Effect of Combinations of Fish Meal and Feather Meal on Milk Fatty Acid Content and Nitrogen Utilization in Dairy Cows

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ABSTRACT

The effect of supplemental fishmeal in combination with feathermeal at two different proportions in the diet on milk docosahexaenoic acid (DHA) content was investigated. Recently, benefits to human health have been attributed to the consumption of this fatty acid, which is normally present in marine lipids. Six Holstein cows past peak lactation were used in a Latin square design with a 2×3 factorial arrangement of treatments. Fish- and feathermeals were prepared as pellets at 4:1 and 1:4 combinations and offered at 3.75, 11.75, and 27% of the diet. The supplements were top-dressed onto a basal diet based on corn silage that was progressively replaced by supplement. Nitrogen balance measures were made during the experiment because of the wide range in crude protein content of experimental diets. Milk protein content increased with level of supplementation in the diet reflecting the protein quality of the supplements used. There was overall higher milk DHA content when cows consumed the supplement containing more fishmeal than feather meal. Milk DHA content increased in a quadratic fashion, as more of either supplement was included in the diet. Apparent transfer efficiency of DHA from diet to milk declined with increasing amount of DHA in the diet. Results from this experiment suggest that transfer of docosahexaenoic acid from diet to milk may depend on diet composition and quantity present in the diet.

(**Key words:** fish meal, feather meal, milk composition, docosahexaenoic acid)

Abbreviation key: CLA = conjugated linoleic acid, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, FA = fatty acid, FM = fish meal, FTM = feather meal, PUFA = polyunsaturated fatty acid.

INTRODUCTION

Modification of ruminant diets to achieve more healthful meat and milk products is an attractive area of research for nutritionists. The nutritional alteration of milk fatty acid (FA) composition for the purpose of improving human health has been a goal of researchers for many years. The use of marine lipids in this pursuit was investigated in dairy cattle diets in the 1970s, but most of this research was abandoned when the concomitant decline in milk fat content was perceived as a negative side effect to dairy producers that were compensated on a milk fat basis (Doreau and Chilliard, 1997). Recently, the potential human health benefits of specific FA, including benefits for docosahexaenoic acid (DHA), conjugated linoleic acid (CLA; see the review by Roche et al., 2001), and eicosapentaenoic acid (EPA) have been identified. Our knowledge about individual FA is more important than the more generic advantage popularly conferred on polyunsaturated fatty acids (PUFA), and has revived interest in the nutritional manipulation of FA in milk. An excellent review of long chain omega-3 FA and their impact on human health was recently written by Lauritzen et al. (2001). Docosahexaenoic acid (C22:6n-3) is a long chain omega-3 PUFA that is found in some marine lipids, but not naturally present in bovine milk above trace levels. Cunnane (2000) has described DHA as appearing to be conditionally indispensable for infants under 2 yr of age for brain functioning and visual acuity.

The rumen environment is an effective site for the biohydrogenation of dietary PUFA, which accounts for the predominantly saturated nature of ruminant fat in meat and milk (Annison and Bryden, 1998). Strategies for the transfer of intact unsaturated FA through this environment into the small intestine have involved supplemental fats and oils in the diet as well as the protection of FA from biohydrogenation. Protection methods have involved the feeding of Ca-salts or formaldehyde-treated coatings (Ashes et al., 1997). Docosahexaenoic acid has been introduced into milk fat in prior experiments (Cant et al., 1997; Wright et al., 1999; Keady et al., 2000) with some success, while other experiments

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have reported apparent transfer efficiencies to milk fat of such low levels (0.04; Mansbridge and Blake, 1997) as to make the application of expensive marine lipids for this purpose impractical. There are also some discrepancies in the literature as to the fate of DHA in the rumen. Ashes et al. (1992) and Gulati et al. (1999) reported that there was negligible or limited biohydrogenation of DHA, respectively, following incubations with rumen microbes. However, a report by Feivez et al. (2000) indicated that there was biohydrogenation of DHA during an in vitro incubation with rumen contents.

The present experiment was conducted to evaluate the potential to enrich milk fat with DHA using fish meal (**FM**; determined to have a high DHA content) in combination with feather meal (**FTM**) based on a similar supplement used in our laboratory which incorporated DHA into milk fat (Wright et al., 1999). The high CP content in these supplements and our desire to test a wide range of DHA intakes necessitated the use of an intentionally low CP-basal diet. Nitrogen balance measures were made simultaneously to quantify the impact of these diets on estimated whole-body N retention.

MATERIALS AND METHODS

Experimental Design and Analyses

Six multiparous Holstein cows past peak lactation (111 ± 9 DIM; 631 ± 23 kg BW, mean and SE respectively) were housed in tie stalls with ad libitum access to water in the Physiology Wing at the Elora Dairy Research Center (University of Guelph). A 6×6 Latin square design balanced for residual effects with a 2×3 factorial arrangement of treatments was used for this experiment. Each period was 21 d long, and data were collected during the last 5 d of each period. The factors were two pelleted supplements having different combinations of FM and FTM. The combinations were 4 parts FM to 1 part FTM (4FM:1FTM) and 1 part FM to 4 parts FTM (1FM:4FTM) on a weight by weight basis. Each supplement was offered at three levels of intake (3.75, 11.75 and 27% as-fed). The supplement ingredients and chemical analysis are shown in Table 1. The diets consisted of a corn silage based diet fed to all cows (Table 2) twice daily at 0800 and 1500 h with the individually weighed supplements top-dressed in equal portions according to treatment;orts were collected every morning. Fatty acid content of the basal diet and supplements is shown in Table 3. DMI was restricted to 95% of its average for the 5 d before data collection. The supplements progressively replaced a portion of the basal diet fed to all animals. Animal use for this

Table 1. Supplement ingredients and analysis (mean of 6 analyses).

Ingredient	Supplement	
	4FM:1FTM	1FM:4FTM
	——— (% As fed basis) ———	
Wheat	25	25
Fish meal (FM)	60	15
Feather meal (FTM)	15	60
Analysis	Mean (SE)	Mean (SE)
DM (%)	89.65 (0.1)	89.28 (0.2)
	——— (% DM basis, mean, and SE) ———	
CP	68.22 (0.47)	60.93 (0.49)
ADIN	0.75 (0.02)	2.02 (0.09)
Soluble protein	11.98 (1.89)	9.32 (0.21)
Crude fat	7.27 (0.56)	7.08 (0.25)
Ca	2.00 (0.03)	0.87 (0.02)
P	1.58 (0.02)	0.63 (0.01)

experiment was approved under the guidelines of the Canadian Council on Animal Care.

The cows were milked at 0600 and 1600 h daily. Milk samples were collected from morning and afternoon milkings and pooled proportionally by weight to obtain daily composite samples on d 17 to 21. A fresh sample was analyzed for standard components by infrared spectroscopy (Laboratory Services Division, University of Guelph), and a sub-sample was kept frozen at -20°C for N analysis by the macro-Kjeldahl technique (AOAC, 1990). Feed samples of the basal mixture and pelleted supplements were taken twice weekly and pooled into a single weekly sample for chemical analysis at an ac-

Table 2. Ingredients and analysis of basal portion of the diet (n = 6).

Ingredient	%, As fed basis	
Straw	4.0	
Corn silage	60.6	
High-moisture corn	33.6	
Dairy premix ¹	0.8	
Cobalt iodized salt ²	0.2	
Ground lime	0.7	
Analysis	Mean	SE
DM (%)	46.77	0.84
	——— %, DM basis ———	
CP	9.12	0.25
ADIN	0.13	0.01
Soluble protein	2.98	0.23
ADF	20.30	0.85
NDF	33.12	1.59
Crude fat	3.25	0.17

¹Premix contained on a DM basis (per kg): 18% Ca, 9% P, 8% Mg, 1.5% K, 3336 mg Mn, 1500 mg Cu, 5000 mg Zn, 450 000 IU vitamin A, 150 000 IU vitamin D, 2 500 IU vitamin E, 20 ppm Se (Floradale Feed Mill Ltd., Floradale, ON, Canada).

²Contained 180 mg/kg of iodine and 129 mg/kg of cobalt.

Table 3. Fatty acid composition (mg/100g) of fish meal (FM) and feather meal (FTM) combinations and the mixed basal ingredients.

Fatty acid	Dietary component				Basal ingredients	
	4FM:1FTM	SD	1FM:4FTM	SD	Basal ingredients	SD
C8:0	0.83	0.47	4.74	0.36	0.00	0.00
C10:0	18.40	1.97	36.07	4.48	18.40	0.89
C12:0	24.49	2.81	33.32	3.34	26.35	0.43
C13:0	13.73	2.29	8.49	3.47	14.92	0.16
C14:0	325.98	12.16	166.32	3.88	28.37	5.22
C14:1	13.38	0.61	11.62	1.16	1.50	0.72
C15:0	30.48	2.78	19.53	2.68	20.39	0.92
C16:0	1204.73	16.33	1532.18	21.31	372.43	10.20
C16:1	398.76	10.23	335.32	9.64	21.46	16.39
C18:0	221.59	3.32	483.79	7.34	75.62	9.22
C18:1	1185.48	19.82	2075.83	27.35	626.27	64.87
C18:2n-6	505.87	6.59	861.31	13.92	1442.39	21.34
C18:3n-6	7.42	0.33	4.24	0.43	2.48	0.75
C18:3n-3	67.25	3.10	68.69	3.97	104.43	5.31
C18:4n-3	65.58	6.72	15.72	5.83	0.00	0.00
C20:0	26.73	2.40	69.15	1.84	17.42	3.70
C20:1	846.67	19.48	214.45	16.99	27.95	21.38
C20:2n-6	8.73	0.73	0.62	0.47	2.00	1.43
C20:3n-6	34.27	2.14	21.13	0.77	0.99	0.01
C20:4n-6	2.62	0.55	9.12	0.55	0.99	0.01
C20:3n-3	5.20	3.88	1.13	0.69	0.50	0.71
C20:4n-3	18.44	1.30	6.42	1.30	0.00	0.00
C20:5n-3	465.04	7.00	145.73	14.84	6.98	2.88
C22:0	ND ¹	ND	25.33	6.23	14.42	0.85
C22:1	1255.09	20.91	355.66	15.29	18.92	5.82
C22:2n-6	1.35	0.24	0.91	0.54	1.50	0.72
C22:4n-6	13.67	1.70	8.06	1.36	2.49	0.73
C22:5n-6	5.75	2.08	1.22	1.08	0.50	0.71
C22:5n-3	58.65	0.97	17.95	0.43	1.50	0.72
C22:6n-3	839.65	12.99	248.37	8.00	7.97	2.90
C24:0	16.88	0.41	39.20	0.60	14.92	1.56
C24:1	58.50	1.07	21.09	0.41	0.50	0.71

¹Not detected.

credited commercial laboratory (Agribands Purina Canada Central Laboratory Services, Strathroy, ON). Sample collection and analyses for estimated N balance were done similarly to the procedures of Wright et al. (1998). After the removal of the bladder catheters following the N collection period cows were treated with 15 ml of antibiotic (Excenel, Pharmacia Canada, Mississauga, ON) to reduce the potential for cystitis. Milk and feed FA content was determined according to the methods used by Wright et al. (1999). Fatty acid intake and N balance related calculations were based on the separate chemical compositions of the basal diet and the supplements, adjusted for the chemical composition of a sample of the feed refusals. Apparent FA transfer efficiency was calculated as output in milk divided by the intake of FA.

present experiment was: $Y_{ijkl} = \mu + C_i + P_j + S_k + L_l + (SL)_{kl} + \epsilon_{ijkl}$, where Y = dependent variable, μ = true mean, C = effect of cow ($i = 1$ to 6), P = effect of period ($j = 1$ to 6), S = effect of supplement ($k = 4\text{FM}:1\text{FTM}$ or $1\text{FM}:4\text{FTM}$), L = effect of level of supplement ($l = 3.75\%$, 11.75% , or 27%), SL = interaction term, and ϵ = random residual error. An effect was initially included in the model for residual effect in the Latin square but was not significant and therefore was removed from the final model. Only the main effects are presented for milk yield and milk composition data because there was no significant interaction for these variables. In this case, the associated degrees of freedom were added to the error term. Significant results were interpreted as $P < 0.05$.

RESULTS AND DISCUSSION

Statistical Analysis

Statistical analysis was conducted using the GLM procedure of SAS (SAS, 2000). The model used for the

Milk Composition

Milk yield and composition data are presented in Table 4. Neither the supplement composition nor the

Table 4. Milk yield (kg/d) and composition data based on the main effects of supplement [different combinations of fish meal (FM) and feather meal (FTM)] and inclusion level.

Variable	Supplement			Level				Effect	
	4FM:1FTM	1FM:4FTM	SE	3.75	11.75	27	SE	Supplement	Level
Milk yield	23.4	22.3	0.5	23.4	22.0	23.1	0.6	0.13	0.24
Fat %	2.69	2.68	0.11	2.77	2.82	2.46	0.14	0.99	0.17
Protein %	3.24	3.02	0.04	3.00	3.16	3.23	0.04	0.004	0.003 ¹
Lactose %	4.45	4.52	0.03	4.54	4.41	4.47	0.03	0.05	0.09

¹Denotes linear effect.

level of inclusion affected milk yield ($P > 0.05$) in the present experiment. The 4FM:1FTM fed cows had a higher milk protein content ($P = 0.004$) compared to cows consuming the 1FM:4FTM supplement. However, milk from cows fed the 1FM:4FTM supplement had a higher lactose content ($P = 0.05$) than milk from the 4FM:1FTM treatments. There was an increase ($P = 0.003$, linear) in milk protein content as the overall level of supplementation increased in all diets.

The absence of a main effect on milk production may not be unexpected given the composition of the diets fed and the low DMI in the present experiment (see Table 6). The synchronization between ruminally available carbohydrate and N was likely suboptimal, and the additional nutrients provided by the supplements were likely not the first limitation for milk production. Wright et al. (1998) reported an increase in milk yield for cows consuming similar diets, but DMI was higher overall in that study compared with the current one. The linear increase in milk protein content likely reflects the improved postruminal supply of AA to support milk protein synthesis. Animal-based protein sources, including FM and FTM, are resistant to rapid degradation in the rumen and can increase the postruminal delivery, of AA (England et al., 1997; O'Mara et al., 1998) to support increased milk protein synthesis. The advantage of relatively more FM compared with more FTM to support increased milk protein content is supported by the work of England et al. (1997), who concluded from their in vitro pepsin-HCl incubations that the RUP value of FTM could be restricted by its lower intestinal digestibility.

Milk Fatty Acid Content

Milk FA content is reported in Table 5. The 4FM:1FTM supplement fed cows had a higher content ($P < 0.05$) of C10:0, C12:0, C14:0, C16:1, C20:1, C20:4n-3, C20:5n-3, C22:0, C22:6n-3 compared to milk FA concentrations from cows consuming the 1FM:4FTM supplement. The content of C18:0 and C18:2n-6 was higher from cows consuming the 1FM:4FTM supplement com-

pared to those consuming the 4FM:1FTM supplement ($P < 0.05$).

The level of supplementation affected ($P < 0.05$) the content of 11 FA in milk, of which only 2 (C10:0 and C14:1) had a chain length less than C16:0. There was a linear decline in the concentration of C10:0 with level of supplementation, while conversely there was a linear increase in the concentration of C14:1 with the addition of either supplement to the diet. Long chain FA ($\geq$ C16) had several specific responses to the graded levels of supplementation used in the present experiment. Concentrations of C16:1, C20:0, C20:1 and C22:0 increased in a linear fashion ($P < 0.05$) with level of supplement in the diet. The increase in C22:0 concentration likely reflects ruminal biohydrogenation of C22 PUFA. A decline in the concentration of C18:2n-6 was detected ($P < 0.05$, linear) as level of supplement increased. Quadratic ($P < 0.05$) responses to level of supplementation were detected for C18:0, C18:3n-3 and C22:6n-3. There was a significant interaction between supplement and diet inclusion level for the concentration of C18:0 in milk. The 4FM:1FTM supplement fed cows had a quadratic decline in C18:0 concentration with increasing level of supplement, whereas the level of C18:0 from the 1FM:4FTM fed cows was relatively constant. Linolenic acid (C18:3n-3) concentration had a significant quadratic response, with the lowest concentration in milk measured from the 11.75% level of supplementation. Docosahexaenoic acid concentration increased in milk with level of supplementation in a quadratic manner ($P < 0.001$). While the content of DHA in milk is still relatively low (less than 1%) in the present experiment, it exceeds levels present in the breast milk of nursing mothers (0.14 to 0.2%; Lucas et al., 1992).

The impact of the dietary supplements in the present experiment is related to the supply of long chain FA present in the FM. Comparisons to, and between, other studies are frequently limited by the differences between laboratories in reporting the composition of FA present in FM, fish oil or milk FA. There remain wide differences between laboratories in their ability to measure individual FA isomers or very long chain ($>$ C20)

Table 5. Least square means of milk fatty acids (FA) from fish meal (FM) and feather meal (FTM) combinations (g/100g of FA).

FA	Supplement						SE	Effect		
	4FM:1FTM			1FM:4FTM				Supp ¹ .	Level	S×L ²
	3.75	11.75	27	3.75	11.75	27				
C8:0	2.10	1.88	2.32	2.09	1.42	0.93	0.39	0.06	0.41	0.22
C10:0	2.45	2.28	2.18	2.39	2.14	1.72	0.11	0.03	0.001 ⁴	0.19
C12:0	2.97	3.28	3.10	2.95	2.60	2.23	0.14	0.001	0.09	0.02
C14:0	10.69	11.77	10.89	10.99	10.27	9.45	0.40	0.01	0.11	0.06
C14:1	1.43	2.23	2.04	1.52	1.48	1.87	0.17	0.06	0.02 ⁴	0.06
C15:0	1.11	1.42	1.43	1.16	1.08	0.90	0.20	0.11	0.84	0.36
C16:0	29.29	31.21	29.35	29.87	31.21	30.87	0.94	0.37	0.23	0.72
C16:1	2.55	3.47	3.80	2.26	2.66	3.21	0.25	0.01	0.001 ⁴	0.58
C18:0	9.94	5.45	5.20	9.54	10.01	9.09	0.54	0.001	0.001 ⁵	0.001
C18:1	33.41	32.07	33.70	33.17	33.40	35.75	1.12	0.27	0.21	0.58
C18:2n-6	2.14	1.98	1.40	2.69	1.95	1.77	0.16	0.04	0.001 ⁴	0.23
C18:3n-6	0.03	0.03	0.03	0.04	0.03	0.03	0.01	0.61	0.45	0.95
C18:3n-3	0.22	0.12	0.24	0.29	0.10	0.27	0.05	0.47	0.01 ⁵	0.59
C18:4n-3	0.02	0.09	0.06	0.07	0.001	0.06	0.03	0.66	0.84	0.09
C20:0	0.24	0.23	0.35	0.13	0.28	0.31	0.05	0.54	0.04 ⁴	0.40
C20:1	0.19	0.63	1.27	0.13	0.16	0.27	0.20	0.01	0.02 ⁴	0.09
C20:2n-6	0.04	0.08	0.07	0.05	0.05	0.06	0.02	0.65	0.46	0.58
C20:3n-6	0.03	0.05	0.03	0.05	0.06	0.05	0.01	0.16	0.50	0.91
C20:4n-6	0.10	0.10	0.07	0.12	0.11	0.05	0.02	0.90	0.09	0.61
C20:3n-3	0.001	0.004	0.001	0.001	0.003	0.003	0.002	0.94	0.45	0.50
C20:4n-3	0.005	0.01	0.02	0.001	0.003	0.002	0.01	0.05	0.49	0.59
C20:5n-3	0.06	0.08	0.11	0.04	0.03	0.05	0.02	0.01	0.25	0.76
C22:0	0.11	0.37	1.03	0.05	0.08	0.11	0.19	0.01	0.05 ⁴	0.09
C22:1	0.24	0.24	0.26	0.18	0.27	0.29	0.03	0.83	0.15	0.32
C22:2n-6	0.003	0.004	0.006	0.009	0.003	0.006	0.005	0.69	0.81	0.74
C22:4n-6	0.001	0.01	0.02	ND ³	0.001	0.02	0.008	0.91	0.03 ⁴	0.83
C22:5n-6	0.001	0.004	0.002	ND	0.001	0.001	0.001	0.14	0.15	0.73
C22:6n-3	0.38	0.68	0.72	0.26	0.34	0.38	0.03	0.001	0.001 ⁵	0.05

¹Supplement.²Supplement × Level interaction.³Not detected.⁴Denotes effect of linear contrast.⁵Denotes effect of quadratic contrast.

FA at low concentrations. Chilliard et al. (2001) in their review also noted that fish oil composition could vary with geography, season, and species.

There is some consistency in the literature when incremental amounts of marine-origin lipids are fed to cows for milk C16:1 content. The linear increase ($P = 0.001$) in C16:1 in the present study agrees with data from Mattos et al. (2002), who fed graded levels (0 to 7.8% of DM) of FM with a corn silage and alfalfa based diet. Abu-Ghazaleh et al. (2001) reported an increase in C16:1 when Menhaden FM was supplemented at 5.8% of DM compared with 3.06% of DM but not compared with a 1.5% level of FM supplementation. In the experiment by Abu-Ghazaleh et al. (2001), there was a replacement of soybean meal by FM, and a small amount of animal fat was fed in all diets, which may account for slightly different trends. Increased C16:1 concentration compared with control (no marine lipids) was also reported by Cant et al. (1997) who supplemented over 300 g/d of fish oil. Keady et al. (2000),

however, found no effect on C16:1 concentrations in milk when cows were fed between 0 and 450 g/d of fish oil on a grass silage based diet. The significant changes to C18:0, C18:2n-6, and C18:3n-3 in the present experiment do not fit a consistent pattern similar to the C16:1 response. Linoleic acid (C18:2n-6) concentrations declined linearly in the experiment by Mattos et al. (2002), however, Cant et al. (1997) reported a significant increase compared with control milk samples. The linear increase in C20:0 in the present experiment agrees with the data of Keady et al. (2000), although this FA is not widely reported in milk fat, so comparisons are few. Increased concentrations of C20:1 in the present experiment agrees with data from Abu-Ghazaleh (2001) and Keady et al. (2000). Chilliard et al. (2001) hypothesized that high concentration of C20:1 could be related to high levels of CLA. The various isomers of CLA were not separated in the present experiment.

Eicosapentaenoic acid and DHA concentrations in milk are increased by feeding marine lipids (Chilliard

et al., 2001). The data of Mattos et al. (2002) and Abu-Ghazaleh (2001) both indicated increased milk concentrations of EPA and DHA when FM was fed to cows. Keady et al. (2000) reported a linear increase in EPA concentration, but there was only a numerical increase for milk DHA concentration, despite the fact that there was a higher dietary supply of DHA than EPA in their 0 to 450 g/d treatments. The quadratic increase of DHA concentration in the present experiment contrasts with the nonsignificant increase in milk EPA concentrations with increasing level of supplement. The reasons for the differences in milk EPA and DHA responses between studies are unclear, but could be related to whole diet differences or specific FA differences in the marine oil. The ratio of DHA to EPA in the two supplements was approximately 1.75:1 in the present experiment, while it was slightly lower (approximately 1.3:1) in the study by Keady et al. (2000). The incorporation of DHA into milk from a study similar to the present one demonstrated beneficial changes to the processing characteristics of DHA enriched dairy products (Wang, 1999).

Apparent FA Transfer

The apparent transfer of FA from diet to milk is difficult to estimate. Grummer (1991), in his review, presented a concise summary of the problems associated with measures of this kind. The estimates for FA transfer are potentially influenced by stage of lactation, basal diet, and level of feed intake (Grummer, 1991). Additionally, factors such as FA digestibility, circulating FA from adipose tissue, ruminal biohydrogenation, and activity by desaturases can affect apparent transfer estimates (Grummer, 1991). Estimates for dietary EPA and DHA transfer from fish oil to milk were discussed in the review by Chilliard et al. (2001). Those authors concluded from their calculations (corrected for EPA and DHA values in control milk) based on data in the literature that 0.026 and 0.041 were reasonable estimates of transfer efficiency for EPA and DHA respectively, although some experiments reported higher transfer efficiencies.

The apparent transfer efficiency for DHA was calculated for the present experiment. The intake of DHA was greater ($P = 0.001$) for cows consuming the 4FM:1FTM supplement compared to the 1FM:4FTM [19.3 and 5.1 (SE = 0.5) g/d, respectively] and reflects the difference in supplement concentration of DHA (see Table 3). Output of DHA to milk was higher ($P = 0.001$) for cows consuming the 4FM:1FTM compared with the 1FM:4FTM supplement [2.7 and 1.5 (SE = 0.1) g/d, respectively]. Apparent transfer efficiency was less ($P = 0.003$) on the 4FM:1FTM compared to the 1FM:4FTM supplement [0.22 and 0.48 (SE = 0.04), respectively].

The difference in transfer efficiency was not affected ($P = 0.12$) by period in the present experiment that lasted 18 wk, with the cows receiving some amount of FM in each experimental period. This is in contrast to the idea of Franklin et al. (1999) that rumen microorganisms could adapt to the presence of unprotected marine oils and reduce apparent transfer to milk through biohydrogenation of the DHA.

The main effect of supplementation level provided DHA in the diet at 3.4, 10.0, and 23.2 (SE = 0.6) g/d for the 3.75, 11.75, and 27% inclusion levels respectively. Docosahexaenoic acid milk output was 1.7, 2.2, and 2.4 (SE = 0.2) g/d for the three increasing levels respectively. Apparent transfer efficiency declined ($P = 0.04$; quadratic) with increasing level [0.63, 0.31, and 0.12 (SE = 0.05), respectively]. The decline in transfer efficiency with increasing level of supplementation is similar to Wright et al. (1999) who detected a linear decline in apparent transfer efficiency.

There are experimental results in the literature where higher transfer efficiencies than calculated by Chilliard et al. (2001) have been reported. Hagemeister et al. (1988) reported apparent EPA and DHA transfer from postruminal infusions (420 g/d) of fish oil to milk of 0.35 to 0.40. The approach of Hagemeister et al. (1988) negates potential biohydrogenation concerns arising with dietary supplementation and might represent an estimate of postruminal effects on apparent transfer. Cant et al. (1997) calculated the transfer efficiency for DHA as 0.16 in their experiment. Cant et al. (1997) noted that the potential for carryover existed in their experimental design, and that apparent transfer efficiency calculations may be overestimates, based on the level of DHA in milk from the control group. Keady et al. (2000) reported apparent transfer efficiency results from the feeding of an unprotected fish oil supplement of 0.61 and 0.19 for EPA and DHA, respectively, averaged from three levels of oil supplementation (150, 300, and 450 g/d). Franklin et al. (1999) fed ruminally-protected or unprotected forms of marine algae (*Schizochytrium* sp.) to cows and indicated a transfer efficiency of 0.16 and 0.08 for DHA originating from the protected and unprotected algae sources, respectively. Franklin et al. (1999) noted that there was a significant decline in DHA concentration in milk over time (d 14 to 42) for cows fed the unprotected algae and suggested that rumen microorganisms may have adapted to its inclusion and increased the proportion of DHA biohydrogenated in the rumen.

The linear decline in DHA transfer efficiency in the present experiment suggests that there could be an optimal level of dietary DHA fed to cows which may prove effective at elevating milk DHA to desirable concentrations for human health purposes. The differences

Table 6. Dry matter intake and N balance.

Measurement	Supplement						SE	Effect		
	4FM:1FTM			1FM:4FTM				Supplement	Level	SXL ¹
	3.75	11.75	27	3.75	11.75	27				
DMI (kg/d)	15.5	14.5	14.3	16.0	14.2	13.8	0.7	0.89	0.03 ³	0.76
	(g/d)									
N intake	272	363	506	301	391	564	19	0.02	0.001 ³	0.66
Fecal N	115	112	97	123	123	143	7	0.001	0.94	0.01
Urine N	59	116	198	53	112	186	13	0.50	0.001 ³	0.95
Milk N	105	102	112	98	94	103	4	0.03	0.12	0.95
N Balance	-6	32	99	32	59	132	15	0.01	0.001 ³	0.94
App. Digest ²	(%)									
DM	66	69	76	65	70	70	1	0.10	0.001 ³	0.09
N	58	69	81	59	68	75	1	0.15	0.01 ⁴	0.02

¹Supplement $\times$ Level interaction.²Apparent Digestibility.³Denotes effect of linear contrast.⁴Denotes effect of quadratic contrast.

in the literature for transfer efficiencies suggest that rumen biohydrogenation for dietary long chain PUFA is quite variable and possibly dependent on feed intake, diet composition, and FA composition of the marine lipid fed. Gulati et al. (1997) indicated that C20 and C22 PUFA biohydrogenation by rumen microbes is affected by the concentration of oil. Gulati et al. (1999) reported that there was more extensive biohydrogenation at oil concentrations of 1 mg/ml of rumen fluid than at concentrations of 5 mg/ml. The discrepancy between those results and the decline in efficiency with increasing dietary FM in the present experiment indicates that optimization of biohydrogenation of long chain PUFA requires further study. The differences between apparent transfer efficiency estimates in the present study between the two supplements may indicate that the composition of the whole diet could influence transfer efficiency. Our understanding of the transfer of specific FA from diet to milk would benefit from experiments in which purified oils with specific FA patterns are fed. The current situation with marine lipid feeding is analogous to the beginnings of protein feeding to ruminants with the progress in our understanding from CP, to RDP and RUP fractions, and finally to individual AA. Information on interactions between individual FA may explain some discrepancies in the literature, which arise from the feeding of different composites of marine lipids.

DMI and Nitrogen Utilization

The corn silage based portion of the diets was approximately 9% CP (Table 2). The 4FM:1FTM supplement contained approximately 12% more CP than the

1FM:4FTM supplement (Table 1). DMI in the experiment (Table 6) was approximately 2.3% of BW. There was no difference ($P > 0.05$) in DMI between cows consuming either supplement, but there was a linear decline ($P = 0.03$) in DMI as the corn silage based portion of the diets was increasingly replaced by one of the supplements. The low level of DMI in the present experiment may reflect overall diet palatability.

The difference in CP content of the supplements was expected based on the ingredients used. The 4FM:1FTM and 1FM:4FTM supplements were offered at identical inclusion levels rather than isonitrogenous rates because of our desire to maintain the basal diet-to-supplement ratio constant at each of the three treatment levels. The difference in CP content of the diets was not expected to affect the long chain PUFA content of milk.

Nitrogen utilization (Table 6) was dependent on both the level of supplementation and the composition of the supplement in most cases. The difference ($P = 0.02$) in N intake between the two supplements is contrary to the N content of the two supplements. It reflects a higher N content ($P < 0.05$; data not shown) in the feed refusals collected from the cows consuming the 4FM:1FTM supplement. Fecal N output differences ($P = 0.001$) related to supplement reflects the lower intake of N for cows consuming the 4FM:1FTM diets as a result of the diet selection by the cows. The benefits towards milk N output for the 4FM:1FTM supplement likely reflects the better RUP value of relatively more FM compared to FTM (England et al., 1997) for supporting milk protein synthesis. The difference in milk protein content from cows fed the 4FM:1FTM compared with 1FM:4FTM might also be attributed to AA composition.

In their review, Santos et al. (1998) indicated that FM was a good source of essential AA, while, FTM is a poorer source of essential AA by comparison and has an inferior ratio of Lys to Met.

There was a linear increase ($P < 0.001$) in urinary N output with increasing level of dietary N that agrees with the data of Castillo et al. (2001) and Wright et al. (1998). Nitrogen balance in the whole animal was greater for cows consuming the 1FM:4FTM containing diets, which reflects the higher N intake at all levels of supplementation. There was a linear increase in N balance ($P = 0.001$) when increasing amounts of the animal-based protein supplements were offered, which agrees with the previous data of Wright et al. (1998). The linear increase ($P = 0.001$) in DM digestibility was probably related to the replacement of the basal diet with RUP from animal sources (O'Mara et al., 1998). The quadratic response for apparent N digestibility and the interaction ($P = 0.02$) between supplement composition and level of feeding probably reflects the conclusion of England et al. (1997), from their in vitro work, that post-ruminal N digestibility of hydrolyzed FTM could limit its value as an RUP source.

CONCLUSIONS

Results from the present experiment indicated that increasing levels of supplemental FM and FTM combinations increased milk DHA content in a quadratic manner. There was no effect on EPA concentrations with increased level of supplementation, although there was higher EPA concentration in milk when cows were fed the supplement that contained more FM than FTM. Apparent transfer efficiency from diet to milk declined with increasing amounts of DHA in the diet.

Jensen (2002), in his thorough review on bovine milk lipids, implied that it is probably easier and less expensive to modify consumers' diets through education than it is to alter milk composition. While we agree with that position, we would suggest that manipulation of milk FA composition for a conditionally essential nutrient such as DHA, and other FA identified for their human health benefits, might actually be the more successful path to take. This could be particularly important, for example, in the case of young growing children who need DHA for brain development yet consume very little or no fish, while having whole milk as their major source of dietary fat intake (Thompson and Dennison, 1994). The inclusion of DHA and other beneficial FA into widely consumed milk and dairy products would potentially benefit many consumers who do not, or will not, regularly consume fish or other sources of very long chain FA in their diet.

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