Short Communication: Indigestible Markers Reduce the Mammary Δ^9 -Desaturase Index and Alter the Milk Fatty Acid Composition in Cows

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ABSTRACT

Accurate determination of the flow of nutrients at the omasum requires the use of a triple marker system. Typically, a system based on ruminal administration of the lithium salt of CoEDTA, ytterbium acetate (Yb-Ac), and chromium-mordanted straw (Cr-S) has been used. However, there is evidence to suggest that product:substrate ratios for stearoyl-coenzyme A desaturase (Δ^9 -desaturase) are lower in milk fat from cows administered a combination of CoEDTA, Yb-Ac, and Cr-S, indicating reduced Δ^9 -desaturase activity. To evaluate this hypothesis, samples of milk were collected 1 d before, and on d 2, 6, and 9 of administering the CoEDTA, Yb-Ac, and Cr-S triple marker system into the rumen of 4 cows. A 4 × 4 Latin square with 28-d experimental periods was used to assess the effects of 0, 75, 150, and 300 g/d of fish oil in the diet on ruminal and mammary lipid metabolism. Irrespective of the amount of fish oil in the diet, concentrations of all milk fatty acids containing a *cis*-9 double bond were reduced after markers were given. Milk fatty acid pairs dependent on Δ^9 -desaturase were decreased over time, with responses reaching a nadir within 6 d of marker administration. Overall, administering markers into the rumen was associated with a mean decrease in milk *cis*-9 10:1/10:0, *cis*-9 12:1/12:0, *cis*-9 14:1/14:0, *cis*-9 16:1/16:0, *cis*-9 17:1/17:0, *cis*-9 18:1/18:0, and *cis*-9,*trans*-11 conjugated linoleic acid/*trans*-11 18:1 concentration ratios of 44.6, 52.7, 58.7, 36.8, 37.2, 44.3, and 43.0%, respectively. In conclusion, one or more of the markers administered altered milk fatty acid composition and may act as an inhibitor of Δ^9 -desaturase in the bovine mammary gland.

Key words: Δ^9 -desaturase, marker, milk fatty acid, conjugated linoleic acid

Because of the potential benefits to human health (Larsson et al., 2005; Lock et al., 2005), there is consid-

erable interest in enhancing concentrations of *cis*-9,*trans*-11 conjugated linoleic acid (CLA) in milk. *Cis*-9,*trans*-11 CLA is formed in the rumen during metabolism of 18:2n-6 and is synthesized endogenously in ruminant tissues from *trans*-11 18:1 via stearoyl-coenzyme A desaturase (Δ^9 -desaturase; Griinari and Bauman, 1999). Owing to both ruminal and mammary synthesis of *cis*-9,*trans*-11 CLA, understanding the mechanisms underlying milk fat CLA responses to changes in diet composition or lipid supplements is reliant on examining the effects of the diet on ruminal lipid metabolism, as well as milk fatty acid composition. Sampling at the omasum rather than the duodenum has the advantage that measurements of nutrient flow are less dependent on endogenous secretions, but accurate determination of omasal digesta flow requires the use of a triple marker system to account for errors caused by unrepresentative sampling (Ahvenjärvi et al., 2003).

The omasal sampling technique was used in combination with CoEDTA, ytterbium acetate (Yb-Ac), and chromium-mordanted straw (Cr-S) as markers to explain the effects of fish oil in the diet on ruminal lipid metabolism and milk fat *cis*-9,*trans*-11 content (Shingfield et al., 2003). Under these circumstances, the mean ratio of *cis*-9,*trans*-11 CLA:*trans*-11 18:1 concentrations in milk of 0.20 was lower than a corresponding value of 0.42 determined in milk from cows not receiving indigestible markers (Shingfield et al., 2005). Furthermore, administration of the same marker system in zero-grazed cows (n = 4) was associated with a 40 to 63% reduction in the ratios of *cis*-9 14:1/14:0, *cis*-9 16:1/16:0, *cis*-9 18:1/18:0, and *cis*-9,*trans*-11 CLA/*trans*-11 18:1 in milk fat compared with a larger number of cows (n = 12) grazing the same grass swards and not given markers [J. M. Griinari, K. V. V. Nurmela, and J. Nousiainen (Dept. Anim. Sci., Univ. Helsinki, Finland), A. Sairanen and H. Khalili (MTT Agrifood Research Finland, Maa-ninka, Finland); unpublished data].

Early work established that copper alters the conversion of 18:0 to *cis*-9 18:1 in the pig (Thompson et al., 1973) and inhibits Δ^9 -desaturase-catalyzed reactions during incubations with chicken liver microsomes (Sreekrishna and Joshi, 1980). Furthermore, cadmium

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chloride is known to suppress the desaturation of 18:0 by rat hepatocytes in vitro (Kudo and Waku, 1996). The evidence from a number of studies is that certain transition metal ions selectively inhibit the terminal desaturase component of the Δ^9 -desaturase enzyme system (Ntambi, 1995). The mechanisms underlying the reductions in Δ^9 -desaturase activity are not known, but it is plausible that specific transition metal ions interfere with the transfer of electrons required for the conversion of Fe^{2+} to Fe^{3+} in cytochrome b_5 and oxidation of acyl-coenzyme A substrates. It is therefore possible that the combination of CoEDTA, Yb-Ac, and Cr-S alters milk fatty acid composition via reductions in mammary Δ^9 -desaturase activity. To evaluate this hypothesis, samples of milk were collected before and during the administration of this marker system from an experiment that was primarily conducted to assess the effects of fish oil in the diet on ruminal biohydrogenation and milk fatty acid composition.

Procedures involving animals were approved by the Animal Experiment Committee of MTT Agrifood Research Finland in accordance with the 1985 *Use of Vertebrates for Scientific Purposes* Act. Four multiparous dairy cows each fitted with a rumen cannula, were randomly assigned to a 4×4 Latin square with 28-d experimental periods. Cows were offered a basal ration containing (g/kg of DM) grass silage (600) and a cereal-based concentrate (400) supplemented with 0, 75, 150, or 300 g/d of ultrarefined herring and mackerel oil (EPAX 3000 TG; Pronova Biocare A.S., Aalesund, Norway). Diets were offered as 2 equal meals at 0600 and 1800 h.

The triple marker system comprised CoEDTA, Yb-Ac, and Cr-S, identical to that used in earlier studies (Shingfield et al., 2003). The Cr-S (40 g/d) was administered into the rumen as 2 equal doses via the cannula at 12-h intervals starting at 1800 h on d 18 of each experimental period. The CoEDTA (12 g/d) and Yb-Ac (4.0 g/d) were dissolved in 6 L of distilled water and infused into the rumen via separate lines at 1800 h on d 19 at a constant rate using a peristaltic pump (Watson-Marlow, High Wycombe, UK). Markers were given to each animal until d 28 of each period to provide 2.9, 1.6, and 1.5 g/d of Cr, Co, and Yb, respectively.

Samples of milk for determination of fatty acid composition were collected over 2 consecutive milkings (0700 and 1645 h) on 4 occasions during each experimental period, starting in the afternoon on d 17, 20, 24, and 27, corresponding to d -1, 2, 6, and 9 relative to the start of marker administration. Samples of unpreserved afternoon and morning milk were composited according to yield and stored at -20°C until analyzed for fatty acid composition. Milk fatty acid composition was determined using a combination of gas chromatog-

raphy-mass spectrometry and silver-ion HPLC (Shingfield et al., 2003, 2005).

Milk fatty acid composition data were statistically evaluated by ANOVA for repeated measures using the mixed linear model procedure of SAS (SAS Inst., Inc., Cary, NC). The statistical model included the fixed effects of diet, time, and their interaction, and the random effects of cow, assuming an auto regressive order one covariance structure. Least square means are reported and significance was declared at $P < 0.05$.

Administration of markers was associated with distinct changes in the milk fatty acid composition, effects that were characterized as an increase ($P < 0.001$) in total saturated fatty acids of 5.8%, and concomitant decreases ($P < 0.001$) in total monounsaturated fatty acids, CLA, and nonconjugated 18:2 of 14.5, 65.7, and 6.0%, respectively. Concentrations of all milk fatty acids containing a *cis*-9 double bond were reduced ($P < 0.001$) following marker administration, effects that were independent of the amount of fish oil in the diet (data not shown). As a result, the ratio of *cis*-9 10:1/10:0, *cis*-9 12:1/12:0, *cis*-9 14:1/14:0, *cis*-9 16:1/16:0, *cis*-9 17:1/17:0, *cis*-9 18:1/18:0, and *cis*-9,*trans*-11 CLA/*trans*-11 18:1 concentrations in milk were decreased ($P < 0.001$) by 44.6, 52.7, 58.7, 36.8, 37.2, 44.3, and 43.0%, respectively. The appearance of 10:0, 12:0, and 14:0 fatty acids in milk is almost exclusively derived from de novo synthesis in the mammary gland, and these fatty acids act as substrates for Δ^9 -desaturase to yield *cis*-9 10:1, *cis*-9 12:1, and *cis*-9 14:1, respectively (Bauman and Davis, 1974). Therefore, the synthesis of *cis*-9 10:1, *cis*-9 12:1, and *cis*-9 14:1 in milk fat is almost solely dependent on mammary Δ^9 -desaturase (Kay et al., 2004). Furthermore, the ratios of product:substrate for Δ^9 -desaturase in milk fat are correlated with Δ^9 -desaturase mRNA abundance and Δ^9 -desaturase activity in the mammary gland of goats (Bernard et al., 2005), indicating that the ratios of *cis*-9 10:1/10:0, *cis*-9 12:1/12:0, *cis*-9 14:1/14:0, *cis*-9 16:1/16:0, and *cis*-9 18:1/18:0 serve as proxies for mammary Δ^9 -desaturase activity. The ratios of all fatty acid pairs dependent on this enzyme were altered over time in response to markers (Figure 1), consistent with an inhibition of the Δ^9 -desaturase enzyme. Significant ($P < 0.001$) decreases in Δ^9 -desaturase product:substrate concentrations in milk were apparent across all diets within 2 d of markers being administered (Figure 1), but the changes in these ratios were not different ($P > 0.05$) between d 6 and 9, indicating that the inhibitory effects on Δ^9 -desaturase reached a nadir within 6 d of marker administration. Previous studies reported comparable reductions in Δ^9 -desaturase product:substrate ratios and temporal changes in milk fatty acid composition in response to 8.8 to 9.7 g of sterculic oil/d (Griinari et

Figure 1. Effect of administering CoEDTA, ytterbium acetate, and chromium-mordanted straw on the ratio of fatty acid concentrations that represent the product and substrate for stearoyl-coenzyme A desaturase in milk of cows fed incremental levels of fish oil in the diet. Numbers on the x-axis indicate the time relative to the start of marker administration (d). Mordanted straw was administered 24 h before ruminal infusions of CoEDTA and ytterbium acetate. Standard errors for the concentration ratio of product:substrate between sampling times for *cis*-9 10:1, *cis*-9 12:1, *cis*-9 14:1, *cis*-9 16:1, *cis*-9 17:1, *cis*-9 18:1, and *cis*-9,*trans*-11 conjugated linoleic acid (*c*-9, *t*-11 CLA) Δ^9 -desaturase products were 0.0090, 0.0019, 0.0098, 0.0148, 0.0042, 0.1139, and 0.0289, respectively.

al., 2000; Kay et al., 2004), which contained 2 known inhibitors (7-2-octyl-1-cyclopropenyl heptanoic acid and 8-2-octyl-1-cyclopropenyl octanoic acid) of Δ^9 -desaturase. The inhibitory effects of sterculic oil were directly related to reductions in enzyme activity rather than a lowered Δ^9 -desaturase gene or protein expression (Palmquist et al., 2005).

Significant time $\times$ treatment interactions were observed for total 18:1 and *cis*-9,*trans*-11 CLA concentrations in milk ($P = 0.03$ and 0.01 , respectively), but not for Δ^9 -desaturase product:substrate ratios. Possible confounding effects of marker administration with the

duration of fish oil supplementation can be accounted for by examining changes in milk fatty acid composition on the control diet. Administration of markers to cows fed the basal ration altered the relationship between *trans*-11 18:1 and *cis*-9,*trans*-11 CLA in milk and caused reciprocal changes in product:substrate concentrations (Figure 2). Similar temporal patterns were observed for changes in the concentration ratios of *cis*-9 10:1/10:0, *cis*-9 12:1/12:0, *cis*-9 14:1/14:0, *cis*-9 16:1/16:0, *cis*-9 17:1/17:0, and *cis*-9 18:1/18:0 (data not shown).

Because 3 markers were used, it is not possible to establish which one or more of the markers adminis-

Figure 2. Effect of administering CoEDTA, ytterbium acetate, and chromium-mordanted straw on temporal changes in the concentrations of *trans*-11 18:1 (○) and *cis*-9,*trans*-11 conjugated linoleic acid (●) in milk of cows fed a basal diet composed of grass silage and cereal-based concentrates. Numbers on the x-axis indicate the time relative to the start of marker administration (d). Mordanted straw was administered 24 h before ruminal infusions of CoEDTA and ytterbium acetate. Standard errors for *trans*-11 18:1 and *cis*-9,*trans*-11 conjugated linoleic acid concentrations between sampling times were 0.61 and 0.21 g/100 g of fatty acids, respectively.

tered caused the reductions in the Δ^9 -desaturase product:substrate ratios in milk fat. Earlier studies have established that the absorption of Cr from Cr-S (Udén et al., 1980) and Yb delivered as Yb-Ac (Siddons et al., 1985) is insignificant within the gastrointestinal tract of ruminant animals, but about 3% of Co administered as CoEDTA in the rumen was recovered in the urine (Udén et al., 1980). Measurements of milk fatty acid composition from cows dosed with chromic oxide (Piperoova et al., 2002), or ytterbium chloride and polyethylene glycol (Lor et al., 2005) indicated comparable *cis*-9 16:1/16:0, *cis*-9 18:1/18:0, and *cis*-9,*trans*-11 CLA/*trans*-11 18:1 concentration ratios as those determined in milk fat in the present experiment before markers were administered. The evidence thus far implicates the lithium salt of the monovalent CoEDTA anion as being responsible for the changes in milk fatty acid composition and reductions in desaturase ratios, but further studies would be required to confirm that this compound is a potent inhibitor of Δ^9 -desaturase activity in the bovine mammary gland and the mode of action.

Use of single markers to estimate DM flow can lead to erroneous estimates of nutrient flow in the gastrointestinal tract caused by unrepresentative sampling because digesta separates during collection (Ahvenjärvi et al., 2003). In cases where the nutrient flow entering the omasal canal and milk fatty acid composition are

to be determined simultaneously, the use of a marker system comprising CoEDTA, Yb-Ac, and Cr-S cannot be recommended. It is possible to circumvent these problems by sampling milk immediately before marker administration. However, a more ideal solution would be to develop an alternative triple marker system that allows for an accurate assessment of nutrient flow without altering milk fatty acid composition.

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