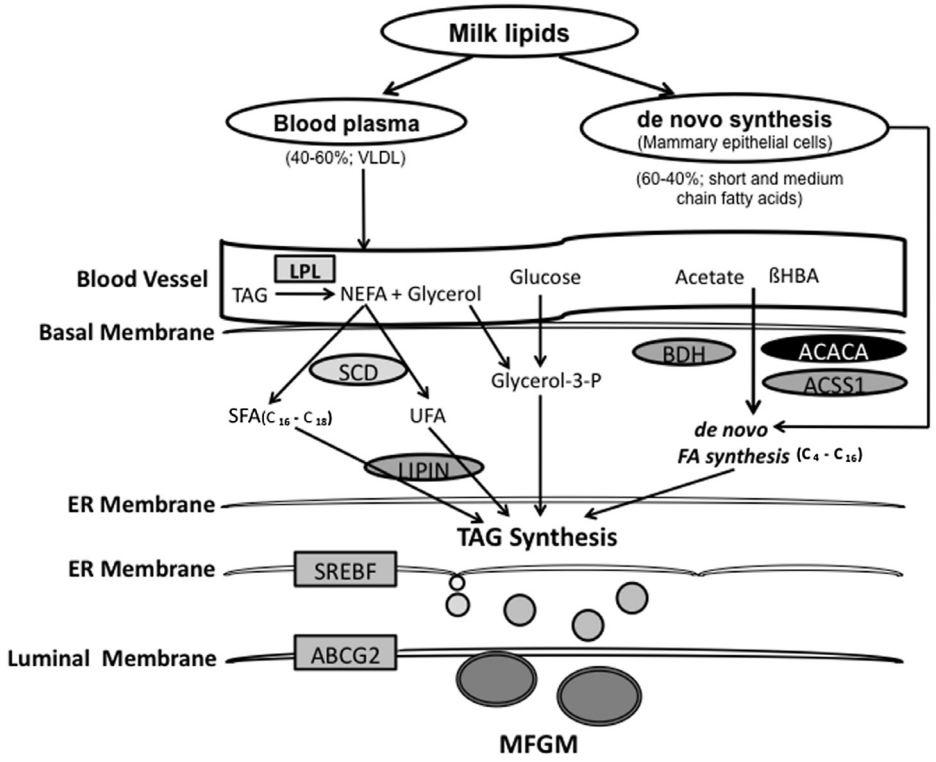

Fig. 1. Process of milk lipid synthesis in mammary gland showing role of different lipogenic genes under study in milk fat synthesis.

and Loor, 2008). Since buffalo milk has the highest fat content among dairy animals, the expression profiles of the lipogenic genes might be an important aspect to understand the mechanism of milk fat synthesis in buffalo. 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 (Table 1 and Fig. 1). Further, studies in different species have highlighted the importance of milk purified MEC over mammary tissue to study expression patterns of genes related to milk synthesis (Annen et al., 2008; Yadav et al., 2014), as expression pattern in MEC would give a real picture of the molecular events involved in milk synthesis during lactation.

2. Methods

2.1. Animal sampling

Five healthy multiparous Murrah buffaloes were selected from cattle yard of National Dairy Research Institute, Karnal. The selection was based on lactation history including milk yield and parity. All selected buffalo were in third parity. The milk samples were collected twice a day (morning and evening) at 15, 30, 45, 60, 90, 120 and 240 days of lactation relative to parturition. The milk was kept at 4 °C until processed for MEC purification.

2.2. Mammary epithelial cell purification from milk

The milk was filtered through fine muslin cloth to remove particulate impurities and centrifuged at 5000 rpm for 20 min at 4 °C. Fat layer was removed and skimmed milk was decanted carefully without disturbing cell pellet, which was resuspended in PBS, washed twice in PBS at 1500 rpm for 7 min and resuspended in 2 ml of 1% PBS-BSA. Dynabeads (Invitrogen, Cergy Pontoise, France) coated with primary mouse monoclonal anti-cytokeratin 8 antibody were used to purify MEC as described (Boutinaud et al., 2008) with some modifications. Briefly, the dynabead solution was washed twice with 1 ml of 1% PBS-BSA to remove preservatives. Dynabead suspension was incubated for 30 min with 2 µl of anticytokeratin 8 antibody (clone

Table 1
Gene symbols, relative mRNA abundances (%), and processes involved of lipogenic genes under study.

S. no.	Gene	Symbol	Relative mRNA abundance	Process involved
1	Lipoprotein lipase	*LPL*	4.25	FA import into cells
2	ATP-binding cassette, sub-family G (WHITE) member 2	*ABCG2*	16.45	Xenobiotic and cholesterol transport
3	Acyl-coA synthetase short-chain family mamber2	*ACSS2*	16.16	Acetate and FA activation and intra-cellular transport
4	Acetyl-coA carboxylase alpha	*ACACA*	6.5	Fatty acid synthesis desaturation
5	Steroyl-CoA desaturase	*SCD*	7.67	Fatty acid synthesis desaturation
6	3-Hydroxybutyrate dehydrogenase	*BDH*	5.5	Ketone body utilization
7	Lipin1	*LIPIN1*	1.75	Triacylglycerol synthesis
8	Sterol regulatory element binding transcription factor 1	*SREBF1*	1.92	Regulation of transcription
9	Peroxisome proliferator activated receptor gamma	*PPARg*	3.58	Regulation of transcription
10	Peroxisome proliferator activated receptor alpha	*PPARa*	32.55	Regulation of transcription