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Effects of pasture versus confinement and marine oil supplementation on the expression of genes involved in lipid metabolism in mammary, liver, and adipose tissues of lactating dairy cows

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ABSTRACT

Research was conducted to evaluate the effects of management system (MS), marine lipid supplementation (LS), and their interaction on the relative mRNA abundance of 11 genes involved in lipid synthesis in mammary, liver, and subcutaneous adipose tissues in lactating dairy cows. These genes included those involved in FA uptake (*LPL*), de novo FA synthesis (*ACACA*, *FASN*), FA desaturation (*SCD1*, *FADS1*, *FADS2*), and transcriptional regulation of lipogenesis (*SREBF1*, *SCAP*, *INSIG1*, *THRSP*, and *PPARG*). Forty-eight peripartal Holstein cows were blocked by parity and predicted calving date and assigned to either a pasture (n = 23) or confinement (n = 25) system. Within each system, cows were allocated randomly (7–9 cows per treatment) to a control (no oil supplement) or 1 of 2 isolipidic (200 g/d) supplements, fish oil (FO) or microalgae (MA), for 125 ± 5 d starting 30 d precalving. The experiment was conducted as a split-plot design, with MS being the whole plot treatment and LS as the subplot treatment. At 100 ± 2 DIM, 4 cows from each treatment combination (24 cows in total) were euthanized and tissue samples were collected for gene expression analysis. No interactions between MS and LS were observed regarding any of the variables measured in this study. Milk production (34.0 vs. 40.1 kg/d), milk fat (1.10 vs. 1.41 kg/d), protein (0.95 vs. 1.22 kg/d), and lactose (1.56 vs. 1.86 kg/d) were lower for pasture compared with confinement. The effect of LS on milk production and milk composition (yields and contents) was significant only for milk fat content that was reduced with MA compared with FO (3.00 vs. 3.40%) and the control (3.56%). The mammary mRNA abundance of *PPARG* (−32%) and *FASN* (−29%) was lower in grazing compared with confined cows, which was accompanied by reduced (−43%) secretion of de novo synthesized fatty acids in milk. Grazing was associated with reduced expression of *ACACA* (−48%), *FASN* (−48%), and *THRSP* (−53%) in subcutaneous adipose tissues, which was consistent with the lower body condition score (i.e., lower net adipose tissue deposition) in grazing compared with confined cows. Feeding either FO or MA downregulated hepatic expression of *FASN*, *SCD1*, *FADS2*, and *THRSP*. Our results suggest that the reduced secretion of de novo synthesized fatty acids in milk of grazing cows compared with confined cows might be related in part to the downregulation of genes involved in lipid synthesis, and that LS have tissue-specific effects on expression of genes involved in lipid metabolism, with liver being the most responsive tissue.

Key words: grazing, total mixed ration, fish oil, microalgae

INTRODUCTION

Feeding plant or marine oils to dairy cows has been shown to improve the milk fat content of beneficial unsaturated FA, including *cis*-9 18:1 (oleic acid; **OA**), n-3 PUFA, and *cis*-9,*trans*-11 CLA (common name rumenic acid; **RA**), and reduce the concentration of detrimental SFA, including 12:0, 14:0, and 16:0 (Chilliard et al., 2007). The latter effect has been attributed mainly to the downregulation of genes involved in milk fat synthesis by specific ruminal biohydrogenation (**RBH**) intermediates such as *trans*-10,*cis*-12 CLA (Harvatine et al., 2009a; Shingfield et al., 2010).

Previous studies (Chilliard et al., 2007; Vahmani et al., 2013) have shown that, compared with cows fed conserved forages and more grain in confinement, grazing cows produce milk fat with a higher content of beneficial unsaturated FA and lower content of detrimental SFA. However, the effect of dairy farm management system (**MS**; pasture vs. confinement) or its interaction with PUFA supplementation on milk fat synthesis has not been studied.

Research in rodents has shown that PUFA supplementation downregulates the expression of genes involved

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in lipid synthesis in liver and adipose tissue, resulting in decreased lipogenesis in these tissues (Jump, 2002; Wang and Jones, 2004). However, studies investigating effects of PUFA supplementation on gene expression in extra-mammary tissues in dairy cattle are scarce. Harvatine et al. (2009b) reported that intravenous infusion of *trans*-10,*cis*-12 CLA reduced mammary expression of lipoprotein lipase (*LPL*), fatty acid synthase (*FASN*), sterol regulatory element-binding transcription factor 1 (*SREBF1*), and thyroid hormone responsive spot 14 (*THRSP*) in dairy cows; however, the opposite effect (i.e., increased expression of these genes) was seen in adipose tissue. A recent study in dairy goats showed that feeding fish oil reduced the expression of *FASN*, stearoyl-CoA desaturase 1 (*SCD1*), and fatty acid desaturase 2 (*FADS2*) in liver, whereas the expression of these genes was not altered in mammary tissue and omental adipose tissue (Toral et al., 2013).

We hypothesized that MS, marine lipid supplementation (**LS**) or their interaction would affect the expression of genes involved in lipid synthesis in mammary, liver, and subcutaneous adipose (**SUBQ**), and that the effects would be tissue-specific. The objective of the current study was to determine the effect of MS, LS, and their interaction on mRNA abundance of genes encoding proteins required for FA uptake, de novo FA synthesis, desaturation, and transcriptional regulation of lipid synthesis in mammary, liver, and SUBQ in lactating dairy cows.

MATERIALS AND METHODS

Experimental Design, Treatments, and Animal Measurements

All procedures performed on cows in the current study were carried out according to the Canadian Council for Animal Care guidelines and were approved by the Animal Care and Use Committee at the Faculty of Agriculture of Dalhousie University. Details of the experimental design and animal management have been reported elsewhere (Vahmani et al., 2013). Briefly, 48 peripartal Holstein cows were blocked by parity and predicted calving date and assigned within block to either a pasture (n = 23) or confinement (n = 25). Within the system, cows were allocated randomly to a control (no oil supplement) or to 1 of 2 isolipidic (200 g/d) marine oil supplements, fish oil (**FO**) or microalgae (**MA**), for 125 ± 5 d starting at 30 d precalving. The number of cows per treatment within each MS varied between 7 and 9. Both supplements provided similar amounts of long-chain (C20–C22) PUFA (~65 g/d), but contained different proportions of eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**). The predominant long-chain PUFA in MA was DHA (24% of total FA), whereas EPA and DHA were equally predominant and comprised about 27% of total FA in FO. Fatty acid composition of lipid supplements is presented in Supplemental Table 1 (http://dx.doi.org/10.3168/jds.2103-7290/). The experiment was conducted as a split-plot design, with MS as the whole plot treatment and LS as the subplot treatment. Both pasture and confinement groups were housed in a tiestall barn from −30 until 28 ± 10 DIM and were fed a TMR with similar formulations. The pasture group was then adapted to a pasture and grazed rotationally on a perennial sward until the end of the experiment (95 ± 5 DIM). The confinement group remained in the tiestall barn and was fed a TMR (forage-to-concentrate ratio of 56:44, DM basis) ad libitum twice daily (0700 and 1500 h). Grazing cows were fed 8.0 kg of concentrate/d (as-fed basis) in equal portions at milking (0600 and 1600 h). The concentrate was similar to that used in the TMR for confined cows. The nutrient and FA composition of feeds are available in Supplemental Tables 1 and 2. Cows had continuous access to water.

Tissue Collection

Four cows were randomly selected from each treatment within each MS (24 cows in total) and transferred to a commercial abattoir at 100 ± 2 DIM, immediately after morning milking, for tissue sampling. Mammary, liver, and SUBQ (between the hook and pin bones) were sampled immediately after exsanguination, snap frozen in liquid nitrogen, and stored at −80°C until RNA extraction.

RNA Extraction and cDNA Synthesis

Total RNA was isolated from 200 mg of mammary and liver tissues using the RNeasy Midi Kit and total RNA in SUBQ was extracted from 400 mg of tissue using the RNeasy Lipid Tissue Midi Kit according to the manufacturer's protocol (Qiagen, Valencia, CA). Concentration of RNA was determined by absorbance at 260 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE), and absorbance was measured to ensure the absorbance ratios of 260:280 and 260:230 were between 1.8 and 2.1 for all RNA samples. Integrity of RNA was confirmed by visualization of the 28s and 18s ribosomal RNA bands after electrophoresis of 500 ng of each RNA sample on a 1% agarose gel stained with ethidium bromide. One microgram of total RNA from each sample was reverse-transcribed in a final volume of 20 μL using the Quantitect Reverse Transcription kit with genomic DNA Wipeout (Qiagen) according to the