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Lipopolysaccharide-Induced Alterations in mRNA Expression of Genes Related to Fatty Acid Metabolism

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Abstract: The study was conducted to evaluate the effect of Lipopolysaccharide (LPS) on milk fatty acid metabolism. The expression of genes for fatty acid metabolism in dairy cows was studied by incubating mammary epithelial cells with LPS at 0, 0.1 or 10 ng mL^{-1} for 24 h using RT-qRCR *in vitro*. LPS increased transporter CD36 mRNA expression ($p<0.01$). FABP3 mRNA expression ($p>0.05$) was not affected by LPS but the FABP4 mRNA expression was affected ($p<0.05$), decreasing 0.79 fold when LPS was provided at 10 ng mL^{-1}. Treatment with 0.1 ng mL^{-1} LPS tend to increased fatty acid synthesis enzyme FASN, ACACA, ACSS2 and fatty acid metabolism gene PPARG, PPARGC1A mRNA expression ($0.10>p>0.05$) compared with the control. LPS alters fatty acid metabolism, functioning as an important factor affecting milk fat synthesis.

Key words: LPS, fatty acids metabolic-related gene, mammary epithelial cells, milk fat, China

INTRODUCTION

LPS, a cell-wall component of all gram-negative bacteria, released from cell lysis of bacteria in the rumen under high-concentrate/low-forage diets (Anderson *et al*., 2007) in dairy cows. It may be carried into the peripheral circulation, mediating enzymes involved in fatty acid synthesis then depressed the milk fat synthesis. Zebeli and Ametaj (2009) reported that there was a strong negative relationship between rumen LPS and milk fat content and yield and the relationship could explained 69% of the variation in milk fat production when cows were fed high-concentrate/low-forage diets. These observations suggest that LPS may function as an important factor affecting milk fat fatty acid synthesis.

Based on these studies, researchers hypothesized that LPS regulates milk fat synthesis. Main objective of this study was to determine the relationship between LPS and milk fat synthesis in dairy cows. Expression profiles of fatty acid metabolic-related gene were measured following exposure of mammary epithelial cells of dairy cows to LPS.

MATERIALS AND METHODS

***In vitro* stimulation:** Dairy Bovine Mammary Gland Epithelial Cell Line (DBMECs), preserved in the laboratory with karyotype of the chromosome and mRNA expression of ACACA, BTN1A1 and CSN2 was resuscitated and cultured as described by the laboratory (Hu *et al*., 2009). Briefly, the cell line was maintained by serial passages in DMEM/F12 medium supplemented with 0.5 μg mL^{-1} insulin (Sigma, USA), 10 ng mL^{-1} epidermal growth factor (Sigma, USA), 5 μg mL^{-1} transferrin (Sigma, USA), 1 μg mL^{-1} hydrocortisone (Sigma, USA), 1 μg mL^{-1} progesterone (Sigma, USA) and 1 mg mL^{-1} FAF-BSA (Sigma-Alsrich, USA). Cells were cultured at 38°C in 5% CO_2 until 90% confluent.

Cultured DBMECs cells were washed 3 times with phosphate buffered saline (pH 7.4) and incubated in 35 mm dishes supplemented with LPS (*E. coli* O111:B4, Sigma, Cat. No. L4391) at 0, 0.1 or 10 ng mL^{-1}. Treated cells were grown for 24 h and then total RNA was isolated using RNeasy® Mini Kit (QIAGEN). The RNA integrity was assessed by electrophoretic analysis of 28 and 18S rRNA subunits and concentration was measured with spectrophotometer (Thermo Sceintific, USA). Purity of

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Table 1: Gene name, genera, accession number and primers for the analysis of gene expression by qRT-PCR

Genes	Genera	Accession No.	Primers (5'-3')
ACACA	*Bos taurus*	NM_174224.2	GGATCCAATAGACAGAGTCATCG
			CCGTAAGCCGTCCACGAT
ACSS2	*Bos taurus*	NM_001105339.1	CATTGAATGGATGAAAGGAGCA
			CTGGGAAACCCTTCTCCTGAC
ACTB	*Bos taurus*	NM_173979.3	AGGGCGTAATGGTGGGC
			GTACATGGCAGGGGTGTTGA
CD36	*Bos taurus*	NM_174010.2	CCTATAACTGGATTTACTTTACG
			GTTTGGGCAGGTGGGAGGGAGTG
FABP3	*Bos taurus*	BT021486.1	CCTGGAAGTTAGTGGACAGCAAG
			GCCTGGTAGCAAAACCGACA
FABP4	*Bos taurus*	NM_174314.2	AGTGGGCGTGGGCTTTG
			AGGTGCTTTCTGATTTAATGGTGA
FASN	*Bos taurus*	NM_001012669.1	AGCACACGCCTGTAGTATTCG
			CAGCTTCTGCTGGTGGGTAG
PPARG	*Bos taurus*	NM_001040533.1	GGTGGGAGTCGTGGCAAAT
			GAGTGGTCATCCATCATAGAAAGG
PPARGC1A	*Bos taurus*	NM_177945.3	TACGAGGAGTACCAGCACGA
			AACATAAATCACACGGCGCT
SCD	*Bos taurus*	AF188710.1	AAGAAGACATCCGCCCTGAA
			GCGCACAAGCAGCCAAC
SREBF1	*Bos taurus*	NM_001113302.1	CTGACGACCGTGAAAACAGA
			AGACGGCAGATTTATTCAACTT

RNA (A260/A280) for all samples was about 1.9. Total RNA was reversely transcriped using a PrimeScript® RT reagent Kit (TaKaRa, Japan). The mRNA of fatty acid translocase (CD36), Fatty Acid-Binding Protein 3 (FABP3), Fatty Acid-Binding Protein 4 (FABP4), Acetyl-Coenzyme A Carboxylase Alpha (ACACA), Stearoyl-CoA Desaturase (SCD), Fatty Acid Synthase (FASN), Peroxisome Proliferators-Activated Receptor Gamma (PPARG), PPAR Gamma Coactivator 1 Alpha (PPARGC1A), Element-Binding transcription Factor 1 (SREBF1) and ACTB were quantified by real-time PCR (ABI 7500). The RT-PCR reaction of genes started with 1 cycle of 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C and at 60°C for 34 sec with SYBR® Premix Ex TaqTM II (TaKaRa, Japan). Primers were designed according to sequences of *Bos taurus*. Sequences were analyzed with multiple alignments by DANMAN 6.0.40. All primers were designed with Primer premier 5.0 (Table 1). The RT-PCR data were analyzed using the 2^{-CT} method (Livak and Schmittgen, 2001).

Data were analyzed using the MIXED procedure of SAS (version 8.2, 2001. SAS Insititute Inc., Cary, NC). Values were expressed as mean±Standard Deviation (SD). Significance was declared at $p<0.05$.

RESULTS

Fatty acid intracellular channel genes mRNA expression in response to LPS was analyzed in DBMECs cells (Fig. 1). Expression ratio of CD36 mRNA elevated as concentration of LPS increased ($p<0.01$). The FABP3 mRNA expression ($p>0.05$) was not affected by LPS but FABP4 mRNA expression was affected ($p<0.05$), decreasing 0.79 fold when LPS was provided at

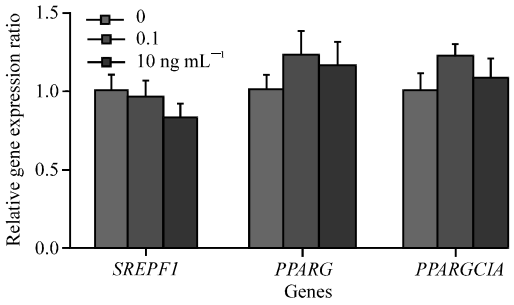

Fig. 1: Effect of external pudic arterial administrated LPS on milk fat concentration in dairy cows

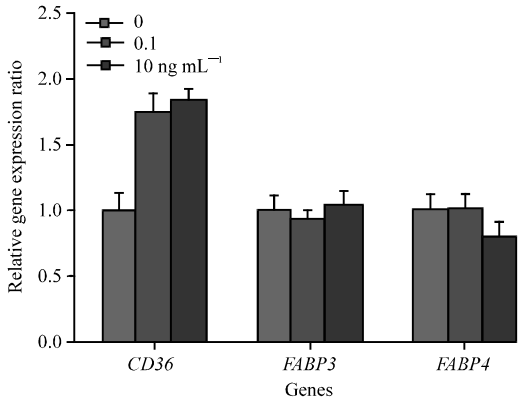

Fig. 2: Effect of LPS on the mRNA expression of fatty acid biosynthesis key enzyme genes in mammary epithelial cells of dairy cows

10 ng mL^{-1}. There was no significant effect of LPS on fatty acid biosynthesis genes (FASN, ACACA or SCD mRNA expression, $p>0.05$, Fig. 2) and fatty acid

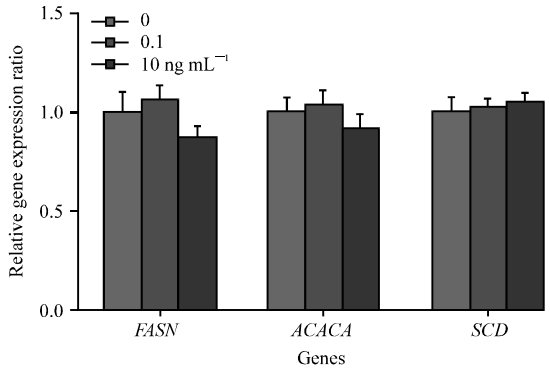

Fig. 3: Effects of LPS on the mRNA expression of fatty acid metabolism genes in mammary epithelial cells of dairy cows

metabolism genes in DBMECs cells (Fig. 3). However, the highest dose of LPS (10 ng mL^{-1}) resulted in a 0.093 fold decrease in SREBF1 mRNA expression compared with the control.

DISCUSSION

Milk fatty acids are from both incorporation and de novo synthesis. Approximately 1 half of 16:0 and all of longer chain fatty acids (>16) are derived from blood (Grummer, 1991). Fatty Acid Binding Proteins (FABPs), Fatty Acid Translocators and Fatty Acid Transport Proteins (FATPs) mediate uptake of Long Chain Fatty Acids (LCFAs) by mammary epithelial cells (Barber *et al*., 1997). Ling and Alcorn (2010) reported that LPS decreased FABP3 mRNA expression and increased FABP4 mRNA expression in the mammary gland of Female Sprague-dawley rats on lactation day 11 after intra-peritoneal injection of LPS at 250 μL kg^{-1} BW. In the study, LPS up-regulated FABP3 expression and down-regulated FABP4 expression in mammary epithelia cells with a low concentration (LPS at 0.1 ng mL^{-1}), however, FABP3 mRNA expression was enhanced and FABP4 mRNA expression was decreased by a higher level of LPS (10 ng mL^{-1}), suggesting that FA uptake by bovine mammary cells involves a complex and coordinated mechanism (Bionaz and Loor, 2008). The results also demonstrate that LPS up-regulates CD36 mRNA expression in mammary epithelia cells ($p<0.01$), agreeing with increased percentages of trans 11C18:1 and PUFA in milk observed in *in vivo* experiment. Interestingly, fatty acid translocase (CD 36) mRNA expression was down-regulated in multiple tissues (adipose, heart, etc.) of male syrian hamsters (Memon *et al*., 1998). This difference might be related to specific experimental materials used. It is also possible that mammary epithelia cells respond to LPS differently dependent on organs.

All short chain fatty acids (LCFAs, C4:0-14:0) and 1 half of 16:0 are synthesized de novo in the mammary gland utilizing fermentation acids produced in the rumen (Grummer, 1991). ACACA is the rate-limiting enzyme in de novo synthesis of FA (Bauman *et al*., 2006) and both acetyl-CoA and butyryl-CoA are primers for FASN that produces SCFAs in ruminants (Palmquist, 2006). Only a fraction of FA taken up by the mammary gland is unsaturated owing to extensive biohydrogenation in the rumen (Bionaz and Loor, 2008). Primary enzyme involved in monounsaturated FA synthesis is SCD (Ntambi and Miyazaki, 2003). The study documented that ACACA and FASN mRNA expression were down-regulated by LPS at a high concentration (0.1 ng mL^{-1}), consistent with decreased C4:0, C12:0 and C14:0 in milk observed *in vivo*. SREBP1 is pivotal in the regulation of milk fat synthesis in the mouse (Anderson *et al*., 2007) and cow (Harvatine and Bauman, 2006; Bionaz and Loor, 2008). In the study, SREBP1 mRNA expression was consistently decreased in response to LPS *in vitro*, consistent with results *in vivo*. PPARG regulates the entire bovine milk fat synthesis machinery (Bionaz and Loor, 2008) whereas PPARGC1A regulates mitochondrial biogenesis and energy metabolism (Liang and Ward, 2006).

CONCLUSION

Increased PPARG mRNA and PPARGC1A mRNA expression in mammary epithelia cells may be a result of enhanced lipid metabolism so as to inhibit the inflammatory response by LPS.

In summary, LPS alters fatty acid metabolism, functioning as an important factor affecting milk fat synthesis.

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REFERENCES

Anderson, S.M., M.C. Rudolph, J.L. McManaman and M.C. Neville, 2007. Secretory activation in the mammary gland: It's not just about milk protein synthesis. Breast Cancer Res., Vol. 9. 10. 1186/bcr 1653.

Barber, M.C., R.A. Clegg, M.T. Travers and R.G. Vernon, 1997. Lipid metabolism in the lactating mammary gland. Biochimica Biophysica Acta, 1347: 101-126.

Bauman, D.E., I.H. Mather, R.J. Wall and A.L. Lock, 2006. Major advances associated with the biosynthesis of milk. J. Dairy Sci., 89: 1235-1243.

Bionaz, M. and J.J. Loor, 2008. Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genom., Vol. 9. 10.1186/1471-2164-9-366

Grummer, R.R., 1991. Effect of feed on the composition of milk fat. J. Dairy Sci., 74: 3244-3257.

Harvatine, K.J. and D.E. Bauman, 2006. SREBP1 and thyroid hormone responsive spot 14 (S14) are involved in the regulation of bovine mammary lipid synthesis during diet-induced milk fat depression and treatment with CLA. J. Nutr., 136: 2468-2474.

Hu, H., J. Wang, D. Bu, H. Wei, L. Zhou, F. Li and J.J. Loor, 2009. *In vitro* culture and characterization of a mammary epithelial cell line from Chinese Holstein dairy cow. PLoS One, Vol. 4. 10.1371/journal.pone. 0007636

Liang, H. and W.F. Ward, 2006. PGC-1á: A key regulator of energy metabolism. Adv. Physiol. Educ., 30: 145-151.

Ling, B. and J. Alcorn, 2010. LPS-induced inflammation downregulates mammary gland glucose, fatty acid and l-carnitine transporter expression at different lactation stages. Res. Vet. Sci., 89: 200-202.

Livak, K.J. and T.D. Schmittgen, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}_{T}$ method. Methods, 25: 402-408.

Memon, R.A., K.R. Feingold, A.H. Moser, J. Fuller and C. Grunfeld, 1998. Regulation of fatty acid transport protein and fatty acid translocase mRNA levels by endotoxin and cytokines. Am. J. Physiol. Endocrinol. Metab., 274: E210-E217.

Ntambi, J.M. and M. Miyazaki, 2003. Recent insights into stearoyl-CoA desaturase-1. Curr. Opin. Lipidol., 14: 255-261.

Palmquist, D.L., 2006. Milk Fat: Origin of Fatty Acids and Influence of Nutritional Factors Thereon. In: Advanced Dairy Chemistry Volume 2 Lipids, Fox, P.F. and P.L.H. McSweeney (Eds.). Springer, USA., pp: 43-92.

Zebeli, Q. and B.N. Ametaj, 2009. Relationships between rumen lipopolysaccharide and mediators of inflammatory response with milk fat production and efficiency in dairy cows. J. Dairy Sci., 92: 3800-3809.