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Case Report–Biohydrogenation of Conjugated Linoleic Acid (CLA) as a Probable Cause of Milk Fat Depression in a Dairy Herd

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Abstract

Milk fat depression (MFD) is a common nutritional disorder on commercial dairy farms. Subacute rumen acidosis (SARA) secondary to inadequate effective fiber is traditionally one of the first areas to investigate when consulting with these herds. A less frequently recognized cause of MFD is the presence of unsaturated oils in the ration, which can have an indirect effect on milk fat synthesis.

One of the predominant fatty acids produced in the rumen is conjugated linoleic acid (CLA). Under certain altered rumen fermentation processes, the biochemical pathway produces an increased amount of the *trans* vs. the *cis*-isomer of CLA. These *trans*-isomers of CLA are potent inhibitors of milk fat synthesis. This case report will detail the process by which there is diet-induced milk fat depression despite adequate forage intake and apparent normal rumen health.

Résumé

Une baisse dans le contenu en gras du lait est un désordre nutritionnel courant dans les fermes laitières commerciales. L'acidose subaiguë du rumen découlant d'un manque de fibres est l'une des causes les plus souvent discutées lors des consultations auprès de ces troupeaux. La présence d'huile insaturée dans la ration, qui peut avoir un effet indirect sur la synthèse du gras dans le lait, est moins souvent reconnue.

L'acide linoléique conjugué (ALC) est l'un des acides gras les plus couramment produits par le rumen. Lorsque les conditions de fermentation dans le rumen sont modifiées, l'isomère *trans* de l'ALC est produit plus fréquemment que l'isomère *cis*. Les isomères *trans* sont de forts inhibiteurs de la synthèse du gras dans le lait. Ce rapport de cas met en lumière une baisse dans le contenu en gras du lait causée par la diète en dépit d'un apport en fourrages adéquat et d'un fonctionnement apparemment normal du rumen.

Introduction

Milk fat depression (MFD) is an economically costly disorder for the commercial dairy. Fat is the major energy component in milk and generally represents the component with the highest economic return.[2] Traditionally, one of the first areas to investigate when milk fat decreases below 3.5% is the forage-to-concentrate ratio.

Microbial fermentation in the rumen produces the primary volatile fatty acids acetate, propionate and butyrate as a source of energy for the dairy cow. Acetate is produced from the fermentation of structural carbohydrates and is utilized by the mammary gland for the synthesis of fatty acids for milk fat. In contrast, propionate is produced from the fermentation of sugars, starches and pectins and is the major precursor for the synthesis of glucose, and ultimately lactose.[4]

High forage rations favor a higher rumen pH (>6.0), which in turn favors normal ruminal production of volatile fatty acids (VFA) and normal milk fat synthesis. In contrast, feeding higher amounts of concentrates can reduce rumen pH below 5.8 and change the rumen VFA pattern. Most commonly there is an increase in the ratio of propionate production over that of acetate.[2] This basic theory of acetate deficiency was traditionally proposed as the cause of MFD; however, it is now recognized that the supply of acetate is not appreciably changed when high concentrate diets are fed. The change in the ratio of acetate to propionate is an indicator that the rumen environment is altered in such a way to potentially cause diet-induced MFD.

A less frequently recognized cause of MFD is the presence of unsaturated oils in the ration, which in the presence of an altered rumen environment, can have an effect on milk fat synthesis. These oils are present in fish oils and plants, such as soy and cottonseed. The major unsaturated fatty acids in typical dairy feeds, linolenic acid (18:3) from forages and linoleic acid (18:2) from plant oils and seeds, can be toxic to the rumen bacteria and cause an alteration in microbial processes.[8]

Bauman and Griinari[1] have proposed the "biohydrogenation theory" to explain the concept that two conditions are required to cause diet-induced MFD. Both a dietary supply of unsaturated fatty acids and an alteration in the ruminal microbial processes are required. When a high concentrate ration is fed, there is often a decline in rumen pH and shift in rumen VFA production characteristic of this altered microbial process. In certain instances of feeding unsaturated oils in the ration, there is an alteration of the microbial processes with no appreciable decline in rumen pH or effect on VFA proportions.

Trans fatty acids are formed as intermediates in rumen biohydrogenation. One of the predominant fatty acid intermediates produced is conjugated linoleic acid (CLA).[1] Under altered rumen fermentation processes, such as subacute rumen acidosis and/or the presence of unsaturated oils in the ration, the biochemical pathway produces an increased amount of the *trans*- vs. the *cis*-isomer of CLA (*trans*-10, *cis*-12 CLA; Figure 1).[6] Bauman has shown that these unique *trans*-isomers of CLA are potent inhibitors of milk fat synthesis.[2] Abomasal infusion of 3-4 g/day of the *trans*-isomer reduced milk fat yield over 25%. Results from several recent studies have suggested the existence of several other fatty acid intermediates that can inhibit milk fat synthesis.[8] This mechanism of MFD is referred to as the "biohydrogenation theory" because it is under these altered rumen conditions that biohydrogenation results in the formation of *trans*-10, *cis*-12 CLA and other intermediates.[2] Feeding a low forage and high concentrate ration can induce MFD; however, this case report will detail a dairy herd with diet-induced milk fat depression despite adequate forage intake and apparent normal rumen health.

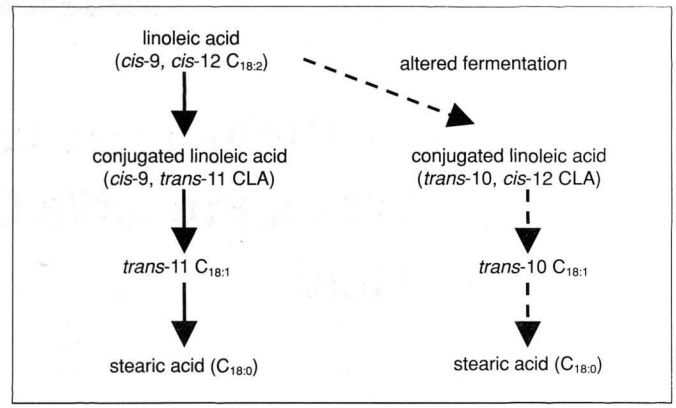

Figure 1. Pathways of rumen biohydrogenation of linoleic acid. Adapted from Bauman & Griinari.[2]

Clinical Report

A 50-cow commercial Holstein dairy, housed in a conventional tie-stall barn and fed a component ration consisting of corn silage, mixed grass/alfalfa haylage and a custom grain mix, reported a history of milk fat depression in the herd. The bulk-tank fat percentage was less than 3.2% for six consecutive months, and had decreased to an average of 2.8% for the three months prior to the investigation. An initial history was obtained and the ration was reviewed.

During the three months prior to the herd investigation, the average herd production ranged from 70-75 lb (32-34 kg)/cow/day. Milk protein averaged 3.0% and milk fat averaged 2.8%. The herd owners did not report any health problems in the lactating herd that would suggest a digestive disturbance, such as rumen acidosis. Likewise, herd records did not indicate any increase in the incidence of treatment for indigestion or other digestive disorders. In the six months previous to the herd investigation there were no recorded cases of displaced abomasum, and the incidence of ketosis was less than 4% of periparturient cows. Records from the hoof trimmer did not reveal any evidence of laminitis or abscess formation.

Initial diagnostics were centered around a ration inadequate in effective fiber or feeding management that allowed for "slug feeding", which led to rumen acidosis. Forage analysis (near infrared reflectance spectroscopy)[a] was performed and the current ration was analyzed with the Cornell Net Carbohydrate and Protein Model (CNCPS[b]; Table 1). The physical ration was also analyzed with a Penn State Particle Separator.[c] The haylage had 70, 9 and 20% of particles on the upper, middle and bottom screens, respectively. The corn silage had 11, 53 and 35% on the upper, middle and bottom screens, re-

Table 1. Recommended vs. sampled forage particle size.[c]

	Haylage recommended (%)	Haylage sampled (%)	Corn silage recommended (%)	Corn silage sampled (%)
Upper sieve (>0.75")	10-15	70	10-15	11
Middle sieve (0.75-0.31")	30-40	9	40-50	53
Bottom pan (<0.31")	40-50	20	40-50	35

spectively (Table 1). Results of these analyses suggested that the ration was adequate in effective fiber and not excessive in non-structural carbohydrates or added fat.[3,5]

Rumenocentesis was performed on 12 lactating cows that were 20-150 days in milk to aid in ruling out rumen acidosis. Ruminal pH was measured,[d] and one out of 12 cows had a rumen pH<5.5. Based on the work of Oetzel,[7] these results did not support a diagnosis of rumen acidosis. Evaluation of the manure using an objective scoring system[e] and screens on repeated visits to the farm did not reveal any abnormalities associated with a herd-based digestive disorder.

Other causes of a low fat test, such as mechanical damage to fat due to a malfunctioning milking system or bulk tank, were also investigated. Individual milk sample results from a monthly testing service[f] affirmed a true depression of milk fat from the individual cow, and ruled out post-harvest mechanical damage.

Although the total fat in the initial ration was 4.3%, it was noted that there were sources of plant oils (roasted soy, high soy oil bypass protein[g]) and bypass fat that could possibly attribute to altered rumen function. A new grain mix was formulated with ground corn, distiller's grains and soybean meal composing the main ingredients. No bypass fats or other sources of plant oils were included in the ration. Dry matter intake of corn silage, haylage and dry hay were kept consistent with the original ration. The nutrient profile and dry matter percentages of this ration are summarized in Tables 2 and 3, respectively.

Within four days of feeding the new grain mix, the bulk-tank fat increased from 2.6 to 3.0%. Over the next four weeks the milk fat percentage continued to increase to an average of 3.9%, and maintained a monthly average over 3.6% for 12 months.

Table 2. Ration summary - nutrient composition.

	Ration type	
Nutrient	Initial	Reformulated
Dry matter (%DM)	47.5	47
NEl (Mcal/lb DM)	.70	.76
Crude protein (%DM)	16.0	17.6
Soluble protein (%CP)	32.65	34.0
NDF (%DM)	36.3	32.6
Forage NDF (%DM)	26.8	21.0
Total NFC (%DM)	38	40
Total fat (%DM)	4.33	3.7

NEl: net energy of lactation
NDF: neutral detergent fiber
NFC: non-fiber carbohydrate

Discussion

The dramatic increase in milk fat following ration changes lends substantial support for the biohydrogenation theory as an explanation for milk fat depression in this dairy herd. Although the total fat percentage in the initial ration was within acceptable levels, the dietary presence of certain unsaturated fatty acids was one condition necessary for altered rumen microbial processes and biohydrogenation to occur. The combination of unsaturated fatty acids and altered rumen microbial processes then led to the formation of *trans*-CLA or other intermediates which directly inhibited milk fat synthesis.[2]

Subacute rumen acidosis is the most commonly recognized cause of altered rumen microbial processes that lead to MFD. In general, high concentrate/low forage rations that are high in non-structural carbohydrates cause rumen pH to decline. Improper feeding techniques, such as "slug feeding" or practices that lead to feed sorting, can also support rumen acidosis. Given the long duration of MFD in this herd, with no apparent clinical signs of rumen acidosis and normal rumen pH values, other causes needed to be considered.

The reformulated ration was higher in NFC as compared to the initial ration (NFC 38 vs. 40% DM). It is

Table 3. Ration summary - percent of diet dry matter (DM).

	Percent of diet DM	
Feed ingredient	Initial ration	Reformulated ration
Corn meal	17.6	10.9
Steam flaked corn	0.0	13.0
Wheat middlings	7.1	0.0
Distillers	6.3	9.8
Soybean meal 47.5%	5.6	6.5
Corn gluten meal	2.7	0.0
Roasted soybeans	2.3	1.1
Homer meal[c]	1.2	0.0
Limestone	0.6	0.8
Salt	0.3	0.4
Sodium bicarbonate	0.5	1.1
Urea	0.1	0.0
Molasses	0.8	0.3
Bypass fat	0.3	0.0
Vitamin/mineral mix	1.1	0.5
Yeast	0.0	0.3
Corn silage	25.8	26.7
Haylage	22.7	22.9
Dry hay	5.2	5.7
	100.0	100.0

unlikely that this change alone resulted in an increase in milk fat percentage. Conversely, one might expect a further depression or no change in milk fat percentage as starch availability in the rumen increased, rumen pH potentially decreased and ruminal microbial processes were altered.

Questions remain as to what components of the ration caused the altered rumen microbial processes. Because the ratios of the forages remained consistent, it can be assumed that one of the grain or byproduct ingredients also had a role in altering normal rumen flora in this herd. The interaction in this herd is somewhat unique, as these ingredients are commonly included in dairy rations with no negative effects. While the rumen environment on the initial ration was not associated with metabolic disorders, it resulted in conditions that favored biohydrogenation pathways leading to MFD.

Conclusions

In dairy herds where milk fat depression is an issue, nutritional factors causing subacute rumen acidosis are most commonly implicated as the cause of altered rumen microbial processes. It must be recognized, however, that high concentrate rations or other feeding issues, such as ration sorting or "slug feeding", that affect rumen heath are not the only cause of altered rumen microbial processes.

It is probable that other factors on an individual dairy affect passage rate or other aspects of rumen microbial processes that alter biohydrogenation pathways to form intermediates that are potent inhibitors of milk fat synthesis. The classical sequela related to ruminal acidosis may, therefore, not be recognized in the presence of depressed milk fat. Nutritional factors other than those suggestive of causing low ruminal pH, such as the presence of unsaturated oils in the ration, must be investigated when consulting in herds with a history of milk fat depression.

Endnotes

[a] Dairy One forage analysis laboratory, Ithaca, NY.
[b] Cornell Net Carbohydrate and Protein System, Version 5.0.33, Cornell University, Ithaca, NY.
[c] Penn State Particle Separator, Nasco Farm & Ranch, Fort Atkinson, WI.
[d] Cardy Twin pH Meter, Spectrum Technologies, Inc, Plainfield, IL.
[e] Manure Evaluation Guide, MB Hall, University of Florida. Available: http://www.animal.ufl.edu/hall/
[f] Dairy Records, Joy Platt, Watertown, NY.
[g] Homer meal (4.5% min. oil bypass soybean meal). Homer Oil Co, Inc, Homer, NY.

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