

Research Article**The effects of supplementing diet with unsaturated oil on productive performances of Holstein dairy cows in early lactation****Toktam Vafa* and Mozhdeh Emadi**¹Department of Agricultural Science,

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ABSTRACT

To examine the effect of including oil in diets on early lactating productive performance parameters including; dry matter intake, milk production and components, body condition score, body weight and negative energy balance; twenty multiparous Holstein cows were randomly assigned to the experimental diets from 21 days before predicted calving time to 50 days in milk. Treatments including 1) control (no supplemented oil in both pre and postpartum, n=10) and 2) supplemented diets (supplemented with fish oil and canola oil in 50:50 ratio in amount 1% DM in prepartum and 2% DM in postpartum). Prepartum and postpartum dry matter intake, body condition score and body weight were not affected by the diets ($P>0.05$). Negative energy balance in early lactation was similar between diets ($P>0.05$). Daily milk production increased in oil supplemented diet ($P<0.05$) and milk fat percentage decreased in the supplemented diet ($P<0.05$), but including oil in diets had no effects on protein percentage and production, fat and lactose production in milk ($P>0.05$). According to results, supplementing diet with fish oil and canola oil from prepartum to early lactation had no adverse effects on the productive parameters in early lactation and also increased milk production.

Keywords; oil, early lactation, milk production, DMI**INTRODUCTION**

Nutrition influences the supply of nutrients to tissues and alters the metabolic status of animals. Supplemental lipid increase the energy density of the diet, and it might improve the energy balance of early lactating dairy cows. However, data on the influence of supplemental lipid on the energy balance of early lactating cows were variable, mostly because of variation in DMI and milk production.

Along with a gradual decline in dry matter intake that starts 2–3 week prepartum, an abrupt increase in nutrient demand with initiation of lactation results in negative energy balance (NEB) and extensive mobilization of body fat reserves as non-esterified fatty acids. Depressed concentrations of glucose, and frequently insulin, are typical during NEB in association with

increased hepatic gluconeogenesis (Duske et al., 2009; Drackley, 1999).

The most common way to decrease the NEB is to increase the energy density of the diet by including carbohydrate and fat in diets (Butler, 2003). Diets fed to dairy cows are originally low in fatty acid (FA) content, approximately 20 g/kg of the total dry matter (DM). However, supplemental fat sources are utilized in rations for dairy cows as a common method to increase the energy density of the diet to support energy demands for milk synthesis (Juchem et al., 2008). A wide range of fat sources is available to be fed in the ration of lactating dairy cows, but the adverse effects of some supplements on rumen fermentation and DM intake might offset the benefits of increasing the energy density of the diet (Allen, 2000). In

addition, declines in voluntary intake may increase body fat mobilization, decrease plasma glucose, which can compromise health and milk production (Juchem et al., 2008).

Most researches on feeding fat supplemented diets in dairy nutrition focused on early lactation supplementation and the productive performance, however, the influence of dietary fat supplied during dry period and late lactation on lactation performance and metabolic changes during the following lactation were less examined. It has been reported that cows supplemented with fat during 60 until 7 d before calving had lower hepatic lipid contents at parturition in comparison to cows fed a control or a high-grain diet, which suggests that cows fed fat prepartum might not be predisposed for fatty liver syndrome (Grum et al., 1996). According to research, fat supplementation during the whole dry period failed to affect peripartal lipid content in liver (Douglas et al., 2004, 2006). In contrast, others reported that hepatic lipid accumulation tended to increase at parturition by supplementation of fat 17 d prepartum (Skaar et al., 1989).

Fish oil which contains relatively high concentrations of two PUFA of the n-3 family, EPA and DHA, is an effective means of increasing CLA, a group of positional and geometrical isomers of linoleic acid (*c*9, *c*12 octadecadienoic acid) with conjugated double bonds, in milk fat, due to an inhibition of *t*-18:1 reduction in the rumen that results in an increased supply of *t*-11 18:1 which would be available for endogenous conversion to *c*9,*t*11-18:2 (CLA) in the mammary gland (Shingfield et al., 2006). Including FO in dairy diets also increased the content of EPA and DHA in milk, even though the transfer efficiencies of DHA (3%) and EPA (4%) into milk fat are very low (Chilliard et al., 2001). The combination of marine lipids and 18:2 rich plant oils is an established strategy to modify ruminal biohydrogenation for increasing CLA in milk fat (Palmquist and Griinari, 2006). Canola seed, containing only about 6% 16:0 and up to 58% *c*-18:1 and with a much higher *c*-18:1 to 16:0 ratio than other common oilseeds, has potentially interesting characteristics to change the fatty acid profile of milk fat (Bayourthe et al., 2000). Thus

the objective of the current study were to examine the effects of supplementing diet with fish oil and canola oil on the milk production, dry matter intake (DMI), energy balance (NEB) and body condition score (BCS) in early lactation.

MATERIALS AND METHODS

Animals, Management, and Diets

Twenty multiparous Holstein dairy cows were blocked by parity, expected calving time, and previous 305-2X milk production and were randomly assigned within block to diets. Cows were housed in tie stalls with individually access to fresh water and were allowed to exercise daily in an outside lot for 4 h. Treatments were no supplemental lipid (control, n = 10) or supplemented with fish oil and canola oil in 50:50 ratio in amount 1% DM in prepartum and 2% DM in postpartum (supplemented, n=10), and cows were fed experimental diets (table 1) two times a day to allow 5 to 10%orts (as-fed basis) from three weeks before to seven weeks after parturition. Immediately after parturition cows were switched to the lactation diet and milked twice daily. Forage to concentrate ratios were 70:30 and 37:63 in prepartum and early lactating periods respectively and total mixed ration were formulated according to NRC 2001. Body weight and BCS were determined at enrollment and every week through the study. The BCS (scale of 1 = thin to 5 = obese; Wildman et al., 1982) were assigned by the same individual throughout the study and NEB was calculated for each cow with the system adopted by the NRC 2001. The amounts of feed offered and refused were measured daily.

Sampling Procedures

The TMR were sampled weekly throughout the experiment and DM content was determined by drying at 110°C for 18 h. Samples of TMR were collected weekly, composited monthly and analyzed by wet chemistry procedures for CP, NDF, ADF and ether extract. The weekly TMR samples were stored in -20°C until the analyses. Ingredient and nutrient composition of the diets are listed in Table 1. Cows were milked 3 times per day at 0100, 0930 and 1700 h and yields were recorded. Milk samples were

collected from each milking on 1 d per week and composited for analysis of milk composition (Micro Scan; FOSS Electric A/s, Denmark).

Statistical Analyses

Milk yield and composition, DMI, BW, BCS and NEB were analyzed by using a mixed model (PROC MIXED, SAS Inst. Inc., Cary, NC) for a completely randomized design with repeated measures using the following model:

$$Y = \mu + T_i + A_{(ij)} + D_k + (T \times D)_{ik} + g_{ijk}$$

Where,

Y_{ijk} = Dependent variable

μ = Overall mean

T_i = Treatment effects

$A_{(ij)}$ = Random effects of animal within treatments

D_k = Effects of sampling day or time

$(T \times D)_{ik}$ = Interaction effects of treatment and sampling day or time

G_{ijk} = Residual error associated with the ijk observations

RESULTS AND DISCUSSION

DMI, BW, BCS and NEB

Ingredient and chemical compositions of the diets are shown in Table 1. The results on DMI, BW, and BCS either on prepartum or postpartum periods are shown in Table 2. BCS and BW were not different between diets in pre and post-partum periods ($P > 0.05$) with a decreasing pattern during the experiment. Postpartum DMI was similar between diets ($P > 0.05$). These findings differ from previous observations in which supplementing fish oil at 1.8 to 2.0% of the diet DM suppressed DMI (Donovan et al., 2000; AbuGhazaleh et al., 2002; Whitlock et al., 2002; Mattos et al., 2004). Donovan et al. (2000) reported that DMI was not affected when 1% FO was supplemented in the diet, and DMI only decreased when supplementing either 2 or 3% FO. Ballou et al (2009) has reported similar results in DMI when they had included fish oil in amount of 250 g/d, but supplementing diet with 2% of diet DM suppressed DMI in other studies (AbuGhazaleh et al., 2002; Whitlock et al., 2002). Supplementing cow's diet with 230 g/d prilled fat and 215 g/d calcium salt of long chain fatty acids had led to an average decrease of 2-3% from 14 to 7 d prepartum, and by 14% from 7 to 1 d prepartum in

comparison with control group (Moallem et al., 2007). It has been suggested that the unsaturated fatty acids which reach the intestine affect the gastrointestinal motility and consequently decrease DMI (Drackly et al., 1992). In the present study, the concentration of prepartum supplemental oil was 1% which was similar to concentrations reported to have no adverse effects on DMI (Donovan et al., 2000). Moreover, numerous studies (Moallem, 2007) have been conducted that provide some insight into how to feed dry cows, particularly during the close-up period. In a review, Grummer (1995) suggested that prepartum DMI was positively correlated with postpartum DMI and that prepartum DMI should be maximized to improve postpartum performance and health. Grummer (1995) suggested that increasing the nutrient density of the diet could increase DMI and thus nutrient intake. In response to this review, several researchers focused on maximizing DMI or energy intake during the close-up period (Minor et al., 1998; Dann et al., 1999; Doepel et al., 2002; Rabelo et al., 2003). One of the probably descriptions for postpartum DMI is the procedure of prepartum DMI. Douglas et al. (2006) reported improved postpartum consumption for cows that were feed restricted prepartum although in their report it has been mentioned that the pattern of changing DMI from pre to post-partum is more important than the kind of DMI offer which could be restricted or ad libitum.

Weekly BCS and BW were not affected by the diet in pre and postpartum periods ($P > 0.05$), but time significantly affected the BCS and BW ($P < 0.05$) which these two decreased by the time after parturition. Similar to our results, including capsulated fatty acids and calcium salts of fatty acids from 21 d prepartum (Castañeda-Gutiérrez et al., 2008) or 1.35% (DM) sunflower oil or Ca-salt of plant oil from 5 week prepartum to 15 week postpartum (Bilby et al., 2006) had no effects on BCS and BW in pre and postpartum periods both. According to the milk production results in the current study, the oil supplemented experimental group with the higher energy intake in comparison with the control group had a higher milk production, but there were no significant

difference in BCS for two groups which might be because of the similar DMI in them that provide the energy required for the higher milk production in this the supplemented group. As lactation begins, milk production increases with the increment in energy demands for milk production which all leads to body weight losses and BCS decreases during the weeks after parturition. The average NEB in the first six weeks of lactation was not affected by the diets (-6.49 and -5.36 for control and supplemented diets respectively, $P > 0.05$) which is in agreement with some other research (Dann et al., 2006; Juchem et al., 2008). The results of milk production and components are presented in table 3. Supplementing diet with fish oil and canola oil increased milk production significantly ($P < 0.05$), but weekly 4% FCM was similar between diets ($P > 0.05$). Milk fat percentage was significantly decreased in fat supplemented diet but the percentage of protein and lactose were not affected by the diet ($P > 0.05$). According to researches, the results for the milk production when including oil in diet is different. Bilby et al (2006) had reported no difference in milk production when supplemented diet with high oleic sunflower oil, Ca salts of *Trans* fatty acids, Ca salts of palm and soybean oil and linseed oil from 5 weeks before expected calving time to 15 weeks after parturition. Similarly, including 2% (DM) Ca salts of palm oil (Ceri et al., 2009), Ca salt of fatty acids (Castaneda-Gutierrez et al., 2009) and 2% DM fish oil (Ballue et al., 2009) from dry period to 10 weeks postpartum had no effects on milk production. In most researches supplementing diets with unsaturated oil decrease DMI and milk production both (Donovan et al., 2000; Mattos et al., 2004). In contrast to the researches with the negative effects of oil supplementation on DMI and milk production, including 1% fish oil in diet (Donovan et al., 2000), increasing fish oil levels from 0 to 450 grams per day (Kidye et al., 2000) increased milk production significantly. One of the proper explanations for this increment is the increase in DMI in these groups. However, it is not clear that the more milk production the more DMI, or the more DMI, the more milk production (NRC, 2001). According to the theories controlling DMI,

energy requirements control DMI, so more milk production finally leads to increased DMI (NRC, 2001). Milk production increases by the time ($P < 0.01$). Milk fat concentration was decreased by including fish oil and canola oil in diets ($P < 0.05$), although milk fat production was similar between diets. Castaneda-Gutierrez et al (2009) has reported that feeding fish oil in diet decreased milk fat significantly in comparison to formaldehyde protected linseed or duodenal injection of linseed oil. Including canola oil in diet and rumen infusion of 330 g of canola oil (DePeters et al., 2001) decreased milk fat significantly. Other researchers (Abughazaleh et al., 2004; Whitlock et al., 2002; Ramaswamy et al., 2001; Donovan et al., 2000) also reported depression in fat percentage of milk when fish oil was incorporated in the diets of cows. Rapid availability of the oil in the diet and its potential effect on fiber digestibility is one of the possible reasons of lower fat percentage in oil containing diets (Abughazaleh et al., 2004). Rumen environment alteration which causes to incomplete biohydrogenation and production of various substrates and conjugated linoleic acid isomers is the other possible reason for milk fat decrease when oil is included in diets (Bauman et al., 2003). Recent studies (Baumgard et al., 2001; Peterson et al., 2002) have established a relation between milk fat depression and the increase *trans*-10, *cis*-12 CLA content in milk fat. Milk protein concentration and production were similar between diets ($P > 0.05$). Milk protein percentage often decreases when supplemental fat is fed to lactating dairy cows (Juchem et al., 2007; Chichlowski et al., 2005; DePeters and Cant, 1992). Even when small amounts of EPA and DHA from fish oil were fed, for example approximately 10 g/cow/day, they decreased milk protein content in relation to diets that contained similar amounts of crude fat but without any fish oil (Petit et al., 2002), suggesting a direct effect of fatty acids from fish oil on protein synthesis or amino acid uptake in the mammary gland because of negative effects of dietary fat on somatotropin release (Casper and Schingoethe., 1989). Energy intake is generally thought to be the major nutritional factor affecting milk protein

concentrations (Lock and Shingfield, 2004), as reported previously DMI was not affected by the experimental diets in the current study. Lactose concentration in milk was similar between diets but lactose production increased significantly in supplemented diet ($P < 0.05$).

Results of this experiment suggested that combination fish oil with canola oil will decrease negative effects of feeding fish oil on DMI and milk production and especially including this oil supplement in dairy diets from prepartum period to postpartum with improve dairy performance in early lactation.

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Table 1. Ingredient component and chemical composition of experimental diets

Ingredients	Experimental diets ¹			
	prepartum		postpartum	
	control	supplemented	control	supplemented
Ingredients,% of DM				
Alfalfa	26	26	20	20
Corn silage	32	32	17	17
Wheat straw	12	12	-	-
Corn grain	7.2	6.3	14.98	13
Barely grain	7.2	6.3	14.98	13
Soybean meal	9.6	9.75	20.2	20.32
Bran	4.95	5.1	10.99	10.70
Oil supplements	-	1	-	2
Limestone	0.39	0.39	0.81	0.81
Vitamin supplement	0.45	0.45	1.01	1.01
Salt	0.06	0.06	0.2	0.2
Chemical composition ,% of DM				
CP	14.4	14.3	17.9	17.8
NDF	39.5	39.3	30.4	30.1
ADF	25.3	25.2	17.8	17.7
OM				
NFC ²	39	38.3	44.3	42.8
Ether extract	2.4	3.2	2.6	4.5
Ca	0.6	0.6	0.7	0.7
P	0.4	0.4	0.5	0.5
NE _L , Mcal/KgDM	1.54	1.59	1.58	1.65

¹control= diet without oil; supplemented= diet supplemented with fish oil and canola oil in 50:50 ratio(1 % DM, prepartum and 2%DM, postpartum), fish oil from khazar Co, Babolsar, Iran and canola oil from Golestan Soybean, Gorgan, Iran.

²NFC = 100 - (NDF + CP + ash + ether extract).

Table 2. Least squares means for DMI, BCS, and BW during the pre- and postpartum periods

variable	Treatments ¹		SED	Main effects ²		
	Control	supplemented		Trt	time	Trt × time
DMI, kg/d						
Prepartum	13.92	14.45	0.33	0.29	0.006	0.17
Postpartum	21.16	20.91	0.50	0.72	<0.0001	0.001
BCS ³						
Prepartum	3.58	3.52	0.07	0.59	0.09	0.18
Postpartum	3.13	3.21	0.09	0.54	<0.0001	0.15
BW, kg						
Prepartum	685.11	698.89	30.6	0.75	0.50	0.92
postpartum	566.18	604.26	18.6	0.16	0.01	0.66
NEB	-6.49	-5.36	1.01	0.23	0.01	0.03

¹Control diet containing no supplemental lipid and supplemented diet containing 1% (DM) fish oil and 1% (DM) canola oil during prepartum and postpartum periods.

²Effects of treatment (Trt) and Trt by day interaction.

³1 = thin to 5 = obese; Wildman et al. (1982)

Table 3. Least squares means for milk production and components in early lactation

variable	Treatments ¹		SED	Main effects ²		
	Control	supplemented		Trt	time	Trt × time
milk, kg/d	34.2 ^a	36.65 ^b	0.77	*	**	ns
Milk 4%FCM, kg/d	28.03	29.81	1.39	ns	ns	ns
Milk kg/week	34.07	35.73	1.4	ns	**	ns
Milk fat						
%	3.23 ^a	2.83 ^b	0.35	*	ns	ns
Production(kg/d)	1.087	1.027	0.04	ns	ns	ns
Milk protein						
%	2.86	2.83	0.35	ns	**	ns
Production(kg/d)	0.95	1.03	0.03	ns	ns	ns
Milk lactose						
%	4.49	4.67	0.06	ns	ns	ns
Production(kg/d)	1.51 ^a	1.70 ^b	0.05	**	ns	ns

¹Control diet containing no supplemental lipid and supplemented diet containing 1% (DM) fish oil and 1% (DM) canola oil during prepartum and postpartum periods.

²Effects of treatment (Trt) and Trt by day interaction.

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.0001$

Ns, non significant