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Case Study – A Reduction of Polyunsaturated Fatty Acid Intake in a Lactating Dairy Cow Herd Increased Milk Fat Percentage

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Abstract

A 100-cow dairy herd was investigated for chronic milk fat depression. Implementation of traditional measures to reduce the impact of altered rumen fermentation by increasing physically effective fiber intake only partially alleviated the problem. A field study was then performed to quantify the effect of reducing polyunsaturated fatty acid intake on milk fat and milk fatty acid profile. A total of 22 cows randomly selected in the herd were enrolled in the study. Dietary intake of linoleic acid was decreased from 0.609 lb (277 g) to 0.519 lb (236 g) per cow per day by modifying the ration. The effect of ration reformulation was analyzed in linear regression models using repeated measures within cows. Milk production, milk composition, and milk fatty acid profile data were considered the outcomes. Ration reformulation was associated with an increase of 0.3 percentage points of milk fat ($P<0.01$) and a concomitant decrease of *trans*-10, *cis*-12 conjugated linoleic acid in milk ($P<0.001$). Results from this case study indicate a small reduction of polyunsaturated fatty acid intake may mitigate milk fat depression in dairy cows.

Keywords: bovine, dairy, milk fat depression, polyunsaturated fatty acid

Résumé

Une étude de cas portant sur un troupeau de 100 vaches laitières souffrant de dépression chronique du pourcentage de matière grasse du lait a été effectuée. La mise en place de mesures visant à augmenter la consommation de fibres physiquement efficaces et à réduire l'altération de la fermentation ruminale n'a amélioré que partiellement la situation. Un essai clinique a été réalisé dans le but de quantifier l'effet qu'aurait une réduction de l'ingestion d'acides gras poly insaturés sur la matière grasse du lait et sur sa composition en acides gras du lait. Vingt-deux vaches du troupeau, choisies au hasard, ont participé à cette étude. L'ingestion quotidienne d'acide linoléique est passée de 277 g à 236 g par vache par jour grâce à une reformulation de la ration. L'effet de la reformulation de la ration a été analysé en utilisant des modèles linéaires à mesures répétées. La production laitière, la composition du lait et le profil des acides gras du lait ont été considérés comme variables dépendantes. La reformulation de la ration a été associée avec une augmentation de 0,3 point du pourcentage de matière grasse du lait ($P<0.01$) et une réduction concomitante de la concentration du lait en acide linoléique conjugué *trans*-10, *cis*-12 ($P<0.001$). Les résultats de cette étude de cas démontrent qu'une petite réduction de l'ingestion quotidienne d'acides gras poly insaturés peut atténuer une situation de dépression de la matière grasse présente dans un troupeau.

Introduction

Milk fat depression (MFD) in dairy cows is defined as a reduction in the concentration and yield of milk fat, while minimal or no change is observed in lactose and protein yields.[6] It is well accepted by the scientific community that milk fat depression occurs as a result of several concurrent diet or management factors, rather than the result of a single factor.[13] The biohydrogenation theory proposes that under specific dietary conditions some biohydrogenation intermediates produced in the rumen can inhibit *de novo* synthesis of milk fatty acids in the mammary gland.[6] This theory proposes that two conditions are necessary to induce MFD, namely, alteration of rumen fermentation and a dietary supply of polyunsaturated fatty acids (PUFA).[7]

Alteration of rumen fermentation is defined as a shift in bacterial fermentation products and can be caused by several factors, including a variation of rumen

pH, an insufficient intake of physically effective fiber, and a high intake of non-fiber carbohydrates.[7,13] Thus, depending whether the rumen fermentation is normal or altered, the biohydrogenation of PUFA such as linoleic acid will vary (Figure 1). When ruminal fermentation is altered, linoleic acid may be hydrogenated to form *trans*-10, *cis*-12 conjugated linoleic acid (CLA), which is a potent inhibitor of milk fat synthesis in the mammary gland.[9] Other biohydrogenation intermediates such as *cis*-10, *trans*-12 CLA and *trans*-9, *cis*-11 CLA have also been shown to inhibit milk fat synthesis, which suggests that other biohydrogenation pathways could be involved.[17,18] Exhaustive review papers on this topic can be consulted elsewhere for further information.[8,12]

The traditional approach for investigating MFD is to suspect subacute ruminal acidosis (SARA) in the herd. After confirming dry matter intake (DMI) and dry matter (DM) content of the feed ingredients, ration modifications usually work toward reducing the alteration of rumen fermentation by increasing effective fiber intake or decreasing non-fiber carbohydrate intake. However, recent findings suggest that this approach should be modified because it has been shown that SARA is not required to induce MFD.[11] In fact, PUFA intake is the major factor that should be addressed because it has been shown that when PUFA intake is low, SARA will not cause milk fat depression.[5,10] On the other hand, high PUFA intake can cause MFD, in which case the presence of SARA exacerbates the problem.[2] Moreover, alteration of rumen fermentation can be more subtle than SARA and can be related to other factors such as grain fermentation rate.[15] The availability of PUFA for rumen biohydrogenation can also influence the amount of intermediates absorbed from the rumen and should be considered as another potential component of MFD.[11]

Although the biohydrogenation theory points out the importance of PUFA intake, it has not traditionally been assessed by veterinarians and nutritionists as part of a MFD investigation. Therefore, the objective of this case study was to quantify the effect of a reduction of PUFA intake on milk fat and milk fatty acid profile in a dairy herd experiencing chronically low bulk tank milk fat percentage.

Rumen biohydrogenation of linoleic acid

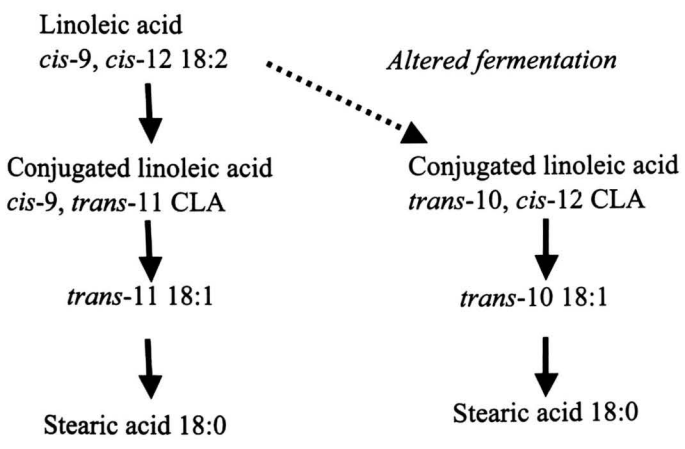

Figure 1. Pathways of ruminal biohydrogenation of linoleic acid. Adapted from Bauman and Griinari.[7]

History

A 100-cow commercial Holstein dairy herd in the vicinity of Guelph (Ontario, Canada) was investigated for lower milk fat percentage than expected by the farmer and nutritionist over the previous three years (2005-2007). Lactating cows were fed a one-group total mixed ration (TMR) and housed in a free-stall barn. In 2005-2006, the average bulk tank milk fat percentage was 3.4% (SD = 0.11; range = 2.97 - 3.72) which made quota production fulfillment difficult. In 2007, an investigation of the factors potentially causing milk fat depression had been performed using a traditional approach for solving MFD. Regular biweekly monitoring of DMI, DM content of feed ingredients, and particle size of ration and feed ingredients, had been implemented for the nine months prior to the present intervention and study. Adjustments were made to increase intake of physically effective fiber and to avoid sorting behavior. Dietary monensin supplementation was also stopped at that time. Implementing all those measures only partially alleviated the MFD, as the average bulk tank milk fat percentage increased only to 3.6% (SD = 0.08; range = 3.45 - 3.85) over that period. In the fall of 2008, it was suggested to reduce the PUFA intake of the cows by reformulating the diet and monitoring the response.

Methods

Animals and experimental design

A total of 22 lactating Holstein cows randomly selected and evenly distributed for stage of lactation were enrolled in the clinical trial. Although all lactating cows in the herd were fed the same ration, only 22 head were enrolled in the study because of the high cost of performing fatty acid composition analysis. The cows were fed an initial diet for 30 days (days -31 to -1), and were then switched to a reformulated diet for 32 days (days 0 to 32) which contained a lower PUFA level.

Feed ingredients and treatments

The initial diet consisted of corn and alfalfa silage, wheat straw, high moisture corn and brewer's grain. The remainder of ingredients were provided as a protein

supplement (Table 1). The goal of feeding the reformulated ration was to reduce the quantity and availability of dietary PUFA as much as possible, specifically linoleic acid. Soybean oil and corn distillers grain were removed from the diet, and the intake of brewers' grain was reduced. To compensate for the decreased protein content, the quantities of soybean meal and bypass soybean meal were increased in the reformulated ration. Barley grain was removed because the amount of corn silage fed, containing 40% corn grain, was increased for farm logistical reasons. Overall, the nutrient composition of both initial and reformulated rations was similar (Table 1), with the exception of a decrease in ether extract fraction and a change in the fatty acid profile of the diets (Table 2). The average daily intake of total lipid (dry matter basis) per cow for the initial and reformulated rations was 1.76 lb (798 g) and 1.55 lb (704 g), respectively. The average daily intake of linoleic acid in the initial and reformulated diets was 0.609 lb (277 g) and 0.519 lb (236 g), respectively.

Experimental measures

Feed analysis and milk production data. Two samples of the TMR and two samples of each feed ingredient from the initial and the reformulated rations were collected (days -1 and 32) and analyzed for nutrient composition[a] and for milk fatty acid profile.[b] Ration formulation data were managed using CPM-Dairy.[c] Milk production data were collected from the individual milk meters in the farm parlor.

Milk samples and fatty acid analysis. Two composite milk samples were collected per cow during each farm visit (days -1 and 32). Milk samples were chilled immediately in a cooler and one set of milk samples were submitted for analysis using a near-infrared analyzer.[d,e] The remaining set of samples were frozen (-2°F; -20°C) and a fatty acid composition analysis was performed.[b] The method used for this analysis has been described elsewhere.[4]

Statistical analyses

Data were summarized in Microsoft Excel.[f] All statistical analyses were performed using SAS.[g] Milk production, milk composition, and milk fatty acids composition parameters were considered the outcomes. Descriptive statistics were obtained by using PROC MEANS and PROC UNIVARIATE. The effect of ration reformulation was analyzed in linear regression models using repeated measures within cows (PROC MIXED). Statistical significance was declared as P-value < 0.10.

Table 1. Ingredient and nutrient compositions on dry matter basis of total mixed rations.

	% dry matter intake	
	Initial ration	Reformulated ration
Ingredient composition		
Corn silage	7.5	20.2
Alfalfa silage	31.9	26.6
Wheat straw	7.2	7.7
Corn, high moisture	23.7	22.3
Brewer's grain	6.5	3.0
Barley	4.8	0.0
Blood meal	1.2	1.6
Soybean meal 48%	3.7	4.9
Bypass soybean meal 50%	3.7	4.9
Molasses	2.4	4.0
Soybean oil	0.4	0.0
Corn distillers	3.4	0.0
Mineral/vitamin premix	1.7	2.2
Limestone	1.0	1.3
Sodium bicarbonate	0.7	1.0
Salt	0.2	0.3
Nutrient composition		
Dry matter	56.0	50.4
Crude protein	19.4	19.2
Neutral detergent fiber	31.5	31.8
Ether extract	3.5	3.2
Non-fiber carbohydrate	38.5	37.5
Net energy of lactation (Mcal/lb)	0.73	0.73

Results and Discussion

Dry matter intake, milk production, and milk composition

Because DMI was measured only at the herd level (100 cows) for logistical reasons, only the average DMI per cow was calculated for both rations (Table 3). Rumen pH data predicted from CPM-Dairy[c] software were 6.46 for both rations, indicating that the cows did not have SARA. Ration reformulation had no effect on daily milk yield, protein yield, and lactose yield. Daily milk fat yield increased 0.22 lb (0.10 kg) per cow following ration reformulation. Milk fat percentage of enrolled cows increased 0.3 percentage points when fed the reformulated diet. At the herd level (bulk tank), the daily milk fat yield increased by 0.22 lb (0.10 kg) per cow (day -1 = 2.48 lb [1.13 kg]; day 32 = 2.70 lb [1.23 kg]) and the milk fat percentage increased 0.35 percentage points during the trial period (day -1 = 3.63%; day 32 = 3.98%). Because the herd-level results are very similar to those from the 22 cows enrolled in the trial, it supports the notion that they were a representative sample of the herd. The milk fat increase could be partly attributed to the ration reformulation which lowered its PUFA content. However, because of our study design, it was impossible to differentiate between the ration reformulation effect, the effect of season, the increased stage of lactation effect, and other unknown changes that may have occurred on the dairy. Since ration reformulation had a similar effect both at the herd and cow level on milk fat, and since the average variation on milk fat in the county during this period was small (estimated seasonal effect: +0.02 lb [0.009 kg] yield; +0.03 percentage points)[h], it is believed that the increase in milk fat yield may have been due mainly to the ration reformulation. Milk protein percentage was increased over the trial period. This could be explained by the ration formulation using a slightly different protein source, leading to a better rumen microbial yield. It could also be hypothesized that some fatty acids might have been detrimental to the rumen microbial population and the ration reformulation would have decreased this effect, thus increasing the supply of metabolized proteins.

Table 2. Fatty acid profiles of total mixed rations.

	% of fatty acids	
Fatty acids	Initial ration	Reformulated ration
8:0	0.034	0.052
10:0	0.020	0.041
12:0	0.148	0.299
14:0	0.259	0.281
16:0	16.522	15.482
16:1 *cis*-9	0.255	0.314
18:0	2.733	3.337
18:1 *cis*-9	18.069	21.347
18:1 *cis*-11	0.855	0.975
18:2 n-6	46.332	45.867
18:3 n-3	12.736	10.220
20:0	0.521	0.559
22:0	0.591	0.555
24:0	0.681	0.520
26:0	0.243	0.150

Milk fatty acids

The ration was reformulated to reduce the amount of plant oil containing PUFA and linoleic acid. A strong positive response in milk fat percentage, both at the cow and herd level, was noticed as well as significant changes in milk fatty acid composition (Tables 4 and 5). Milk concentration of *trans*-10, *cis*-12 CLA was significantly decreased when the reformulated ration was fed. This is consistent with *trans*-10, *cis*-12 CLA inhibiting milk fat synthesis.[9] The concentration of another CLA isomer reported in the literature to have similar detrimental effect on milk fat synthesis (*trans*-9, *cis*-11 CLA) was not influenced by ration reformulation.[17] Diet reformulation

Table 3. Milk production and milk composition parameters of 22 lactating Holstein cows enrolled in a clinical trial investigating the impact of a ration reformulation.

	Rations			
	Initial	Reformulated	SEM	*P*-value
Dry matter intake (lb/d)	50.2	48.4	N/A	N/A
Days-in-milk (days)	141.3	173.3	N/A	N/A
Milk yield (lb/d)	71.35	72.27	3.65	0.82
Fat (%)	3.50	3.80	0.11	< 0.01
Fat (lb/d)	2.51	2.73	0.13	0.09
Protein (%)	3.10	3.16	0.06	< 0.01
Protein (lb/d)	2.20	2.27	0.11	0.55
Lactose (%)	4.71	4.72	0.02	0.99
Lactose (lb/d)	3.37	3.41	0.04	0.92

Table 4. Milk fatty acid profile (% of fatty acids) of 22 lactating Holstein cows enrolled in a clinical trial investigating the impact of a ration reformulation.

	Rations			
	Initial	Reformulated	SEM	*P*-value
4:0	4.591	3.531	0.136	< 0.001
6:0	2.062	2.224	0.072	0.034
7:0	0.029	0.036	0.002	0.036
8:0	1.275	1.444	0.054	0.004
9:0	0.033	0.045	0.003	0.002
10:0	2.722	3.237	0.132	< 0.001
11:0	0.055	0.070	0.006	0.024
12:0	2.953	3.649	0.147	< 0.001
13:0 *iso*	0.024	0.027	0.001	0.026
13:0 *anteiso*	0.068	0.113	0.007	< 0.001
13:0	0.169	0.232	0.013	< 0.001
14:0 *iso*	0.116	0.117	0.009	0.105
14:0 *anteiso*	10.114	11.263	0.300	0.001
14:1 *cis*-9	0.916	1.276	0.072	< 0.001
15:0 *iso*	0.171	0.187	0.005	0.007
15:0 *anteiso*	0.387	0.413	0.016	0.110
15:0	1.057	1.213	0.053	0.012
16:0 *iso*	0.352	0.341	0.024	0.644
16:0	27.649	32.506	0.682	< 0.001
16:1 *cis*-9	1.734	1.940	0.081	0.008
17:0 *iso*	0.314	0.292	0.009	0.016
17:0 *anteiso*	0.146	0.120	0.007	0.005
17:0	0.655	0.678	0.014	0.204
18:0 *iso*	0.068	0.041	0.005	< 0.001
18:0 *anteiso*	0.235	0.201	0.013	0.020
18:0	10.621	8.445	0.374	< 0.001
18:1 *cis*	22.971	19.358	0.732	< 0.001
18:1 *trans*	4.227	3.122	0.118	< 0.001
18:1 total	27.198	22.480	0.811	< 0.001
18:2[1]	2.201	1.877	0.082	< 0.001
CLA[2]	0.590	0.596	0.018	0.758
18:3 n-3	0.433	0.372	0.014	< 0.001
18:3 n-6	0.043	0.031	0.002	< 0.001
19:0	0.032	0.026	0.002	0.055
20:0	0.164	0.151	0.004	0.011
20:1 *cis*-9	0.109	0.122	0.004	< 0.001
20:1 *cis*-11	0.063	0.038	0.003	< 0.001
20:2 n-6	0.037	0.034	0.001	0.031
20:3 n-3	0.027	0.017	0.001	< 0.001
20:3 n-6	0.107	0.111	0.006	0.361
20:4 n-3	0.026	0.023	0.001	0.043
20:4 n-6	0.164	0.187	0.008	< 0.001
20:5 n-3	0.040	0.037	0.002	0.188
22:0	0.054	0.051	0.002	0.250
22:3 n-3	0.013	0.009	0.001	< 0.001
22:4 n-6	0.032	0.037	0.002	0.011
22:5 n-3	0.081	0.089	0.004	0.013
22:6 n-3	0.011	0.012	0.002	0.734
23:0	0.016	0.017	0.001	0.535
24:0	0.035	0.029	0.002	0.004
26:0	0.020	0.013	0.001	< 0.001
Summation by source				
<16	26.732	29.078	0.730	0.004
16:0 and 16:1	28.735	34.787	0.711	< 0.001
>16	44.533	36.136	1.135	< 0.001
Total SFA[3]	65.828	70.365	0.861	< 0.001
Total MUFA[4]	30.019	25.856	0.797	< 0.001
Total PUFA[5]	4.153	3.779	0.117	< 0.001

[1]Sum of 18:2 fatty acids excluding conjugated linoleic acid.
[2]Sum of conjugated linoleic acid isomers.
[3]Saturated fatty acids.
[4]Monounsaturated fatty acids.
[5]Polyunsaturated fatty acids.

Table 5. Milk concentration of 18:1 and 18:2 fatty acid isomers (% of fatty acids) of 22 lactating Holstein cows enrolled in a clinical trial investigating the impact of a ration reformulation.

	Rations			
	Initial	Reformulated	SEM	*P*-value
18:1 *cis*-9	21.718	18.357	0.688	< 0.001
18:1 *cis*-11	0.629	0.530	0.039	0.015
18:1 *cis*-12	0.524	0.411	0.021	< 0.001
18:1 *cis*-13	0.100	0.061	0.008	< 0.001
18:1 *trans*-4	0.037	0.025	0.002	< 0.001
18:1 *trans*-5	0.035	0.019	0.002	< 0.001
18:1 *trans*-6+7+8	0.389	0.305	0.010	< 0.001
18:1 *trans*-9	0.407	0.336	0.013	0.001
18:1 *trans*-10	0.520	0.418	0.025	< 0.001
18:1 *trans*-11	1.184	0.881	0.053	< 0.001
18:1 *trans*-12	0.527	0.421	0.018	< 0.001
18:1 *trans*-13+14	0.754	0.429	0.029	< 0.001
18:1 *trans*-16	0.374	0.288	0.011	< 0.001
18:2 n-6	2.066	1.792	0.082	< 0.001
18:2 *trans*-9, *cis*-12	0.048	0.034	0.002	< 0.001
18:2 *trans*-11, *cis*-15	0.086	0.052	0.003	< 0.001
CLA[1] *cis*-9, *trans*-11	0.484	0.490	0.018	0.688
CLA[1] *trans*-9, *cis*-11	0.016	0.016	0.001	0.914
CLA[1] *trans*-10, *cis*-12	0.012	0.006	0.001	< 0.001
CLA[1] *trans*-11, *trans*-13	0.024	0.021	0.002	0.308
CLA[1] *trans*, *trans*[2]	0.056	0.062	0.003	0.055

[1]Conjugated linoleic acid.
[2]Unresolved peak of *trans*-10, *trans*-12 + *trans*-9, *trans*-11 conjugated linoleic acid.

significantly reduced milk concentration of *trans*-10 18:1, a fatty acid frequently associated with MFD, as shown in Figure 2 and demonstrated by Overton *et al.*[16] Even though it is known that *trans*-10 18:1 does not cause MFD, it is relatively easy to measure compared to *trans*-10, *cis*-12 CLA and can generally serve as an indicator of the alteration of rumen biohydrogenation.[12,14]

It has been shown that feeding plant oil can decrease milk fat percentage by impairing ruminal biohydrogenation and generating some fatty acid intermediates that can be transferred to milk.[1,4,19] Results of this study support the biohydrogenation theory by demonstrating that a modification of the fatty acid content of a diet can influence the concentration of biohydrogenation intermediates found in milk. Thus, depending on their concentration, some intermediates such as *trans*-10, *cis*-12 CLA, can inhibit milk fat synthesis and induce a depression of the milk fat percentage.[7]

Laboratory analyses of PUFA content in feed and milk are expensive and not routinely available. Therefore, prudent use of this procedure should be considered for investigating MFD situations. Alternatively, some software packages[c] offer theoretical values of PUFA composition and may be sufficiently accurate to estimate PUFA intake. However, it should be kept in mind that a small decrease in PUFA intake may have an important impact on milk fat percentage, as demonstrated in this study.

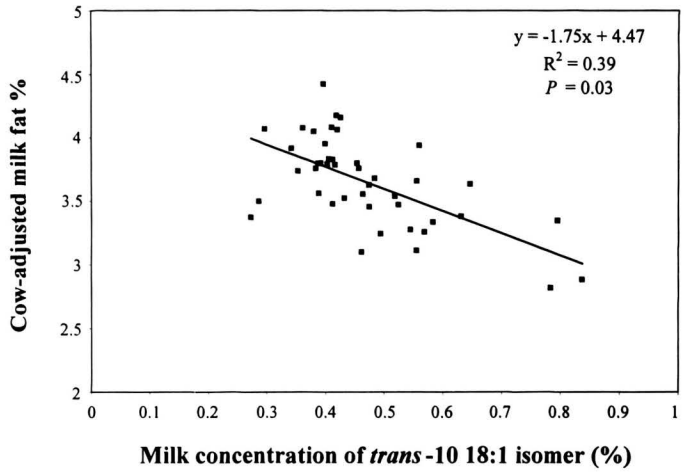

Figure 2. Relationship between milk concentration of *trans*-10 18:1 fatty acid isomer and cow-adjusted milk fat percentage. Calculation method of cow-adjusted values was adapted from Alzahal *et al.*[3]

Conclusions

Subacute ruminal acidosis has traditionally been the first condition suspected when a dairy herd experiences MFD. Because MFD is caused by an interaction of dietary factors, the impact of polyunsaturated fatty acid intake should be assessed. A decrease in PUFA and linoleic acid intake may mitigate MFD.

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Endnotes

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[c]CPM-Dairy, version 3.0.8, Ithaca, NY
[d]Laboratory Service Division, University of Guelph, Guelph, ON, Canada
[e]Foss System 4000, Foss Electric, Hillerd, Denmark
[f]Microsoft Corporation, Richmond, WA
[g]SAS, version 9.1.3, Cary, NC
[h]CanWest DHI, Guelph, ON, Canada

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