Effects of lipid supplementation on the yield and composition of milk from cows with different β -lactoglobulin phenotypes

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ABSTRACT

Responses to lipid supplementation differ between dairy breeds and genetic lines suggesting nutrition by genotype interactions. β -Lactoglobulin phenotype is associated with changes in yield and composition of milk. The response of cows with different β -lactoglobulin phenotypes to lipid supplementation has not been examined. Furthermore, we examined whether lipid supplementation alters milk protein composition. By using a randomized block design, we fed Holstein cows for 3 wk either a control diet containing 2.8% crude fat ($n = 19$) or an experimental diet that was supplemented with 4.2% tallow ($n = 20$). Before randomization, all cows were fed the supplemental tallow diet for at least 2 wk. Dry matter intake, body weight, milk yield, and milk composition were measured in the last week before and during the experimental period. Feeding supplemental tallow increased dry matter intake and yields of milk and milk components, including casein content, without decreasing milk component content or altering milk protein composition. On the low-fat control diet, cows with the β -lactoglobulin allele B had a greater milk and milk component yield than cows with the A allele, whereas no differences by β -lactoglobulin phenotype were observed in cows on the tallow supplement diet. Our results suggest that cows that differ in β -lactoglobulin phenotype respond differently to a low-fat diet and that feeding cows 4.2% of additional tallow increases milk yield without affecting milk component content and milk protein composition.

Key words: β -lactoglobulin phenotype, dairy cow, fat feeding, protein composition

INTRODUCTION

Dietary lipid supplementation has been extensively researched and used as a management tool to increase milk and milk component yields (Chilliard et al., 2001). Lipid type and form influences responses to dietary lipid supplementation. Generally, supplementation with protected lipids of animal origin increased milk and milk component yields and fat content and decreased protein content (Wu and Huber, 1994; Chilliard et al., 2001). The responses to increasing levels of yellow grease differed between breeds at greater supplementation levels. Yellow grease supplementation increased milk and milk component yields of Holstein cows to a larger extent than of Jersey and Brown Swiss cows (Carroll et al., 2006), which suggests that the genotype of the cow alters the response to lipid supplementation. Support for a genotype $\times$ diet interaction is provided by studies that show that the difference in milk and milk component yields between cows of greater and lesser genetic merit increases with dietary energy levels (Veerkamp et al., 1994; Beerda et al., 2007).

β -Lactoglobulin phenotype is associated with changes in yield and composition of milk. Cows with the β -LG B allele produce less β -LG than those with the A allele, which increases their casein content and, thus, the cheese yield of their milk (Ng-Kwai-Hang and Grosclaude, 2003; Wedholm et al., 2006). The effect of β -LG phenotype on milk yield is variable, which could partly be the result of a β -LG phenotype $\times$ diet interaction. The evidence for a β -LG phenotype $\times$ diet interaction is inconclusive. In a pasture system, grazing ad libitum versus restricted allowance resulted in similar milk and milk component responses in cows with β -LG phenotype AA and BB (Auldist et al., 2000), whereas concentrate supplementation increased protein and noncasein N content more in cows with β -LG phenotype BB (Mackle et al., 1999). To our knowledge, milk responses to lipid supplementation in cows with different β -LG phenotypes have not been reported.

In contrast to the effects of lipid supplements on general milk composition, knowledge about the effects on individual milk proteins is limited (DePeters and Cant,

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Table 1. Dietary ingredient and nutrient composition¹

Item	Diet	
	Control	Fat
Ingredient (% of DM)		
Corn silage	25	25
Alfalfa hay	25	25
Corn grain, ground	5.88	2.16
Tallow	0	4.21
Soybean meal (56% CP)	12.67	12.53
Soybean hulls	16.47	16.28
Wheat middlings	12.63	12.48
Dicalcium phosphate	0.56	0.55
Calcium carbonate	0.73	0.72
Magnesium oxide	0.37	0.37
Sodium chloride	0.11	0.11
Vitamin mineral premix ²	0.60	0.59
Nutrient		
NE _L (Mcal/kg)	1.57	1.68
CP	21.1	20.6
RUP	6.3	6.1
MP (g/kg)	66.0	66.1
Ether extract	2.8	6.8
NDF	35.0	34.5
ADF	22.3	22.0
NFC ³	37.1	34.1
Ca	0.94	0.93
P	0.46	0.45

¹Nutrients were calculated using the NRC (2001) model and observed forage nutrient values.

²The vitamin mineral premix contained cobalt (135 mg/kg), copper (12,389 mg/kg), iodine (743 mg/kg), iron (61,946 mg/kg), manganese (49,556 mg/kg), selenium (334 mg/kg), zinc (49,556 mg/kg), vitamin A (394,685 IU), vitamin D (264,819 IU), and vitamin E (5,460 IU) and was from Land O'Lakes/Purina Mills (Gray Summit, MO).

³NFC = 100 - (% NDF + % CP + % ether extract + % ash).

1992). Determining the effects of lipid supplementation on individual milk proteins is important because a decrease in the proportion of CN proteins is associated with decreased cheese yield (Wedholm et al., 2006). Several studies evaluated the effect of lipid supplementation on the N composition of milk. Feeding supplemental whole cotton seed, soybeans, sunflower oil, or safflower oil to cows did not alter milk N composition (DePeters et al., 1985; Kim et al., 1991; Stegeman et al., 1992) except when provided in combination with niacin (Horner et al., 1986). Feeding formaldehyde-treated or not-treated tallow or yellow grease supplement at 6% or more of the diet decreased the casein N fraction and increased the whey protein N and NPN fractions (Dunkley et al., 1977; DePeters et al., 1987, 1989). The effect of supplemental lipids on individual milk proteins has been examined only in one study, which did not find an effect in bST-treated dairy cows when sunflower or safflower seeds were fed at 20% of the diet (Stegeman et al., 1992).

The objectives of this study were a) to evaluate the effect of feeding supplemental tallow (4.2% of DM) on

yield and composition of milk in mid to late lactation cows with different β -LG phenotypes, and b) to evaluate the effect of supplemental lipids on individual milk proteins.

MATERIALS AND METHODS

Experimental Design and Sample Collection

The study design was a randomized block design (3 blocks) with a 2-wk covariate period followed by a 3-wk experimental period. The study was conducted at the Land O'Lakes/Purina Feed Research Center (Gray Summit, MO). Cows were managed and treated in accordance with institutional Animal Care and Use guidelines. Mid to late lactation dairy cows ($n = 39$) averaging 238 DIM and in their first to fifth lactation were used in the study. Cows were randomly assigned to a control low fat (control; $n = 19$ cows) or a high fat (fat; $n = 20$ cows) diet. Cows were not blocked by β -LG and κ -CN phenotypes as the β -LG and κ -CN phenotypes were not determined before the study; therefore, β -LG and κ -CN phenotypes were unevenly distributed across diet. All but 6 cows had κ -CN AA (κ -CN AB: 5 cows, κ -CN BB: 1 cow), and only 1 out of the 6 cows were randomized to the control low-fat diet; therefore, the effect of lipid supplementation on the yield and composition of milk from cows with different κ -CN phenotypes could not be tested. All cows were fed the high fat diet for at least a 2-wk covariate period followed by the respective dietary treatments for 3 wk during the treatment period. Diets were formulated to meet nutrient requirements of dairy cows according to NRC (2001). The ingredient and chemical compositions of the diets and the forages are provided in Tables 1 and 2. Intake, milk yield, and milk composition data were collected during the final week of each period and averaged for the week before being subjected to statistical analyses.

Cows were housed in tie stalls and fed a fresh TMR twice daily for ad libitum intake with continuous access to feed except during milkings. Refused feed was removed before the evening feeding. Amounts fed and refused were recorded daily. Cows were milked twice

Table 2. Observed forage nutrients (% of DM)

Item	Alfalfa hay	Corn silage
DM	82.8	35.7
Protein	18.0	7.5
NDF	59.2	51.1
ADF	42.4	30.2
Lignin	10.4	5.1
Starch	—	22.5
Ash	8.8	4.6

daily (a.m. and p.m.) 12 h apart. Milk production and BW were measured daily at the a.m. and p.m. milking. Milk samples were taken from 5 consecutive p.m. milking periods during collection weeks for analysis of milk composition.

Chemical Analyses

Milk samples were analyzed for total solids, fat, lactose, and protein by mid-infrared spectrophotometry (Milk-O-Scan 134AB, Foss Food Technology Corp., Eden Prairie, MN; AOAC, 1990, method 972.16). Protein content was based on total N content and did not exclude NPN. A second aliquot of each milk sample was composited into a weekly sample and analyzed for the 6 major milk proteins (α_{s1} -CN, β -CN, κ -CN, β -LG, α_{s2} -CN, α -LA, β -LG) and κ -CN and β -LG phenotypes (AA, AB, BB) by reversed-phase HPLC (Bobe et al., 1998).

Statistical Analyses

Statistical analysis was done by using the general least-squares means procedure (PROC GLM; SAS Institute, 2002). The fixed effects in the model were block ($n = 3$), diet (control, fat), β -LG phenotype (AA, AB, BB), diet $\times$ β -LG phenotype interaction, and as covariates the linear effects of DIM at the beginning of the study and the value of the y-variable in the last week before the experimental period. Statistical differences were considered significant at $P \leq 0.05$ and were considered to trend toward significance at $P \leq 0.10$. Means and their SEM are presented as least-squares means.

RESULTS AND DISCUSSION

Feeding supplemental tallow at 4.2% on a DM basis resulted in increased DMI, and yields of milk and milk components without decreasing fat, protein, and lactose concentrations in milk (Table 3). Our results are consistent with those of Chilliard et al. (2001) wherein feeding 1.48 kg/d of additional tallow (80% in a ruminally inert form) resulted in increased milk yield (29.9 vs. 27.6 kg/d) and numerical improvements in milk fat content (3.38 vs. 3.28%). We had anticipated that protein content would decrease in response to tallow supplementation because the increased milk yield would dilute the constant amount of protein synthesized (Wu and Huber, 1994). However, milk protein yield was stimulated by fat feeding with no change in protein content (3.38 vs. 3.34%). Potential reasons why we did not observe the decrease in protein content with tallow supplementation are that, in contrast to most other studies (Dunkley et al., 1977; DePeters et al.,

1987, 1989), most cows were in late lactation (average DIM: 238) and were adapted to the tallow supplement. Our results suggest that feeding supplemental tallow at 4.2% on a DM basis increases DMI, and yields of milk and milk components in late lactation cows without detrimental effects on milk component content. However, it cannot be excluded that the supplementation period (3 wk) was too short to observe the decrease in milk protein content because decreases in milk protein content associated with supplemental fat feeding can take 5 wk or longer to become apparent (Casper et al., 1990; Kim et al., 1991).

On the control low-fat diet, cows with the β -LG B allele produced more milk and milk components than did cows with the β -LG A allele. On the supplemental tallow diet, no significant differences in milk and milk component yields of cows with different β -LG phenotypes were observed (Table 3). Our results suggest a β -LG phenotype by cow diet interaction, which was significant for protein yield ($P = 0.01$), and tended to be significant for lactose content ($P = 0.07$), and lactose ($P = 0.10$) and total solids ($P = 0.06$) yield (Table 3). A β -LG phenotype $\times$ diet interaction could partly explain the inconsistent results for the effect of a β -LG phenotype on milk and milk component yield in the literature (Ng-Kwai-Hang and Grosclaude, 2003).

We did not anticipate that cows with different β -LG phenotypes would differ in milk and milk component yields on the control diet because such differences were not observed when evaluating responses to various pasture feeding systems for cows with β -LG phenotype AA and BB (Mackle et al., 1999; Auldist et al., 2000). Furthermore, cows with β -LG phenotypes BB and AA responded similarly to atropine infusions, which decreased DMI and protein content in milk (Prosser and McLaren, 1997). One potential explanation why cows with different β -LG phenotypes responded differently to dietary fat is that the β -LG structural differences associated with the different phenotypes (Qin et al., 1999) affect lipid metabolism in the mammary gland. β -Lactoglobulin binds fatty acids (Burczynski et al., 1990; Frapin et al., 1993) and can, as a fatty-acyl carrier, increase de-novo fatty acid synthesis (Knudsen and Grunnet, 1982). It has previously been observed that the β -LG B allele was associated with greater proportions of primarily de-novo synthesized fatty acids (C14:0, C16:0, C16:1) and lesser proportions of preformed fatty acids (C18:0, C18:2) in milk fat in comparison with the A allele (Bobe et al., 2004). If this is a consequence of a lesser ability to transport and metabolize preformed fats, the ability to respond to the greater preformed fat supply associated with a high fat diet may be dampened with the β -LG B allele. Conversely, cows with the β -LG A allele may spare energy by shifting fat synthesis more

toward the use of preformed fatty acids, which would free up energy for synthesis of additional lactose and protein.

It could be that a gene close to the β -LG locus affects the utilization of the diet by the cow. We are not aware of a QTL near the β -LG locus that affect milk production traits (Khatkar et al., 2004); however, a diet $\times$ genotype interaction would make it difficult to detect such a QTL if it exists. It cannot be excluded that our results are spurious because the number of cows on each diet within each β -LG phenotype was limited. However, the observed linear responses for milk and component yields of cows with respect to the number of A alleles are not consistent with a random event (Table 3). Furthermore, the effect of β -LG phenotype on milk protein content and composition (Tables 3 and 4) is consistent with literature values (Jakob, 1994; Ng-Kwai-Hang and Grosclaude, 2003). Replications of our findings in other studies with larger number of cows within each β -LG phenotype are warranted before definite conclusions can be drawn.

Feeding supplemental lipids affected the synthesis of individual milk proteins similarly (Table 4). The only difference was that the proportion of α_{s1} -CN tended to be less in cows fed supplemental lipids (35.3 vs. 35.7% of total milk proteins; $P = 0.07$). We do not expect any negative impact of supplemental lipids on cheese yield because no changes in milk protein concentrations in response to lipid supplementation were observed (Table 4). Furthermore, the proportion of total CN (86.1 vs. 86.3% of total milk proteins) was not altered by lipid supplementation because the proportion of β -CN (27.4 vs. 27.3% of total milk proteins) and κ -CN (14.4 vs. 14.1% of total milk proteins) increased numerically in response to lipid supplementation and thereby balanced out the decrease in α_{s1} -CN (Table 4). These results are consistent with those of Stegeman et al. (1992) wherein dietary supplementation with sunflower or safflower seeds (20% of diet DM) did not affect milk protein composition in bST-treated dairy cows. However feeding tallow (formaldehyde-treated or untreated) or yellow grease at 6% or more of the diet has been observed to decrease the casein N fraction and increase the whey protein N and NPN fractions (Dunkley et al., 1977; DePeters et al., 1987, 1989), but such high levels of fat feeding can negatively affect DMI and milk protein content (Wu and Huber, 1994; Chilliard et al., 2001).

Heat stress (Bernabucci et al., 2002), atropine infusions (Auldist et al., 2003), or glucagon infusions (She et al., 1999; Bobe et al., 2009), which all decrease milk protein content and to a smaller extent DMI and milk yield, have been observed to affect milk protein composition. However, most feeding studies have reported only minor changes in milk protein composition and its CN

Table 3. Effect of supplemental fat feeding on dry matter intake, milk yield, and milk composition of cows with different β -lactoglobulin phenotypes

Item	β -LG AA		β -LG AB		β -LG BB		Type III SS $P > F$ for main comparisons			
	Control (n = 4)	Fat (n = 8)	Control (n = 9)	Fat (n = 8)	Control (n = 6)	Fat (n = 4)	SEM	Fat (F)	β -LG	F $\times$ β -LG
DMI, kg/d	20.43 ^a	22.19 ^{cd}	21.10 ^{ab}	22.36 ^{cd}	21.67 ^{bc}	22.58 ^d	0.28	***	*	NS
Weight gain, kg/d	0.121	0.240	0.164	0.480	0.296	0.100	0.325	NS	NS	NS
Milk, kg/d	25.19 ^a	29.93 ^b	28.13 ^{ab}	29.15 ^b	29.38 ^b	30.70 ^b	1.10	**	NS	NS
3.5% FCM, ¹ kg/d	21.83 ^a	26.48 ^b	24.64 ^{ab}	25.75 ^b	26.14 ^b	27.02 ^b	1.13	*	NS	NS
Fat, kg/d	0.780 ^a	0.974 ^b	0.890 ^{ab}	0.943 ^b	0.953 ^b	0.991 ^b	0.051	*	NS	NS
% milk	3.29	3.48	3.26	3.31	3.30	3.36	0.12	NS	NS	NS
Protein, kg/d	0.796 ^a	0.979 ^b	0.935 ^b	0.957 ^b	0.968 ^b	0.976 ^b	0.030	**	†	*
% milk	3.24	3.40	3.41	3.38	3.38	3.37	0.07	NS	NS	NS
Lactose, kg/d	1.213 ^a	1.475 ^b	1.372 ^{ab}	1.410 ^b	1.444 ^b	1.483 ^b	0.057	*	NS	†
% milk	4.82 ^a	4.93 ^b	4.88 ^{ab}	4.84 ^{ab}	4.92 ^{ab}	4.88 ^{ab}	0.03	NS	NS	†
Total solids, kg/d	2.800 ^a	3.420 ^b	3.199 ^b	3.304 ^b	3.370 ^b	3.434 ^b	0.123	**	NS	†
% milk	11.31	11.69	11.57	11.54	11.67	11.48	0.144	NS	NS	NS

^{a-d}Least squares means within a row with different superscripts differ ($P \leq 0.05$).

¹3.5% FCM = (0.432 $\times$ milk production [kg/d]) + (16.2 $\times$ fat yield [kg/d]).

NS > 0.10; † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Table 4. Effect of supplemental fat feeding on individual milk proteins of cows with different β-lactoglobulin phenotypes

Milk protein	β-LG AA		β-LG AB		β-LG BB		SEM	Type III SS $P > F$ for main comparisons		
	Control (n = 4)	Fat (n = 8)	Control (n = 9)	Fat (n = 8)	Control (n = 6)	Fat (n = 4)		Fat (F)	β-LG	F × β-LG
Total casein										
g/d	724.5 ^a	896.3 ^{bc}	830.1 ^{ab}	882.1 ^b	998.1 ^c	901.6 ^{bc}	38.4	NS	**	*
g/L of milk	29.15 ^{ab}	30.40 ^{ab}	29.43 ^a	30.26 ^{ab}	33.62 ^b	30.91 ^{ab}	1.47	NS	NS	NS
% of protein	84.9 ^a	85.3 ^{ab}	86.2 ^{bc}	86.2 ^{cd}	87.2 ^{cd}	87.3 ^d	0.41	NS	*	NS
α _{s1} -Casein										
g/d	305.5 ^a	372.4 ^{bc}	348.9 ^{ab}	362.7 ^{bc}	409.9 ^c	369.0 ^{bc}	16.7	NS	*	*
g/L of milk	12.18	12.49	12.22	12.39	13.68	12.67	0.60	NS	NS	NS
% of protein	35.7	35.2	35.8	35.4	35.5	35.3	0.26	†	NS	NS
β-Casein										
g/d	231.0 ^a	288.2 ^{bc}	261.4 ^{ab}	280.6 ^{bc}	315.8 ^c	282.0 ^{bc}	14.4	NS	†	*
g/L of milk	9.41	9.83	9.27	9.61	10.61	9.69	0.51	NS	NS	NS
% of protein	27.2	27.6	27.2	27.3	27.6	27.2	0.43	NS	NS	NS
κ-Casein										
g/d	114.3 ^a	143.9 ^b	135.3 ^b	146.3 ^b	166.5 ^c	152.2 ^{bc}	6.30	†	***	*
g/L of milk	4.58 ^a	4.97 ^{ab}	4.89 ^{ab}	5.12 ^{ab}	5.69 ^b	5.20 ^{ab}	0.28	NS	NS	NS
% of protein	13.5 ^a	14.0 ^{ab}	14.2 ^{ab}	14.5 ^b	14.5 ^b	14.8 ^b	0.29	NS	*	NS
β-Lactoglobulin										
g/d	86.65 ^a	114.36 ^b	96.73 ^{ab}	100.51 ^{ab}	98.58 ^{ab}	85.11 ^a	6.92	NS	NS	*
g/L of milk	3.98 ^b	4.03 ^b	4.50 ^{ab}	3.59 ^{ab}	3.39 ^{ab}	3.06 ^a	0.26	NS	NS	NS
% of protein	11.5 ^b	11.3 ^b	10.2 ^{ab}	10.1 ^{ab}	8.8 ^a	8.7 ^a	0.55	NS	†	NS
α _{s2} -Casein										
g/d	73.50 ^a	90.28 ^{bc}	84.40 ^{ab}	91.81 ^{bc}	106.09 ^d	97.73 ^{cd}	4.53	NS	***	†
g/L of milk	3.04 ^a	3.10 ^a	3.09 ^a	3.18 ^{ab}	3.56 ^b	3.32 ^{ab}	0.16	NS	†	NS
% of protein	8.70 ^a	8.70 ^a	9.01 ^{ab}	8.90 ^{ab}	9.52 ^b	9.37 ^{ab}	0.25	NS	*	NS
α-Lactalbumin										
g/d	31.84 ^a	36.84 ^{abc}	35.70 ^{ab}	38.42 ^{bc}	48.02 ^d	42.31 ^{cd}	2.22	NS	**	†
g/L of milk	1.15 ^a	1.16 ^a	1.24 ^a	1.27 ^a	1.67 ^b	1.50 ^b	0.07	NS	***	NS
% of protein	3.52 ^{ab}	3.37 ^a	3.66 ^b	3.68 ^b	4.17 ^c	4.05 ^c	0.09	†	***	NS

^{a-d}Least squares means within a row with different superscripts differ ($P \leq 0.05$).

NS > 0.10; † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

content (Coulon et al., 1998, 2001; Leiber et al., 2005; Summer et al., 2005). Based on this previous work and observations reported herein, it would seem that under most commonly used dietary regimens, large changes in milk protein composition would be unlikely.

Consistent with the literature (Jakob, 1994; Ng-Kwai-Hang and Grosclaude, 2003), β -LG phenotype affected milk protein composition (Table 3). Milk from cows with the β -LG B allele had a lesser β -LG content and a greater κ -CN, α_{s2} -CN, total CN, and α -LA content than milk from cows with the β -LG A allele (Table 4). These changes are favorable for cheese yield (Wedholm et al., 2006). The effects of β -LG phenotype on milk protein composition were larger than those of lipid supplementation (Table 4), which supports our previous observations that β -LG phenotype has a larger impact on milk protein composition than diet (Bobe et al., 1999). β -Lactoglobulin phenotype did not alter milk protein compositional responses to lipid supplementation (Table 4), which is consistent with results reported for atropine-infused cows differing in β -LG phenotype (Prosser and McLaren, 1997).

CONCLUSIONS

In conclusion, the current study demonstrates that feeding supplemental tallow at approximately 1.5 kg/d increased DMI and yields of milk and milk components, including total CN, without decreasing milk component content or altering milk protein composition. Because our study was conducted in mid- to late-lactation cows that were adapted to supplemental tallow feeding, further studies will determine whether our findings are generalizable across lactation stages and feeding regimens. The larger responses to supplemental tallow feeding in cows with the β -LG A allele suggest a β -LG phenotype $\times$ diet interaction. Such an interaction needs to be confirmed in independent studies with larger cow numbers within each β -lactoglobulin phenotype before definite conclusion can be made.

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