



# Milk fat secretion in Holstein dairy cows: Insights from grain type and oil supplement



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## ABSTRACT

Effects of grain type and dietary oil supplement on milk fat depression and milk fatty acid (FA) composition of dairy cows were evaluated using eight multiparous Holstein cows ( $77 \pm 22.1$  days in milk; mean  $\pm$  SD) in a replicated  $4 \times 4$  Latin square design with a  $2 \times 2$  factorial arrangement of diets. Experimental diets contained either ground barley or ground corn supplemented with either fish oil or soybean oil at 2% of dietary dry matter (DM). Experimental periods were 25 d, with the final 7 d used for sample and data collection. Dry matter intake tended ( $P=0.09$ ) to be greater for barley- vs. corn-based diets (23.2 vs. 22.3 kg/d), but was reduced for the fish oil compared to soybean oil supplemented diets (21.1 vs. 24.3 kg/d;  $P < 0.001$ ). Total FA intake was greater in corn-based diets and also in soybean oil supplemented diets. Regardless of type of the diet fed, MFD occurred. Although milk fat yield was not affected, the barley-based diets increased ( $P < 0.001$ ) the concentration of mixed-origin FA (C16:0 plus *cis*-9 C16:1) but decreased the concentration of preformed FA ( $P < 0.001$ ) as compared with corn-based diets. Corn-based diets increased concentration of both *trans*-11 C18:1 ( $P=0.03$ ) and *cis*-9, *trans*-11 C18:2 ( $P=0.01$ ) which was a reflection of greater intake of *cis*-9, *cis*-12 C18:2 as substrate for rumen biohydrogenation. Severity of MFD was greater for fish oil than for soybean oil which was evidenced by the increased concentration and yield of biohydrogenation intermediates (especially *trans*-10 C18:1) associated with MFD ( $r = -0.61$ ;  $n = 32$ ) in milk fat. However, fish oil increased concentration of both *trans*-11 C18:1 ( $P=0.001$ ) and *cis*-9, *trans*-11 C18:2 ( $P < 0.001$ ) as compared with soybean oil. Grain type and oil supplement did not interact to affect milk odd- and branched-chain FA (OBCFA) concentration. Cows fed barley-based diets ( $P < 0.001$ ) and soybean oil supplemented diets ( $P < 0.001$ ) produced greater OBCFA in milk fat. Overall, there was no interaction between the type of grain and oil supplement on induction of MFD and milk fat yield. However, milk fatty acid composition was differently modified by the grain type and an increase in the concentration of mixed-origin FA of fish oil supplemented diets was unable to attenuate the severity of MFD because of the concomitant decrease in concentrations of *de novo* synthesized- and preformed-FA as compared with soybean oil supplemented diets.

## 1. Introduction

During the past decade, major progress has contributed to our understanding of the link between rumen lipid metabolism and milk fat biosynthesis. Risk factors involved in MFD in dairy cows include decreased ruminal pH and a high concentration of unsaturated FA in the diet (Bauman et al., 2011). Several dietary factors may influence

ruminal pH and the microbial population and therefore alter the extent and rate of ruminal biohydrogenation of dietary FA. These include type of grain (Casper et al., 1990; Mohammed et al., 2010), oil supplement (Alizadeh et al., 2012; Kargar et al., 2012), forage to concentrate ratio, forage type and composition of basal diet (Loor et al., 2005b, 2005c; Kargar et al., 2012). Modification of ruminal FA biohydrogenation may alter duodenal flow of FA and consequently milk FA composition (Loor

Abbreviations: FA, fatty acid; DM, dry matter; MFD, milk fat depression; OBCFA, odd- and branched-chain FA; PUFA, poly unsaturated FA; TMR, total mixed ration

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et al., 2005c). As rumen pH is mainly affected by the fermentable carbohydrate in the diet, it is possible that the source of starch and/or the rate of ruminal degradation of starch could play a role in altering rumen pH, which in turn could influence biohydrogenation intermediates in the rumen. Mohammed et al. (2010) showed that the barley-based diets resulted in greater ruminal concentration of PUFA (C18:2+C18:3) and less C18:0 than the corn-based diets despite greater C18:2 intake for corn-based diets. This has led to increased ruminal concentrations of the total *trans*-C18:1 and *trans*-C18:1 intermediates including *trans*-10- and *trans*-11 C18:1, but not C18:0, suggesting a greater inhibition of the reduction of *trans*-10- or *trans*-11 C18:1 to C18:0 relative to corn-based diets (Mohammed et al., 2010). A similar type of inhibition was also observed when fish oil was included in dairy rations (Whitlock et al., 2002; Shingfield et al., 2003; Alizadeh et al., 2012). Therefore, the inclusion of a highly fermentable starch source is also considered a risk factor for MFD (Casper et al., 1990; Mohammed et al., 2010). In our recent experiment performed with dairy cows feeding barley- compared with corn-based diets (which were differed in ruminal fermentability) supplemented with a mixture of soybean oil and fish oil (5:1) at 2% of dietary DM did not affect milk fat yield (Kargar et al., 2014). The concentration of the intermediates in the rumen depends on the extent to which PUFA substrates are metabolized and are influenced by the initial concentration of dietary PUFA and rumen pH (Loor et al., 2005b). Oil supplements which are either substrate (plant lipids like soybean oil) or modifier (marine products like fish oil) of rumen biohydrogenation have been shown to induce the formation of several *trans* and conjugated FA isomers (*trans*-10, *cis*-12 C18:2, *trans*-6/*trans*-7/*trans*-8 C18:1 and *trans*-10 C18:1) during ruminal biohydrogenation, which negatively affects *de novo* milk FA synthesis (Loor et al., 2005a, 2005c; Kadegowda et al., 2008; Shingfield et al., 2009). Previous research demonstrated that the feeding of fish oil depresses milk fat yield as compared with extruded soybeans, roasted safflower seeds, and/or soybean oil (Whitlock et al., 2002; Alizadeh et al., 2012; Kargar et al., 2013a). However, AbuGhazaleh et al. (2002) showed no MFD in cows fed 2% fish oil as compared to cows fed 2% soybean oil from extruded soybeans. To the best of our knowledge, however, the effect of grain type has not been characterized yet in combination with oil supplements (either as substrate or as modifier of rumen biohydrogenation) to investigate MFD. We hypothesized that both barley-based diets and fish oil supplemented diets would cause a greater shift in biohydrogenation toward pathways that produce FA intermediates that cause MFD. Therefore, the objective of this experiment was to determine the effects of and interactions between, grain type and oil supplement on milk fat yield and milk fatty acid profile in Holstein cows fed total mixed rations.

## 2. Materials and methods

This paper is a part of a larger study evaluating the effects of grain type (barley compared with corn) and oil supplement (soybean oil compared with fish oil) and those interactions on performance, feeding and chewing behavior, bioenergetic status, insulin sensitivity and oxidative stress status in early- to mid-lactating Holstein dairy cows. This paper discusses diet effects mainly on milk fat yield and milk fat composition, and the companion papers which were reported elsewhere focus on nutrient intake, feeding and chewing behavior and lactational performance (Kargar et al., 2013a), energy balance and partitioning and blood metabolic and antioxidant parameters (Kargar et al., 2015b) and apparent total tract nutrient digestion and rumen fermentation characteristics (Kargar et al., 2015a).

### 2.1. Environment, animals and experimental diets

The average maximum temperature-humidity index, relative humidity, and maximum temperature were 77.7, 11%, and 36 °C for data collection week 1; 78.5, 10%, and 37 °C for data collection week 2;

73.3, 20%, and 30 °C for data collection week 3; and 73.1, 9%, and 31 °C for data collection week 4, respectively (Kargar et al., 2015b). Experimental details have been presented in a companion manuscript (Kargar et al., 2013a) and are summarized in the following. Eight Holstein cows were used in a replicated 4×4 Latin square design with a 2×2 factorial arrangement with 25 d periods. Each experimental period consisted of an 18 d diet adaptation period and a 7 d collection period. Diets were two-grain types (barley relative to corn) and two oil sources (fish oil relative to soybean oil). The cows were multiparous (parity=3.3 ± 1.3; mean ± SD; 77 ± 22.1 days in milk) and producing 43.9 ± 4.9 kg milk per day with 3.00% fat and 2.90% protein. This pretty low milk fat and protein concentrations may be, in part, attributed to a mild degree of heat stress that cows experienced during covariate- and trial periods. The cows were assigned to two squares such that milk yield and days in milk were similar for both squares. Cows within square were randomly assigned to four experimental diets: 1) **BF**=barley-based diet supplemented with fish oil at 2% of dietary DM, 2) **BS**=barley-based diet supplemented with soybean oil at 2% of dietary DM, 3) **CF**=corn-based diet supplemented with fish oil at 2% of dietary DM and 4) **CS**=corn-based diet supplemented with soybean oil at 2% of dietary DM (Table 1; Kargar et al., 2013a). Diets were formulated to meet or exceed the Cornell Net Carbohydrate and Protein System (version 5.0) nutrient allowance for a lactating dairy cow weighing 673 kg and producing 43 kg/d of milk with 3.0% milk true protein and 3.2% fat and consuming 24.1 kg of DM. Feed was supplied twice daily at 0900 and 1500 h in amounts that allowed 10% refusals.

**Table 1**

Ingredients and chemical composition of experimental diets on DM basis.

	Diet <sup>a</sup>			
	BF	BS	CF	CS
Ingredient composition, % of DM				
Corn silage	19.00	19.00	19.00	19.00
Alfalfa hay	21.00	21.00	21.00	21.00
Beet pulp	4.22	4.22	4.22	4.22
Barley grain, ground	33.20	33.20	–	–
Corn grain, ground	–	–	28.50	28.50
Soybean meal	14.02	14.02	2.45	2.45
Canola meal, pelleted	2.50	2.50	17.80	17.80
Corn gluten meal	0.96	0.96	1.93	1.93
Fish oil	2.00	–	2.00	–
Soybean oil	–	2.00	–	2.00
Mepron <sup>b</sup>	0.05	0.05	0.05	0.05
Sodium-bicarbonate	0.75	0.75	0.75	0.75
Calcium carbonate	0.65	0.65	0.65	0.65
Vitamin E premix <sup>c</sup>	0.10	0.10	0.10	0.10
Vitamin A, D <sub>3</sub> , and E premix <sup>d</sup>	1.18	1.18	1.18	1.18
Salt	0.42	0.42	0.42	0.42
Chemical composition				
DM, %	54.61	55.42	54.05	54.48
NDF, % of DM	32.23	32.05	29.92	29.48
Ether extract, % of DM	4.08	4.08	4.94	4.85
NE <sub>L</sub> , <sup>6</sup> Meal/kg of DM	1.70	1.70	1.72	1.72
Selected dietary FA composition, g/100 g FA				
Palmitic acid (C16:0)	19.21	16.17	16.38	13.48
Stearic acid (C18:0)	3.31	3.51	3.14	3.26
Oleic acid (C18:1 <i>n</i> -9)	18.36	16.71	24.61	22.91
Linoleic acid (C18:2 <i>n</i> -6)	28.63	47.53	28.17	45.48
Linolenic acid (C18:3 <i>n</i> -3)	10.20	12.20	9.13	10.70
Eicosapentaenoic acid (C20:5 <i>n</i> -3)	3.08	0.00	2.69	0.00
Docosahexaenoic acid (C22:6 <i>n</i> -3)	7.39	0.00	6.46	0.00

<sup>5</sup>Calculated from NRC (2001)

<sup>a</sup> BF=barley-based diet supplemented with fish oil; BS = barley-based diet supplemented with soybean oil; CF = corn-based diet supplemented with fish oil; and CS = corn-based diet supplemented with soybean oil.

<sup>b</sup> Mepron<sup>®</sup> M85 (M85; Degussa AG, Hanau, Germany).

<sup>c</sup> Contains 500,000 IU of vitamin E per kilogram.

<sup>d</sup> Contains 15,000,000 IU of vitamin A; 400,000 IU of vitamin D<sub>3</sub>, and 6000 IU of vitamin E per kilogram.

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