MILK PRODUCTION AND MILK COMPOSITION OF HIGH PRODUCING DAIRY COWS AS AFFECTED BY PROTECTED AND UNPROTECTED FAT SUPPLEMENTATION

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SUMMARY

This study was conducted at North Carolina State University, USA. Primiparous (n=9) and multiparous (n=17) Holstein cows were utilized in a completely randomized block design. Three treatments were as follows : (1) control treatment (CON) without fat supplementation ; (2) unprotected fat treatment (UF), which fat supplemented as soybean oil; (3) protected fat (PF), which fat supplemented as Megalac . Fats were supplemented to the cows diets beginning at 21 days prior to expect calving date through 60 days postpartum. Supplemental fats were fed at 2% and 3% dietary dry matter (DM) during prepartum and postpartum periods, respectively. Milk production measured daily. Milk fat, protein, lactose and solids not fat (SNF) were analyzed at day 0, 30 and 60 postpartum. The results indicated that, prepartum dry matter intake (DMI) was not significantly different across treatments. However, fat supplementation decreased postpartum DMI compared to the CON treatment. Cows fed PF beginning in the prepartum period produced more milk and higher FCM than cows fed UF and CON treatments. Feeding PF numerically increased milk fat percentage compared to the CON and UF diets, but the difference was not significant among treatments. However, PF treatment tended to decreased (P=0.10) milk protein percentage compared to CON and UF treatments. Feeding fat did not alter milk lactose and solids not fat percentages in comparison with CON diet. Feed efficiency, expressed as kg of 3.5% FCM per kg of DM intake, was significantly higher when feeding fat treatments than feeding CON. Concentration of C8:0 in milk fat tended to be higher (P=0.10) in cows fed fat compared to CON treatment. Fat supplementation had no significant effect on production of C12:0 , C14:0 , C15:0 , C18:1 and C18:2 in milk fat . However, significant differences were found in C10:0, C16:0 and C18:0 in UF and PF treatments compared to CON treatment. From the present results it can be concluded that supplementation of protected fat in the rations of high producing dairy cows during prepartum and postpartum led to positive effect on milk production and milk composition.

Keywords: *Milk production, milk composition and protected fat.*

INTRODUCTION

Extensive mobilization of body fat is a key factor in meeting energy demands in early lactation of high producing dairy cows. Fat supplementation during prepartum and postpartum periods is a strategy that has been evaluated to enhance productive performances of high producing dairy cows. Addition of fat to the diet of high producing dairy cows may improve their energy status and increase energy supply. Milk yield (Salado *et al.,* 2004; Tyagi *et al*., 2010 and Reis *et al.,* 2012) and milk fat concentration (Titi and Obeidat, 2008) have increased by addition of fat in the diet. The ruminal microorganisms transform unsaturated fatty acids in a process called biohydrogenation to saturated end products. Therefore, delivery of unsaturated fatty acids to the mammary tissue is limited even when their dietary concentration is high (Jenkins and McGuire, 2006). There is considerable interest in finding ways to protect dietary unsaturated fatty acids from biohydrogenation to enhance their absorption and delivery to the mammary gland. Nutritionists commonly recommend feeding dairy cows calcium salt of fatty acid, protected against ruminal biohydrogenation by microbes to modify milk fatty acid composition (Lounglawan *et al.,* 2008 and Purushothaman *et al.,* 2008). The aim of this study investigated the effect of fat supplementation (protected and unprotected) during prepartum and postpartum periods on milk production and its composition.

MATERIALS AND METHODS

Experimental design:

Experiment was conducted at North Carolina State University (USA). Periparturient Holstein primiparous (n = 9) and multiparous cows (n = 17) were classified into three groups by predicted calving date, parity (primiparous or multiparous), body weight and milk production of the previous year for multiparous cows. Cows were randomly assigned to three treatments at approximately 21± 5 days prior to their expected calving data.

The cows were fed the experimental dry cows diets and fed lactating cows diets from calving until 60 day after calving. Three diets were prepared and fed individually as a total mixed ration (TMR) in ad-libitum amounts once daily at 11:00 a.m. Individual dry matter intake (DMI) was measured daily. Cows were fed to cover the requirement of dry matter (DM) and total digestible nutrient (TDN) according to NRC (2001) and the rations were adjusted biweekly. Each group of cows was randomly assigned to one of the three experimental treatments as the following: 1) control treatment (CON, 8 Cows) without fat supplementation; 2) unprotected fat treatment (UF, 9 Cows) which fat supplemented as soybean oil; 3) protected fat (PF, 9 Cows) which fat supplemented as calcium salt of long chain fatty acids (CSLCFA, Megalac®). Supplemental fats were added at 2% and 3% of dietary DM during prepartum and postpartum periods, respectively. The basal diet during the prepartum period consisted of: corn silage, normal oats silage, soybean hulls, whole cottonseed and concentrate mixture. The basal diet during the postpartum period consisted of: corn silage, normal oats silage, whole cottonseed, soybean hulls, corn gluten feed and concentrate mixture. Fat was added at the top of the fat treatments separately for each individual cow diet for complete consumption of supplemental fat.

Samples collection and analysis:

Diets samples:

Representative samples of the experimental diets were collected on a weekly basis and composited on a monthly basis. Representative samples were analyzed for DM, CP, EE, NDF, ADF, NFC, Ca, P and TDN (Table, 1) according to AOAC (1990).

Table (1). Proximate analysis and TDN of experimental diet fed to Holstein cows during experimental period.

Item	Proximate analysis								TDN
	DM	CP	NDF	ADF	EE	*NFC	Ca	P	
Prepartum	31.47	14.89	53.2	34.19	3.08	22.75	0.43	0.25	64.73
Pospartum	53.70	17.12	37.67	25.71	3.15	33.33	0.67	0.49	72.46

**NFC = Non fiber carbohydrate.*
1- Fat was added separately to the UF and PF treatments and chemical composition does not reflect addition of fat.
2- CP, NDF, ADF, EE, NFC, Ca, P and TDN % of DM.

Milk samples:

All cows were milked twice daily at 07:00 and 18:00 h. Milk yield was recorded individually at each milking time. Individual milk samples from consecutive a.m. and p.m. milkings were collected on week 1 (days 0), week four (day 30), and week 8 (day 60) postpartum and composited according to milk weight at each milking time (3 mL/kg milk at each milking time). One aliquot of 10 mL composite samples were analyzed individually for fat, protein and solid not fat. Milk fat, protein, lactose and solid not fat (SNF) yields were calculated by multiplying milk yield from the respective day by fat, protein, lactose and SNF content of the milk for an individual cow. Another aliquot of the milk samples was frozen at -20°C for fatty acids (FA) determination. Weighted composite milk samples from a.m. and p.m. milkings at day 60 postpartum were analyzed for fatty acid (FA) composition using gas-liquid chromatography according to (Kramer *et al*., 1997). Fat-corrected milk (FCM; 3.5%) was calculated according to Tyrrell and Reid (1965) by using the following equation:

3.5% FCM (kg/day) = (16.22milk fat, kg/day) + (0.43milk yield, kg/day)

Statistical analysis:

Data for milk production, milk constituents and milk fatty acid composition were analyzed according to a randomized complete block design using SAS (2005). Results were presented as least square means ± standard error of the mean.

RESULTS AND DISCUSSION

Dry matter intake (DMI):

The present results (Table, 2) showed that prepartum DMI was not significantly different among the dietary treatment groups. This may be due to the low amount of fat (2% of dietary DM) offered to experimental cows during the prepartum period. Many studies have reported no changes in dry matter intake with supplemental fat (Andersen *et al*., 2008; Castañeda-Gutiérrez *et al*., 2009; Tyagi *et al*., 2010 and Shelke *et al*., 2012). Other studies have reported a decrease in dry matter intake (DMI) with supplemental fat (Moallem *et al*., 2007a and Moallem *et al*., 2010). During the postpartum period, dry matter intake was significantly higher (P < 0.0001) in cows fed the CON treatment without any fat supplementation than those cows fed the fat treatments (Table 2). A comparison between fat treatments showed that cows fed PF supplemented diet had significantly lower (P < 0.05)) dry matter intakes than cows fed UF treatment. In addition, postpartum dry matter intake expressed as % of BW showed lower values in PF and UF groups compared to CON group. In a review of several studies Moallem *et al*., (2007b); Purushothaman *et al*., (2008); Tyagi *et al*., (2010), Shelke *et al*., (2012) and Ganjkhanlou *et al*., (2009) reported that DMI was decreased with supplementation of rumen protected fat to the lactating cows diets compared to control diet. Variation in response to adding fat to the diets can be dependent on the type and amount of fat added and stage of lactation.

Table (2). Dry matter intake (DMI) and postpartum body weight (BW) of Holstein cows fed control diet (CON) or diets supplemented with unprotected fat (UF) or protected fat (PF).

Item	Treatments				Contrast, *P* values	
	CON	UF	PF	SEM	CON vs. FAT	UF vs. PF
Measurements:						
DMI (kg/day)						
Prepartum	7.97	7.79	7.75	0.19	0.56	0.54
Postpartum	20.41	18.87	17.99	0.23	<.0001	0.005
DMI (%BW)						
Postpartum	3.82	3.68	3.38	0.04	<.0001	<.0001

Orthogonal contrast of means were the following 1= CON vs. FAT, 2 = UF vs. PF.
SEM = standard error of the mean.

Milk yield:

In the present study, cows fed PF treatment beginning at prepartum period (21 day before calving) through the first 60 days in milk produced 18% and 15% more milk yield than CON and UF treatments, respectively (Table, 3). In addition, milk yield of UF cows was higher than milk yield of CON cows. Generally, significant differences in milk yield and 3.5% FCM were detected among cows fed CON and those cows fed fat (UF and PF). Supporting to our results, the previous studies also reported enhanced milk production (Salado *et al*., 2004 and Reis *et al*., 2012) when Ca salts of fatty acids were offered to dairy cows. The superiority of milk yield in added fat groups may be attributed to that fat supplementation increasing the energy density of the diets in which resulting in reducing the negative effect of negative energy balance. In addition, feeding PF during the prepartum period increased after parturition milk production may be due to increase energy intake and minimal stress of adjusting to a new dietary ingredient early in lactation. The present results are in agreement with the findings of Bu *et al*., (2007); Tyagi *et al*., (2010) and Shelke *et al*., (2012) showed that supplementation of bypass fat increased milk production when compared to control diet and this may be due to bypass fat supplementation increased the energy density of the ration and resulting in reducing the deleterious effect of negative energy balance. However, other studies (Aguilar-Pérez *et al*., 2009 and Ganjkhanlou *et al*., 2009) showed no effect of fat supplementation on milk production of high producing dairy cows .

Feed efficiency:

Feed efficiency, expressed as kg of 3.5% FCM per kg of DM intake was significantly higher when feeding fat treatments than feeding CON treatment (Table, 3). In addition, a significant difference was found between UF and PF treatments. These results suggest that cows fed fat supplementation were more able to efficiently convert feed nutrients into milk. The present study are in agreement with those of Ganjkhanlou *et al*., (2009) and Moallem *et al*., (2010).

Table (3). Milk yield, feed efficiency and milk composition of lactating Holstein cows fed control diet (CON) or diets supplemented with unprotected fat (UF) or protected fat (PF).

Item	Treatments				Contrast, *P* values	
	CON	UF	PF	SEM	CON vs. FAT	UF vs. PF
Measurements:						
Milk yield, kg/d	27.80	28.47	32.70	0.60	0.0003	<.0001
3.5% FCM, kg/d	26.94	27.08	34.34	0.59	<.0001	<.0001
Feed efficiency	1.42	1.60	2.03	0.32	<.0001	<.0001
Milk composition:						
Milk fat,						
%	3.27	3.20	3.81	0.30	0.51	0.18
(Kg/d)	0.91	0.91	1.25	0.02	<.0001	<.0001
Milk protein,						
%	2.91	2.99	2.79	0.08	0.87	0.10
(Kg/d)	0.81	0.85	0.92	0.02	0.001	0.01
Lactose						
%	4.78	4.69	4.69	0.08	0.36	0.99
(Kg/d)	1.34	1.34	1.53	0.03	0.005	<.0001
SNF						
%	8.62	8.60	8.41	0.11	0.40	0.22
(Kg/d)	2.41	2.45	2.75	0.05	0.003	<.0001

Orthogonal contrast of means were the following 1= CON vs. FAT, 2 = UF vs. PF
SEM = standard error of the mean.
Feed efficiency = kg of 3.5 % FCM of DMI.

Milk fat content:

No significant differences were found in milk fat percentage among groups, although value of concentration of fat was numerically higher in milk from PF cows than in milk from CON or UF cows (Table, 3). Milk fat content was 17% and 19% greater for cows fed PF treatment versus CON and UF treatments, respectively. The observed decrease in milk fat content in CON and UF treatments are generally attributed to altered rumen function, biohydrogenation of unsaturated fat sources and ruminal fermentation (Bauman and Griinari, 2003). Also, Zheng *et al*., (2005) observed that milk fat percentage was lower when cows received supplemental soybean oil diet than control diet. Milk fat yields were greater for cows fed PF treatment than those cows fed CON or UF treatments. Milk fat yield of PF cows was higher than milk fat yield of CON or UF cows. The higher fat production observed in cows fed PF treatment may be explained by higher milk production.

Milk protein content:

Milk protein percentage did not significantly differ among cows fed CON and those cows fed the two fat treatments (Table, 3). However, milk protein percentage tended to decrease (P = 0.10) when cows received PF treatment compared to cows receiving UP treatment. The change may be attributed to increase in milk yield rather than decrease in milk protein synthesis (Appeddu *et al*., 2004; Schroeder *et al*., 2004). Generally, Petit *et al*., (2007) and Reis *et al*., (2012) reported that milk protein concentration did not decrease when fat was added to lactating cows diets. In addition, previous studies have reported no effect of soybean oil supplementation on milk protein content (Zheng *et al*., 2005; Bu *et al*., 2007). In another study Afzalzadeh *et al*., (2010) revealed that feeding fat at prepartum period may help animals to use fat sources for energy requirements and consequently other sources of energy such as amino acids (glucogenic precursors) could be used for other functions like protein synthesis and milk production. Likely due to increase milk production, milk protein yield was significantly (P < 0.05) greater by cows fed fat treatments than cows fed CON treatment. In addition, a significant difference (P<0.05) was observed in milk protein yield between cows fed the two fat treatments.

Milk lactose and solids not fat (SNF) contents:

Concentrations of milk lactose and SNF were not significantly different among treatments, whereas yields of milk lactose and SNF were significantly ($P < 0.05$) higher for cows fed fat treatments than those cows fed CON. This may be due to the difference in milk yield among all treatments (Table, 3). The present results are in agreement with those reported by Afzalzadeh *et al*., (2010) and Shelke *et al*., (2012).

Milk fatty acid composition:

Fatty acids composition of milk as affected by the different treatments groups are presented in Table (4). The production of fatty acids with 14 carbons or less and 50% of C16:0 formations is a result of de novo fatty acid synthesis in the mammary gland and from mammary uptake of preformed fatty acids. Fat source, time of initiation of the supplementation, inclusion rate, period length of supplementation and biohydrogenation extent will differentially affect the incorporation of the long chain fatty acids into milk fat of dairy cows. The depression of these fatty acids can be explained by inhibitory roles of fat supplementation on fiber digestion and de novo fatty acid synthesis (Gaynor *et al*., 1995). Concentration of C8:0 in milk fat tended to be higher ($P = 0.10$) in cows fed the fat treatments than the CON treatment. Fat supplementation had no significant effect on production of C12:0, C14:0, C15:0, C18:1 and C18:2 in milk fat. However, significant differences in C10:0, C16:0 and C18:0 when compared to CON treatment. A comparison between fat treatments showed that addition of PF to the ration increased the proportion of saturated fatty acids from C8 to C16 and decreased the proportions of C18:0, C18:1 and C18:2 in milk fat compared to UF treatment. Increase level of C16:0 in milk fat of cows fed PF treatment may be due to the fact that the protected fat (PF) consisted mainly of palm oil fatty acids (C16). Increased levels of unsaturated fatty acids in the milk can only occur when unsaturated fatty acids present in feed or supplements escaped microbial action in the rumen. Concentration of C18:2 and C18:3 were decreased when fat was added to the diets (Onetti *et al*., 2001). The low concentration of C18:2 and C18:3 in cows fed Ca salts of unsaturated fatty acids may be as a result of Ca salts dissociating in the rumen and fatty acids being saturated by ruminal microorganisms (Chouinard *et al*., 1998). In another study, Warntjes *et al*., (2008) showed that cows fed rumen inert fat as palmitic acid (C16:0) led to increase concentration of C16:0 in milk fat and decreased concentration of C17:0 and C18:0. However, concentration of C18:2 was not affected when compared to the control diet. Obeidat *et al*., (2011) reported that except C18:1 and C 20:0 dietary treatments had no effects on profile of short or medium chain fatty acids when lactating ewes were fed control or fat supplemented diets. The increase in C18:1 can be the result of partial biohydrogenation of C18:2 and C18:3 FA and desaturation of C18:0 in the mammary gland (Kennelly, 1996). However, Warntjes *et al*., (2008) reported a decrease in milk short and medium chain fatty acids with palmitic acid (C16:0) supplementation. The authors attributed that C16:0 in the diet may have decreased de novo synthesis of short and medium chain FA in the mammary gland. In the present study, the data revealed that there was no negative effect of PF supplementation on short or medium chain fatty acids.

Table (4). Milk fat fatty acids composition of lactating Holstein cows fed control diet (CON) or diets supplemented with unprotected fat (UF) or protected fat (PF).

Item, % of total fatty acids	Treatments				Contrast, *P* values	
	CON	UF	PF	SEM	CON vs. FAT	UF vs. PF
Fatty Acids						
Caprylic acid (C8:0)	0.24	0.50	0.57	0.13	0.10	0.71
Capric acid (C10:0)	0.72	1.29	1.95	0.28	0.02	0.12
Lauric acid (C12:0)	1.74	1.52	2.13	0.29	0.81	0.16
Myristic acid (C14:0)	6.99	7.21	8.02	0.96	0.61	0.56
Pentadecylic acid (15:0)	0.64	0.56	0.63	0.14	0.80	0.72
Palmitic acid (C16:0)	27.45	27.39	35.69	1.24	0.02	0.0003
Stearic acid (C18:0)	19.59	17.72	14.65	1.33	0.06	0.12
Oleic acid (C18:1)	36.56	37.65	31.50	1.67	0.36	0.02
Linoleic acid (C18:2)	4.58	4.55	4.02	0.56	0.68	0.52

Orthogonal contrast of means were the following 1= CON vs. FAT, 2 = UF vs. PF.

From the present study, it can be concluded that the supplementation of fat during prepartum and postpartum specially protected fat led to positive effects on milk production and milk composition. SEM = standard error of the mean.

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انتاج وتركيب اللبن في الابقار عالية الادرار نتيجة اضافة الدهن المحمى وغير المحمي

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تم اجراء هذه الدراسة في جامعة كارولينا فى الولايات المتحدة الامريكية وقد استخدم في هذه الدراسة عدد 26 بقرة (عدد 9ابقار في اول ولادة و عدد17 متعددة الولادات) من الابقار الهولشتين وقد وزعت الابقار علي ثلاث معاملات عشوائية واتبع التصميم الاحصائي للقطاعات العشوائية الكاملة في التوزيع وكانت المعاملا ت الثلاثة كالاتى: المجموعة الاولى (مقارنة دون اضافة اى دهن الى العليقة) المجموعة الثانية (تم اضافة دهن فول الصويا الي العلائق) المجوعة الثالثة تم اضافة الدهن الحمى (ميجا لاك) الي العلائق وقد تم اضافة الدهن الي المجموعة الثانية والثالثة لمدة 21 يوم قبل التاريخ المتوقع للولادة ولمدة 60 يوم بعد الولادة وكان نسبة الدهن المضاف 1% و2% من المادة الجافة لكل فترة علي الترتيب وتم تسجيل انتاج اللبن بعد الولادة يوميا كما تم تقدير كل من الدهن والبروتين واللاكتوز والجوامد الصلبة الغير دهنية في كل عينات اللبن فى اليوم صفر و 30 و60 يوم بعد الولادة اظهرت النتائج انه لا توجد فروق معنوية في كمية المادة الجافة الماكولة في فترة قبل الولادة في حين ادى اضافة الدهن الي العلائق بعد الولادة الى نقص معنوى في كمية المادة الجافة الماكولة كما ادى اضافة الدهن المحمى الى العلائق قبل الولادة الى زيادة كمية اللبن المنتج يوميا بعد الولادة او زيادة كمية اللبن المعدل به نسبة الدهن عند مقارنة ذلك بالعلائق المضاف اليها دهن غير محمى او عليقة المقارنة كما ادى ايضا اضافة الدهن المحمي الي العلائق الي زيادة نسبة الدهن في اللبن للعلائق المضاف اليها دهن محمى بالمقارنة بالعليقة المضاف اليها دهن غير محمى وعليقة المقارنة ولكن الفروق كانت غير معنوية كما ان اضافة الدهن الغير محمى ادى الى حدوث نقص معنوى في نسبة البروتين في اللبن في العلائق المضاف اليها دهن محمى بالمقارنة بالعليقة المضاف اليها دهن غير محمى او عليقة المقارنة واظهرت النتائج ان اضافة الدهن الى العلائق لم يؤدى الى تغيير محتوى اللبن من سكر اللاكتوز او مجموع الجوامد الصلبة الغير دهنية واظهرت النتائج ان متوسط الكفاءة الغذائية كيلو جرام مادة جافة لكل كيلو جرام لبن معدل مرتفع معنويا فى العليقة المضاف اليها دهن مقارنة بعليقة المقارنة . واظهرت النتائج ان تركيز الحمض الدهنى C18:0 في لبن العلائق المضاف اليها دهن اعلي معنويا من عليقة المقارنة في حين ان اضافة الدهن لم يوثر معنويا علي تركيز الأحماض الدهنيه الأتيه في اللبن (C12:0 -C14:0 -C15:0 -C18:1 -C18:2) في حين ان هناك فروق معنويه في تركيز الاحماض الدهنية C18:0– C16:0 -C10:0 في العلائق المضاف إليها دهن محمي أو غير محمي بالمقارنة بالعليقة الكنترول. ومن هذه النتائج نستخلص ان اضافة الدهن المحمى الي علائق الابقار عالية الادرار ادى الي تاثير ايجابى علي كمية اللبن وتركيبة.