Use of algae or algal oil rich in n-3 fatty acids as a feed supplement for dairy cattle

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ABSTRACT

Fish oil is used as a ration additive to provide n-3 fatty acids to dairy cows. Fish do not synthesize n-3 fatty acids; they must consume microscopic algae or other algae-consuming fish. New technology allows for the production of algal biomass for use as a ration supplement for dairy cattle. Lipid encapsulation of the algal biomass protects n-3 fatty acids from biohydrogenation in the rumen and allows them to be available for absorption and utilization in the small intestine. Our objective was to examine the use of algal products as a source for n-3 fatty acids in milk. Four mid-lactation Holsteins were assigned to a 4 × 4 Latin square design. Their rations were supplemented with 1× or 0.5× rumen-protected (RP) algal biomass supplement, 1× RP algal oil supplement, or no supplement for 7 d. Supplements were lipid encapsulated (Balchem Corp., New Hampton, NY). The 1× supplements provided 29 g/d of docosahexaenoic acid (DHA), and 0.5× provided half of this amount. Treatments were analyzed by orthogonal contrasts. Supplementing dairy rations with rumen-protected algal products did not affect feed intake, milk yield, or milk component yield. Short- and medium-chain fatty acid yields in milk were not influenced by supplements. Both 0.5× and 1× RP algae supplements increased daily milk fat yield of DHA (0.5 and 0.6 ± 0.10 g/d, respectively) compared with 1× RP oil (0.3 ± 0.10 g/d), but all supplements resulted in milk fat yields greater than that of the control (0.1 ± 0.10 g/d). Yield of *trans*-18:1 fatty acids in milk fat was also increased by supplementation. *Trans*-11 18:1 yield (13, 20, 27, and 15 ± 3.0 g/d for control, 0.5× RP algae, 1× RP algae, and 1× RP oil, respectively) was greater for supplements than for control. Concentration of DHA in the plasma lipid fraction on d 7 showed that the DHA concentration was greatest in plasma phospholipid. Rumen-protected algal biomass provided better DHA yield than algal oil. Feeding lipid-encap-

sulated algae supplements may increase n-3 content in milk fat without adversely affecting milk fat yield; however, preferential esterification of DHA into plasma phospholipid may limit its incorporation into milk fat.

Key words: micro-algae, n-3 fatty acid, rumen protection

INTRODUCTION

Supplements containing n-3 FA are purported to improve reproductive efficiency of dairy cattle by inhibiting prostaglandin F_{2α} synthesis through competition with arachadonic acid as a precursor for prostaglandin synthesis (Staples et al., 1998; Mattos et al., 2000, 2004). In some studies, feeding n-3 FA during lactation has been found to reduce prostaglandin secretion and improve fertility and embryo survival (Santos et al., 2008). Additionally, the health benefits associated with consuming n-3 FA encourage development of functional foods for humans. Therefore, perceptions of milk fat composition and quality may be improved by dietary supplements that increase the milk fat content of n-3 FA (Lock and Bauman, 2004).

Fish oil is the most common ration additive used to provide very long chain n-3 FA to dairy cows. Marine fish do not synthesize n-3 FA; they consume microscopic algae or other algae-consuming fish to obtain n-3 FA. New technology allows for heterotrophic production of algal biomass in bulk fermenters that do not require the lighting and electricity previously needed for phototrophic algae growth (Harel et al., 2002). Algal biomass produced in these facilities provides a consistent, high quality source of docosahexaenoic acid (DHA; C22:6 n-3) in triglyceride (TG) oils that could be used as a ration supplement for dairy cattle.

Polyunsaturated FA undergo extensive hydrolysis and biohydrogenation in the rumen, if included directly, which may adversely affect intake and milk fat yield (Palmquist et al., 2005). Increased DHA content of fish oil causes greater rumen accumulation of vaccenic acid due to partial hydrogenation of linoleic acid (AbuGhazaleh and Jenkins, 2004; Klein and Jenkins, 2011). Importantly in their review of the effects of long-chain

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FA on reproduction, Santos et al. (2008) described the need to develop methods to improve the delivery of specific FA for absorption. Lipid encapsulation is a method that allows PUFA to remain protected in the rumen, avoid biohydrogenation, and be available for absorption and utilization (Perfield et al., 2004). Our objective was to examine use of prototype lipid-encapsulated rumen-protected (**RP**) algal biomass or algal oil to enrich milk fat with n-3 FA in lactating dairy cattle.

MATERIALS AND METHODS

Animals and Experimental Design

The Virginia Tech Institutional Animal Care and Use Committee approved all procedures involving animals. Four lactating Holstein cows (mean $\pm$ SD: 193 $\pm$ 20 DIM) were randomly assigned to treatment in a 4 $\times$ 4 Latin square design. Cows were housed in tie stalls and fed individually at the Virginia Tech Dairy Center during each treatment period. Cows were milked in their stalls at 0600 and 1800 h and were allowed exercise for 2 h each day. Cows were acclimated to tie stalls for 24 h before supplementation and treatments were supplemented for 7 d. Between each period, cows were returned to freestall housing with the main herd for a washout period of 9 to 14 d. Cows were fed a TMR formulated according to NRC (2001) recommendations to meet or exceed nutrient requirements (Table 1). Fresh feed was mixed daily and cows were fed over half the ration at 0930 h and the remaining portion at 1900 h.

Algal biomass (Gold Value Fat) and algal oil (Martek DHA-S) were obtained from Martek Biosciences Corporation (Columbia, MD). Supplements were lipid encapsulated by Balchem Corporation (New Hampton, NY) to provide protection against FA biohydrogenation in the rumen. At each feeding, rations were top dressed with 1 $\times$ or 0.5 $\times$ RP algal biomass supplement (**RP-Bio**), 1 $\times$ RP algal oil supplement (**RP-Oil**), or no supplement (control). Top-dressed supplements were fully consumed when the fresh feed was offered. The 1 $\times$ supplements provided 29 g/d of DHA and 0.5 $\times$ provided half of this amount (Table 2). The total amount of supplemented fat was 112 g/d for 0.5 $\times$ RP-Bio, 224 g/d for 1 $\times$ RP-Bio, and 145 g/d for 1 $\times$ RP-Oil. The total amount of supplement fed was 150, 300, and 194 g/d for 0.5 $\times$ RP-Bio, 1 $\times$ RP-Bio, and 1 $\times$ RP-Oil, respectively.

Data Collection, Sampling Procedures, and Analysis

Samples of TMR were collected on alternate days during each period and stored at -20°C until chemical analysis (Table 1). Samples were composited by period

Table 1. Composition of the TMR

Item	Amount
Ingredient, % DM ¹	
Corn silage	22.6
Concentrate ²	18.1
Ground corn	3.5
Alfalfa silage	2.9
Alfalfa hay	2.3
Barley straw	0.5
Chemical composition	
CP, % of DM	14.6
NDF, % of DM	34.4
ADF, % of DM	20.7
Crude fat, % of DM	4.4
Ca, % of DM	0.69
P, % of DM	0.37
NE _L , Mcal/kg of DM	1.71

¹Diet DM averaged 46.9%.

²Concentrate contained 34.2% soybean meal (48%), 26.4% dehydrated citrus pulp, 6.8% distillers grains, 6.5% wheat middlings, 6.6% Pro-Lak (H.J. Baker & Bro. Inc., Westport, CT), 2.9% animal fat, 2.1% Megalac Plus (Church & Dwight Co. Inc., Princeton, NJ), 3.4% dehydrated molasses, 1.7% urea (45%), 3.9% limestone, 2.15% sodium bicarbonate, 0.71% magnesium oxide, 1.06% Dyna-Mate (Mosaic USA LLC, Plymouth, MN), 1.08% white salt, 0.20% Availa-4 (Zinpro Corp., Eden Prairie, MN), 0.13% selenium (0.06%), 0.07% vitamin E-6000, 0.02% Rumensin 90 (Eli Lilly and Company, Indianapolis, IN), and 0.17% vitamin ADE.

on an equal weight basis and submitted to Cumberland Valley Analytical Service Inc. (Hagerstown, MD) for analysis. Intake was recorded daily.

Milk yield was recorded at each milking and samples were collected for 14 consecutive milkings (starting after 12 h of supplementation) on d 1 through 7 of the treatment period. Milk samples were submitted for analysis of milk fat and true protein contents (DHIA, Blacksburg, VA; Foss 4000 Combi North America, Eden Prairie, MN). A second sample was frozen at -20°C until analysis of fatty acid methyl esters (**FAME**).

Blood was sampled from a coccygeal vessel 1 h before the morning feeding on d 7. Blood was drawn into evacuated tubes (Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin and immediately placed on ice. Plasma was collected by centrifugation (1,300 $\times g$ for 10 min) and stored at -20°C in polypropylene tubes until analysis.

Milk lipids were extracted and methylated according to the method of Kelsey et al. (2003). Lipids were extracted from 3 mL of plasma using the method of Corl et al. (2002) adapted for bovine plasma as described by Perfield et al. (2006). Internal standards (Nu-Chek Prep Inc., Elysian, MN) unique to each lipid fraction were added to the plasma lipid extract for method quality control and to determine recoveries. Plasma was fractionated using aminopropyl columns (Bond Elut LRC-NH₂, Varian Inc., Palo Alto, CA), and FAME were prepared from plasma lipid fractions using 1%

Table 2. Fatty acid composition of lipid supplements

Fatty acid, %	Treatment ¹			
	RP-Bio	RP-Oil	Biomass	Oil
14:0	5.1	4.2	9.4	8.4
15:0	0.2	0.2	0.3	0.3
16:0	22.5	22.5	22.7	23.5
16:1	0.0	0.1	0.4	0.1
17:0	0.1	0.1	0.0	0.1
18:0	27.0	29.9	0.6	0.5
18:1 <i>trans</i> -6–8	1.0	1.0	0.0	0.0
18:1 <i>trans</i> -9	0.9	1.0	0.0	0.0
18:1 <i>trans</i> -10	1.0	1.0	0.0	0.0
18:1 <i>trans</i> -11	0.8	0.9	0.0	0.0
18:1 <i>trans</i> -12	0.9	0.9	0.0	0.0
18:1 <i>cis</i> -9	3.5	1.5	4.2	0.2
18:1 <i>cis</i> -11	0.4	0.4	0.2	0.1
18:2 <i>cis</i> -9, <i>cis</i> -12	0.4	0.2	0.5	0.0
20:0	0.3	0.3	0.1	0.1
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.1	0.0	0.1	0.0
20:4	0.2	0.2	1.2	1.1
20:5	0.6	0.6	1.0	1.4
22:5	7.2	7.4	13.2	15.4
22:6	19.3	18.9	35.3	39.5
Others	8.4	8.6	10.8	9.2

¹Algal biomass (0.5× RP-Bio or 1× RP-Bio; Gold Value Fat, Martek Biosciences Corp., Columbia, MD), or algal oil (1× RP-Oil; Martek DHA-S, Martek Biosciences Corp.). Supplements were lipid encapsulated by Balchem Corp. (New Hampton, NY) to provide protection against FA biohydrogenation in the rumen.

methanolic sulfuric acid, as described by Perfield et al. (2006).

The FAME were analyzed by gas chromatography (Agilent 6890N GC, Agilent Technologies, Palo Alto, CA) using a CP-Sil 88 capillary column (100 m × 0.25 mm i.d. with 0.2 µm thickness; Varian Inc.). The oven temperature, initially 80°C, was increased at 2°C/min to 190°C and maintained for 13 min, followed by an increase of 2°C/min to 210°C and held for 14 min. Inlet and detector temperatures were 250°C, and the split ratio was 100:1. The hydrogen carrier gas flow rate was 1 mL/min. Hydrogen flow to the detector was 25 mL/min, airflow was 400 mL/min, and the flow of nitrogen make-up gas was 40 mL/min. Fatty acids were identified using pure standards (Nu-Chek Prep Inc.). A butter reference standard (BCR 164; Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was analyzed at regular intervals to determine recoveries and correction factors for individual fatty acyl composition in milk fat. Milk fatty acyl yields were calculated according to Stamey et al. (2010).

Statistical Analysis

Data were analyzed as a 4 × 4 Latin square using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Fixed effects included treatment and period, and the random effect was cow. Plasma data also included

the fixed effect of lipid fraction. The REPEATED statement was used for response variables with repeated measurements to account for effect of day and included fixed effects of day, period by day, and treatment by day. The error term for the REPEATED statement was cow. The covariance structure that yielded the lowest Akaike's information criterion was used (Littell et al., 1998). Orthogonal contrasts were used to separate treatment means: (1) control minus RP supplements, (2) both RP-Bio supplements minus RP-Oil, and (3) 0.5× RP-Bio minus 1× RP-Bio. Significance was declared at $P < 0.05$.

RESULTS

As shown in Table 3, DMI and milk yield were not affected by lipid supplementation ($P > 0.05$). Milk fat content and yield and milk true protein content and yield did not differ among treatments.

Feeding RP algal biomass or oil did not alter de novo FA synthesis as indicated by short- and medium-chain fatty acyl yields (Table 4). Fatty acyl yields of *trans*-C18:1 isomers were affected by lipid supplements. Both *trans*-4 and *trans*-5 were increased ($P < 0.01$) with supplementation compared with control. The RP-Bio supplements resulted in greater yields of *trans*-4 and *trans*-5 than did RP-Oil. For *trans*-5 yield, 1× RP-Bio (0.7 g/d) was greater ($P < 0.05$) than 0.5× RP-Bio (0.5 g/d). Yields of *trans*-6–8 and *trans*-9 were also

Table 3. Performance of lactating Holstein cows receiving no supplement (Con), rumen-protected (RP) algal biomass (0.5× RP-Bio or 1× RP-Bio; Gold Value Fat, Martek Biosciences Corp., Columbia, MD), or RP algal oil (1× RP-Oil; Martek DHA-S, Martek Biosciences Corp.) for 7 d¹

Variable	Treatment				SEM	P-value
	Con	0.5× RP-Bio	1× RP-Bio	1× RP-Oil		
DMI, kg/d	20.1	18.4	20.8	19.4	0.65	0.12
Milk yield, kg/d	29.1	27.2	30.9	29.8	0.85	0.07
Milk fat						
kg/d	1.02	1.07	1.01	0.98	0.067	0.83
%	3.5	4.0	3.3	3.3	0.20	0.11
Milk true protein						
kg/d	0.88	0.84	0.95	0.92	0.030	0.11
%	3.0	3.1	3.1	3.1	0.05	0.60

¹Data presented are least squares means and standard errors of the mean.

greater for cows receiving a lipid supplement compared with control ($P < 0.05$). Although the yield of *trans*-11 C18:1 was greater ($P < 0.05$) for cows fed lipid supplements and greater for cows receiving 1× RP Bio (27 g/d) than for those receiving 0.5× RP Bio (20 g/d), it is notable that milk yield of *trans*-10 C18:1 was not changed by lipid supplementation. *Cis*-9,*trans*-11 conjugated linoleic acid (CLA) in milk was also increased for cows receiving a lipid supplement compared with control ($P < 0.05$).

Milk yield of DHA increased ($P < 0.001$) for cows receiving supplements compared with control (Table 4). Fatty acyl yield of DHA in milk was also greater for cows receiving RP Bio compared with RP Oil ($P < 0.001$). The temporal pattern of DHA yield in milk (Figure 1) shows that incorporation of DHA increased daily ($P < 0.05$), but we did not observe a treatment × time interaction ($P = 0.45$). Transfer efficiencies for DHA were 3.4, 2.0, and $1.0 \pm 0.22\%$ for 0.5× RP-Bio, 1× RP-Bio, and 1× RP-Oil, respectively. Protection of algal biomass rather than extracted algal oil allowed greater transfer of DHA into milk ($P < 0.0001$) and 0.5× RP-Bio allowed greater transfer than 1× RP-Bio ($P = 0.0007$).

Concentration of DHA in plasma lipid fractions on d 7 showed that DHA was greatest in plasma phospholipid ($P < 0.0001$; Figure 2). Carryover of DHA was noted in plasma phospholipid of control cows despite the washout period, thus the interaction of treatment and plasma lipid fraction was not significant ($P = 0.2$). Docosahexaenoic acid was not detected in plasma cholesterol esters (Supplemental Table S1; supplemental tables available online: <http://www.journalofdairyscience.org/>). Changes in FA within plasma cholesterol ester (Supplemental Table S1), FFA (Supplemental Table S2), and phospholipid (Supplemental Table S3) concentration were not observed with lipid supplementation. In plasma TG (Supplemental Table S4),

trans-6–8 C18:1 was increased with lipid supplementation compared with control ($P < 0.02$).

DISCUSSION

Supplements of RP algal biomass and RP algal oil did not detrimentally influence DMI, milk yield, or milk fat yield although decreases in intake have been observed when unprotected marine algae supplements are fed (Franklin et al., 1999; Offer et al., 2001a; Boeckaert et al., 2008). Unsaturated TG in unprotected supplements are predominantly hydrolyzed in the rumen, and the FFA are biohydrogenated to saturated FFA. The difference in lipids may influence responses in DMI. Other studies indicate that reductions in DMI may be more pronounced with unsaturated FFA rather than unsaturated TG reaching the duodenum (Litherland et al., 2005; Castañeda-Gutiérrez et al., 2007). Although Franklin et al. (1999) offered a xylose-protected marine algae supplement, their results suggest that this form of protection was not adequate to prevent rumen outflow of unsaturated FFA, which may have caused the decreased DMI observed in that study. Lipid encapsulation of PUFA provides rumen protection and might allow rumen outflow of unsaturated TG rather than the unsaturated FFA from the unprotected supplements used in previous studies, which might explain the lack of effect on DMI with these supplements (Perfield et al., 2004).

Feeding unprotected PUFA can alter rumen biohydrogenation pathways, resulting in production of *trans*-10,*cis*-12 C18:2, a fatty acid known to reduce milk fat synthesis in the mammary gland (Bauman and Griinari, 2003), and reductions in milk fat yield have been observed when diets were supplemented with unprotected marine algae (Franklin et al., 1999; Offer et al., 2001a; Boeckaert et al., 2008). Also associated with reductions in milk fat yield in these studies were decreases in de

Table 4. Milk fatty acyl yields of lactating Holstein cows receiving no supplement (Con), rumen-protected (RP) algal biomass (0.5× RP-Bio or 1× RP-Bio; Gold Value Fat, Martek Biosciences Corp., Columbia, MD), or RP algal oil (1× RP-Oil; Martek DHA-S, Martek Biosciences Corp.) for 7 d¹

Fatty acyl, g/d	Treatment				SEM	P-value	Contrast ²		
	Control	0.5× RP-Bio	1× RP-Bio	1× RP-Oil			Con – RP	Bio – Oil	0.5× – 1×
4:0–12:0	116.0	111.3	102.3	91.4	11.58	0.49			
14:0	90.1	85.5	84.5	75.9	7.02	0.57			
14:1	6.5	6.4	6.7	6.8	1.07	0.99			
15:0	7.2	6.0	7.1	5.9	0.93	0.68			
16:0	262.1	253.9	240.9	228.3	17.21	0.55			
16:1	11.6	12.6	12.0	12.8	1.71	0.95			
17:0	8.0	8.0	7.8	7.3	0.48	0.71			
18:0	102.6	98.0	76.6	86.2	10.98	0.38			
18:1 <i>trans</i> -4	0.3	0.5	0.6	0.4	0.05	0.01	**	*	†
18:1 <i>trans</i> -5	0.3	0.5	0.7	0.4	0.06	<0.01	**	*	*
18:1 <i>trans</i> -6–8	4.6	7.0	10.9	7.2	1.29	0.05	*		†
18:1 <i>trans</i> -9	3.1	4.7	7.1	4.8	0.76	0.03	*		†
18:1 <i>trans</i> -10	10.2	14.4	33.3	19.6	7.29	0.20			
18:1 <i>trans</i> -11	13.0	20.0	27.0	14.6	2.99	0.02	*	*	
18:1 <i>trans</i> -12	5.6	8.1	11.5	7.0	0.96	0.01	*	*	*
18:1 <i>cis</i> -9	206.9	199.6	169.1	191.1	13.59	0.29			
18:1 <i>cis</i> -11	2.8	3.8	4.6	3.1	0.40	0.05			
19:0	3.8	4.8	5.4	4.1	0.39	0.07			
18:2 <i>cis</i> -9, <i>cis</i> -12	29.9	30.4	26.6	28.0	2.61	0.73			
20:0	1.2	1.2	1.1	1.1	0.12	0.72			
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	2.4	2.4	2.1	2.3	0.19	0.72			
18:2 <i>cis</i> -9, <i>trans</i> -11	5.4	7.5	10.2	6.4	0.98	0.03	*	†	†
18:2 <i>trans</i> -10, <i>cis</i> -12	0.1	0.1	0.4	0.2	0.12	0.36			
20:2	0.3	0.3	0.3	0.2	0.04	0.71			
20:4	1.3	1.4	1.0	1.1	0.16	0.29			
20:5	0.1	0.2	0.3	0.4	0.13	0.37			
22:6	0.1	0.5	0.6	0.3	0.05	<0.001	***	**	
Others	20.6	21.6	23.5	20.0	1.01	0.07			
Glycerol	151.5	150.1	143.3	134.1	9.59	0.58			
Total	1,067.3	1,060.8	1,017.4	960.9	63.09	0.63			

¹Data presented are least squares means and standard errors of the mean.²Orthogonal contrasts: Control minus RP supplements, 0.5× and 1× RP-Bio minus RP-Oil, and 0.5× RP-Bio minus 1× RP-Bio, respectively.† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

novo synthesized FA and increased production of *trans*-C18:1 isomers and CLA caused by alterations in rumen biohydrogenation pathways. In the current experiment, lipid encapsulation of both RP-Bio and RP-Oil supplements did not cause reductions in milk fat content and yield while providing a similar amount of dietary DHA. Furthermore, protection of algal biomass rather than extracted algal oil allowed greater transfer of DHA into milk, and 0.5× RP-Bio allowed greater transfer than 1× RP-Bio.

The specific FA present in the unprotected PUFA supplements are the factors that influence rumen biohydrogenation of dietary FA. Klein and Jenkins (2011) demonstrated that DHA itself is not converted to *trans*-11 C18:1, but it modifies biohydrogenation of other long-chain PUFA present in the rumen, such as linoleic and linolenic acids. Whereas DHA promotes accumulation of vaccenic acid during linoleic acid biohydrogenation, other fatty acids in unprotected PUFA supplements interact with dietary linoleic acid to in-

crease *trans*-10,*cis*-12 CLA production (Whitlock et al., 2002; AbuGhazaleh and Jenkins, 2004; Whitlock et al., 2006). In the current study, milk fat yield of *trans*-C18:1 isomers was increased with supplementation of RP-Bio and RP-Oil. Of the *trans*-C18:1 isomers, production of *trans*-11 C18:1, and subsequently, *cis*-9,*trans*-11 CLA, was increased rather than *trans*-10 C18:1, which is correlated with milk fat depression (Lock et al., 2007). Feeding strategies incorporating supplemental lipids must provide protection to prevent interactions among other long-chain PUFA present in the diet to prevent milk fat depression. In the present study, the increase in milk *trans*-C18:1 FA indicates that microalgae supplements influenced rumen biohydrogenation intermediates. The lipid coating also contained *trans*-C18:1 FA (Table 2) and might have contributed minimally to changes in *trans*-C18:1 FA. Milk fat depression was not observed because linoleic acid biohydrogenation did not shift toward *trans*-10,*cis*-12 CLA production, as evidenced by no change in the isomer's content in

Figure 1. Temporal pattern of docosahexaenoic acid (DHA) yield in milk fat from lactating Holstein cows receiving no supplement (◇), 0.5× rumen-protected (RP) algal biomass (□), 1× RP algal biomass (■), or 1× RP-algal oil (●) for 7 d. Data presented are least squares means and standard errors. A significant main effect of time was detected ($P < 0.05$).

milk. The increased variation in yield of *trans*-10 C18:1 in milk suggests that a longer feeding period or altered ration formulation might initiate altered rumen biohydrogenation and increased *trans*-10,*cis*-12 CLA outflow.

Other studies using the same source of marine algae observed similar transfer efficiencies with unprotected supplements (Offer et al., 2001a; Boeckaert et al., 2008; Abughazaleh et al., 2009). Toral et al. (2010) observed DHA transfer efficiencies ranging from 1.6 to 2.5% in sheep supplemented with sunflower oil and incremental levels of marine algae. Using the same algal species (*Schizochytrium*), Franklin et al. (1999) reported DHA transfer efficiencies as high as 8.4 and 16.7% in unprotected and protected algal supplements. Greater availability of preformed FA in early lactation modify milk fat composition (Palmquist et al., 1993), and indeed, lower transfer efficiencies (<5%) were consistently observed for studies that started algal supplementation in mid to late lactation. Boeckaert et al. (2008) reported experiments in both early and late lactation with transfer efficiencies of 5.9 and 3.1%, respectively. Transfer efficiency in the present experiment may have been improved by supplementing earlier in lactation. It is also possible that lipid encapsulation decreased digestibility of the TG in the RP-Bio and RP-Oil supplements.

Mammary uptake of FA from plasma is dependent upon the action of mammary lipoprotein lipase on TG transported in chylomicrons and very low density lipoproteins (Palmquist, 1976; Moore and Christie, 1979).

Accordingly, FA incorporated into phospholipid or cholesterol esters in the small intestine or liver are not available for mammary uptake; however, increased fat feeding can overwhelm the normal esterification capacity of the small intestine, allowing increased incorporation of DHA into the TG fraction (Bauchart, 1993). In the present experiment, DHA was preferentially incorporated into plasma phospholipid, as it is normally found in this lipid fraction, preventing mammary uptake and reducing transfer. Offer et al. (2001b) support this observation, noting that supplemental long-chain PUFA, such as DHA, are not present in plasma TG.

CONCLUSIONS

Feeding lipid-encapsulated algae supplements may improve milk fat composition. Supplements did not significantly affect milk production or milk fat yield. Increased milk fat yield of *trans*-C18:1 isomers indicated that supplements were altered by rumen biohydrogenation. Preferential esterification of DHA into plasma phospholipid also limited its incorporation into milk fat. It is possible that long-chain PUFA such as DHA must be delivered as plasma TG for maximal transfer to milk fat. Long-term feeding of lipid-encapsulated algae supplements may allow for increased DHA in plasma TG by saturating plasma phospholipids.

Figure 2. Docosahexaenoic acid (DHA) concentration in plasma from lactating Holstein cows after receiving no supplement (Con), rumen-protected (RP) algal biomass (0.5× RP-Bio or 1× RP-Bio), or RP algal oil (1× RP-Oil) for 7 d by lipid fraction: FFA, phospholipid (PL), and triglyceride (TG); DHA was not detected in cholesterol ester. Data presented are least squares means and standard errors. A significant main effect of lipid fraction was detected ($P < 0.0001$).

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