Journal of Agricultural Science and Technology A 2 (2012) 930-936
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Practical Application of Diets Rich in Ground Linseed to Enhance Omega-3 Milk Production in Holstein Dairy Farms Located in Northeast Iran

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Received: March 26, 2012 / Published: July 20, 2012.

Abstract: In an attempt to enhance organic omega-3 milk production, a practical study was performing in 7 Holstein dairy farms in Northeast Iran. The experiment was conducted at the "ShafashirToos industrial dairy farmers production and distribution cooperative" located in Northeast Iran, between June 2010 and December 2010. In Golshid-Mashhad Co., cows were fed with no linseed containing diets (NO-Lin), while in the other farms (Hanaei Co., Ghasemi Co., Taheri Co., Teliseh Co., Arasp Co. and Navingholshid-Khorasan Co.), cows were fed diets containing 9% ground linseed (Lin). Cows were used in a randomized complete block experimental design. Diets were provided as TMR. Concentration of fat, protein and lactose in milk was measured. Milk fat extraction and fatty acid separation were determined. There were no differences in the most of the milk fatty acid profiles of cows in farms that received ground linseed but feeding Lin diets compared with NO-Lin, significantly decreased ($P < 0.05$) C8:0-C16:0 concentration and it increased those of C18:1 n9trans, C18:1 n9cis, C18:2 trans and C18:3 n3. As a result, farms with Lin diets had better milk fatty acid profiles with doubled in α-Linolenic acid concentration and increased in mono and polyunsaturated fatty acids and decrease in medium chain and saturated fatty acids compared with those of the NO-Lin diet. These results clearly demonstrate the possibility for sustainable production of organic omega-3 milk with levels of C18:3 FA up to 2.4 times higher than typical levels in dairy fat. The manufacture of omega-3 enriched milk and milk products could supply dietary fatty acids at levels that may benefit health, without the need for unrealistic changes to eating habits.

Key words: Linseed, α-linolenic acid, milk yield, fatty acid profile.

1. Introduction

Dietary polyunsaturated fatty acids (PUFA) are perceived to be healthier than saturated fatty acids (SFA). Therefore, there has been a great deal of interest in manipulating the fatty acid profile of milk fat to respond to consumer demand. On the contrary, within the group of PUFA, n-3 fatty acids possess a protecting effect against cardiovascular diseases [1]. Therefore, an enrichment of milk fat with n-3 fatty acids is considered desirable from a human health perspective. Diet is the main factor that influences milk fatty acid concentrations. Supplementing dairy rations with oilseed and/or other dietary fat sources has a potential for changing milk yield, fat content and fatty acid composition [2]. Feeding trials indicate that lipids available as supplementary energy sources for dairy cattle markedly influence the fatty acid composition of milk fat, thus improving milk quality [3-5]. Linseed is an excellent source of omega-3 fatty acids, which is known to be anti-carcinogenic, to prevent cardiovascular diseases, and to increase visual activity [6]. Feeding up to 15% of the total dry matter (DM) as linseed has no effect on dry matter intake (DMI) of mid lactating dairy cows [7]. Linseed is also known to increase the concentration of PUFA in milk, but usually they do not exceed 3% to 4% of total fatty acids [3]. Feeding linseed generally results in the

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lowest omega-6 to omega-3 FA ratio in milk fat [8], which may improve milk FA profile and result in better human health. Processing flaxseed may also improve milk FA profile from a human health perspective due to increased proportions of conjugated linoleic acid and omega-3 FA and decreased proportions of medium-chain and saturated FA in milk fat of cows fed ground compared with those fed whole flaxseed [9]. The objective of this experiment was to determine the effect of diets rich in linseed to enhance organic omega-3 milk production in Holstein dairy farms which are member of "ShafashirToos industrial dairy farmers production and distribution Cooperative", located in Northeast Iran.

2. Materials and Methods

2.1 Procedure and Chemical Analysis

In Northeast Iran, a cooperation consisting of 7 dairy farms has been formed and named "ShafashirToos industrial dairy farmers production and distribution cooperative". The dairy farms are Golshid-Mashhad Co., Hanaei Co., Ghasemi Co., Taheri Co., Teliseh Co., Arasp Co. and Navingholshid-Khorasan Co. with lactating cows of 89, 76, 134, 142, 202, 67 and 68, respectively and average 685 ± 17 kg, 700 ± 12 kg, 692 ± 15 kg, 680 ± 13 kg, 705 ± 13 kg, 687 ± 11 kg and 698 ± 17 kg body weight, respectively. Cows were used in a randomized complete block experimental design. All farms had same management programs and located in similar climatic condition. The cooperation is mainly involved in producing organic omega-3 milk. In Gholshid-mashhad Co. farm, cows were fed with no linseed containing diets (NO-Lin) while in the other farms; cows were fed diets containing 9% ground linseed (Lin). All diets were provided to achieve a rate of forage: concentrate as 39:61, 40:60 and 42:58 for early, mid and late lactation, respectively. In each farm, animals were divided into 3 groups based on their days in milk (DIM) as early (up to 120 DIM), middle (up to 220 DIM) and late (up to drying). Experimental diets are presented in Table 1 and provided in 3 groups for early, middle and late lactating cows. In all farms diets were provided as TMR and fed to the animals for 6 months. Diets were fed twice daily at 07:00 and 18:00 at adlibitum rates to allow 100 g/kg refusals. To determine dry matter intake, orts were weighed before commencement of each feeding in the each collection experimental period. Each feed ingredient and samples of TMR were collected, and composited. Composited samples were mixed thoroughly and sub samples were provided for next chemical analysis.

Samples of milk from each farm were collected monthly, started 2 months after cows received the experimental diets. The samples were collected at 3 consecutive milking from the bulk tank, mixed in proportion to yield appropriate composted samples, and then analyzed to determine the milk chemical composition (fat, protein and lactose) and parts of each sample were stored at -20 °C until analyzed for milk fatty acid profile. Dry matter of each feed and TMR samples was determined using air forced oven drying at 60 °C for 48 h [10] and ground to pass through a 1mm screen. Determinations of N were conducted using the Kjeldahl method in an automated Kjelfoss apparatus (Foss Electric, Copenhagen, Denmark). Neutral detergent fiber was measured according to the method of Goering and Van Soest [11].

Concentration of fat, protein and lactose in milk was determined using milkoscananalyser (Foss Electric, Conveyor 4000). Procedure for fatty acid analysis of the oilseeds used in the present experiment was as described by Sukhija and Palmquist [12]. Milk fat extraction and fatty acid separation were performed according to a procedure described by Chouinard et al. [13]. Fatty acid methyl esters were prepared by trans-methylation, then were quantified by using a gas chromatograph (GC system 6890, Hewlett-Packard, Wilmington, DE) equipped with a flame-ionization detector and a CP-7489 fused-silica capillary column (100 m × 0.25 mm i.d. with 0.2 μm

Table 1 Ingredients of total mixed diets (% DM) of Holstein lactating cows of dairy farms which are members of "ShafashirToos industrial dairy farmers production and distribution cooperative" located in Northeast Iran fed no linseed (NO-Lin) or linseed (Lin).

Ingredients (% DM)	Early lactation		Mid lactation		Late lactation	
	NO-Lin	Lin	NO-Lin	Lin	NO-Lin	Lin
Corn silage	15	18	15	20	15	26
Alfalfa hay	26	21	26	20	27	16
Wheat straw	1	2	1	5	7	10
Barley grain	20	13	20	14	21	12
Corn grain	10	12	10	11	5	8
Linseed[1]	-	9	-	9	-	9
Cottonseed	4	5	4	-	-	-
Soybean meal	12	10	10	12	8	6
Wheat bran	11	6	13	8	16	12
Fish meal	-	3	-	-	-	-
Vitamin and mineral premix	1	1	1	1	1	1
Chemical composition						
Crude protein (g/kg)	172	171	164	164	148	148
Neutral detergent fiber (g/kg)	358	344	379	369	410	391
ME (MJ/kg DM)	12.1	12.3	11.1	11.3	10.6	10.7

1 Linseed oil is contain: (C16:0 = 7.45%, C18:0 = 4.39%, C18:1 = 18.29%, C18:2 = 16.47%, C18:3 = 52.23% of total fatty acids).

film thickness; Varian, Walnut Creek, CA). Initial oven temperatures (50 °C) was held for 1 min then ramped at 5 °C/min to 160 °C where it was held for 42 min, and then ramped at 5 °C/min to 190 °C and held for 22 min. Inlet and detector temperatures were maintained at 250 °C, and the split ratio was 100:1. Hydrogen carrier gas flow rate through the column was 1 mL/min. Hydrogen flow to the detector was 30 mL/min, airflow was 400 mL/min, and nitrogen make-up gas flow was 25 mL/min. Peaks in the chromatogram were identified and quantified using pure methyl ester standards.

2.2 Statistical Analysis

Data were analyzed with ANOVA for a randomized complete block design. Repeated measures milk yield and composition, and that of milk fatty acids were analyzed using the MIXED procedure of SAS (SAS Institute, 2000), with the following model: $Yikj = \mu + \alpha i + \beta j + TK + (\alpha T)\ ik + eijk$, where μ is the population mean, αi is a population parameter corresponding to treatment (diet) i, βj is the fixed effect of block j, Tk is the fixed effect of time k, $(\alpha T)\ ik$ is the effect of treatment by time interaction, and eijk is the residual error. The Dunnet procedure was used to test the mean significant difference at $P < 0.05$.

3. Results and Discussion

Dry matter intake, milk yield and composition are presented in Table 2. Milk yield for the cows were fed NO-Lin diets was significantly lower than for those fed Lin diets ($P < 0.05$). Milk fat content was higher in cows fed NO-Lin diet compared with those fed Lin ($P < 0.05$). Protein and lactose contents did not vary among the cows of the farms evaluated. The increase in milk production observed when feeding the Lin diets might be attributed to the greater energy supply of these rations compared with the NO-Lin diets [14]. This increase in milk yield was not accompanied by the greater ($P < 0.05$) in fat concentration. Percentage of milk fat was significantly reduced when diets were supplemented with linseed.

A similar effect of linseed on milk fat has been observed by others [9]. Previous work indicates that a diet-induced change in rumen environment, such as the change induced by a low level of effective fiber is

Table 2 Feed intake, milk production and milk composition of Holstein lactating cows of dairy farms which are members of "ShafashirToos industrial dairy farmers production and distribution Cooperative" located in northeast Iran fed no linseed (NO-Lin) or linseed (Lin) diets at different lactation periods.

Items	NO-Lin	Experimental diets						S.E.M[1]	P^2
		Lin							
	Golshid-Mashhad Co.	Hanaei Co.	Ghasemi Co.	Taheri Co.	Teliseh Co.	Arasp Co.	Navingholparvar-Khorasan Co.		
Feed intake (kgDM/d)	18.3	20.6	21.1	21.1	20.3	19.5	20.4	-	-
Milk yield (kg/d)	31.4	36.5*	37.2*	38.6*	36.8*	35.2*	37.3*	2.1	< 0.05
Milk composition									
Fat (g/kg)	37.2	36.1*	35.3*	35.2*	32.7*	36.1*	36.2	0.5	< 0.05
Protein (g/kg)	30.9	30.8	30.7	30.0*	31.7*	30.8	32.3*	0.28	< 0.1
Lactose (g/kg)	48.2	46.9*	46.1*	45.1	47.6	46.8*	48.5	0.45	< 0.05

* Superscript signify means within rows differ from means for NO-Lin at $P < 0.05$.

1 S.E.M: Standard Error of Mean, 2 P: Probability ($P < 0.05$).

required to observe a significant reduction in milk fat synthesis when plant oils are fed [15]. Kalscheur et al. [16] also provide an example of minimal effect of plant oil supplementation on milk fat of diets that maintain a normal rumen environment.

Milk fatty acid profiles of cows are shown in Table 3. Cows were fed NO-Lin had higher proportion of C8:0, C10:0, C11:0, C12:0, C14:0, C14:1, C15:0, C15:1, C16:0, C16:1, C17:1, C22:0, C22:1 n9 and C24:1 in milk fat than those fed Lin diets. In general, there were no differences in most of the milk fatty acid profiles of cows in farms that received ground linseed but feeding Lin diets compared with NO-Lin, decreased significantly ($P < 0.05$) C8:0-C16:0 concentration and it increased those of C18:1 n9trans, C18:1 n9cis, C18:2 trans and C18:3 n3 fatty acids. As a result, there was a decrease in concentration of medium-chain and saturated fatty acids and a higher concentration of long chain fatty acids following the use of ground linseed in all farms. An increase in long-chain fatty acid concentration of milk was observed in the cows of farms that fed ground linseed in our study. These results confirm the data reported in previous studies [9, 17]. Similar differences in concentration of milk fatty acids were generally observed when rolled linseed was compared with whole linseed [3]. Feeding unprotected fat in the form of ground linseed is known to depress milk fat percentage [18], which likely results from the generation of high levels of trans-fatty acids in the rumen [19]. A greater dietary supply of C18:2 and C18:3 fatty acids in ground linseed compared to those of fed NO-Lin diet is also known to increase milk C18:1 concentration through ruminal bio-hydrogenation [20], which would cause an increase in proportion of C18:1 in the milk. There were significant increase of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) concentrations in cows fed linseed compared with those in the animals fed NO-Lin treatments. These data confirmed the results of da Silva et al. [9]. Substantial increase in milk MUFA and PUFA in our experiment, implying that sufficient quantities of PUFA present in the linseed escaped bio-hydrogenation in the rumen and ultimately were transferred to the milk fat by physical breakdown of the seed. In Petit and Cortes [21] experiment, although data shown no differences in PUFA concentration between whole and ground linseed, but they reported significant increase in PUFA concentration of whole and ground linseed compared with the control diet.

In our study, significant increase occurred in the proportion of both α-linolenic and linoleic acids in the fat milk of farms that cows fed with linseed diets. Most notable being α-linolenic acid (n-3), which nearly doubled in milk of cows fed ground linseed.

Table 3 Fatty acid profile in milk (g/kg milk containing 35 g fat/lit) of Holstein lactating cows of dairy farms which are members of "ShafashirToos industrial dairy farmers production and distribution cooperative" located in Northeast Iran fed no linseed (NO-Lin) or linseed (Lin) diets at different lactation periods.

Items	Experimental diets							S.E.M[1]	P^2
	NO-Lin	Lin							
	Golshid-Mashhad Co.	Hanaei Co.	Ghasemi Co.	Taheri Co.	Teliseh Co.	Arasp Co.	Navingholparvar-Khorasan Co.		
C4:0	0.664	0.722	0.784	1.007*	1.019*	0.88	1.765*	0.14	< 0.05
C6:0	0.737	0.46*	0.567	0.747	0.77	0.725	1.301*	0.1	< 0.05
C8:0	0.574	0.347*	0.344*	0.46	0.495	0.477	0.773*	0.05	< 0.05
C10:0	1.404	0.717*	0.867*	0.88*	1.074*	1.07*	1.438	0.1	< 0.05
C11:0	0.16	0.074*	0.083*	0.083*	0.121*	0.12*	0.141*	0.01	< 0.05
C12:0	1.608	0.788*	0.951*	0.854*	1.167*	1.189*	1.298*	0.1	< 0.05
C13:0	0.058	0.037*	0.052	0.027*	0.069	0.037*	0.04*	0.005	< 0.05
C14:0	4.715	2.845*	3.289*	2.588*	3.658	4.037*	3.567*	0.27	< 0.05
C14:1	0.693	0.351*	0.407*	0.341*	0.503*	0.509*	0.46*	0.04	< 0.05
C15:0	0.455	0.312*	0.342*	0.275*	0.401*	0.384*	0.36*	0.02	< 0.05
C15:1	0.193	0.088*	0.102*	0.064*	0.102*	0.123*	0.119*	0.01	< 0.05
C16:0	11.389	8.332*	8.442*	7.641*	8.507*	9.174*	8.112*	0.46	< 0.05
C16:1	1.011	0.659*	0.718*	0.591*	0.713*	0.719*	0.957	0.05	< 0.05
C17:0	0.23	0.212*	0.207*	0.207*	0.243*	0.234	0.214*	0.005	< 0.05
C17:1	0.108	0.086*	0.121	0.075*	0.097*	0.09*	0.119	0.006	< 0.05
C18:0	2.576	5.699*	4.871*	4.91*	3.959*	4.182*	3.796*	0.37	< 0.05
C18:1n9trans	0.52	1.042*	0.979*	1.484*	0.94*	0.827*	0.72*	0.11	< 0.05
C18:1n9cis	5.953	9.137*	9.05*	9.418*	8.477	7.806*	7.41*	0.46	< 0.05
C18:2trans	0.325	0.876*	1.166*	1.163*	0.868*	0.697*	0.556	0.11	< 0.05
C18:2n6cis	0.885	1.179*	0.99*	1.095*	0.914	0.793	0.869	0.05	< 0.05
C20:0	0.081	0.087	0.061*	0.097*	0.076	0.068*	0.064*	0.004	< 0.05
C18:3n3	0.205	0.484*	0.4*	0.473*	0.469*	0.485*	0.473*	0.03	< 0.05
C20:1	0.168	0.216*	0.217*	0.305*	0.201*	0.2	0.205*	0.01	< 0.05
C22:0	0.155	0.064*	0.073*	0.07*	0.049*	0.053*	0.06*	0.01	< 0.05
C22:1n9	0.0718	0.055	0.135*	0.062	0.057	0.053	0.05	0.01	< 0.05
C24:0	0.0008	0.088*	0.058*	0.035*	0.018*	0.036*	0.039*	0.01	< 0.05
C24:1	0.06	0.039*	0.016*	0.0456	0.032*	0.033*	0.087*	0.008	< 0.05
MUFA[3]	8.778	11.67*	12.61*	12.38*	11.12*	10.36*	10.132*	0.51	< 0.05
PUFA[4]	1.415	2.54*	2.55*	2.731*	2.253*	1.975*	1.898*	0.14	< 0.05
C18:2	1.21	2.06*	2.16*	2.26*	1.78*	1.49	1.43	0.15	< 0.05
C18:3	0.205	0.485*	0.4*	0.474*	0.469*	0.485*	0.473*	0.03	< 0.05
n6:n3	4.316	2.436*	2.477*	2.313*	1.948*	1.634*	1.839*	0.33	< 0.05
SCFA[5]	3.38	2.25*	2.56*	3.09	3.36	3.15	2.8*	0.16	< 0.05
MCFA[6]	18.61	12.6*	13.37*	11.68*	14.16*	15.17*	13.73*	0.84	< 0.05
LCFA[7]	2.81	5.94*	5.07*	5.11*	4.1*	4.34*	3.96*	0.38	< 0.05

* Superscript signify means within rows differ from means for NO-Lin at $P < 0.05$.

1. S.E.M: Standard Error of Mean, 2. P: Probability ($P < 0.05$).

3. MUFA (monounsaturated FA): C10:1, C14:1, C16:1, C17:1, C18:1 trans, C18:1cis, C20:1, C22:1, C24:1.

4. PUFA (polyunsaturated FA): C18:2 trans, C18:2 cis, C18:3n-3.

5. SCFA (short-chain FA): C4:0 to C10:0.

6. MCFA (medium-chain FA): C11:0 to C17:0.

7. LCFA (long-chain FA): > C18:0.

According to Glasser et al. [22], the proportion of 18:3 in milk fat increases by 79% with linseed supplementation compared with un-supplemented diets. Cows in farm that fed NO-Lin had the lowest proportion of C18:3 in milk fat, as previously reported [17, 21, 23]. Physical breakdown of linseed may contribute to an increase of availability of FA for absorption and transfer in milk partly as a result of more rapid passage rate out of the rumen with ground than whole seed, which would increase the concentration of α-linolenic acid and n-3 FA in milk [9].

4. Conclusions

The experiment demonstrated that the inclusion of linseed was effective at increasing both milk yield and milk polyunsaturated fatty acids, notably C18:3. While, it decreased the level of total C18:2. However, the addition of linseed to the diets decreased milk fat and might be associated with the addition of oils to the dairy diet. The ground linseed caused to decrease the ratio of milk C18:2 to C18:3 concentrations compared with the cows fed no linseed diets. In addition, to the increase in omega-3 fatty acid, diets containing linseed resulted in other favorable changes in the FA profile such as a decrease in saturated fatty acid concentrations. Therefore, these results clearly demonstrate the possibility for sustainable production of organic omega-3 milk with levels of C18:3 FA up to 2.4 times higher than typical levels in dairy fat. The manufacture of omega-3 enriched milk and milk products could supply dietary fatty acids at levels that may benefit health, without the need for unrealistic changes to eating habits.

Acknowledgments

The authors gratefully acknowledge the financial support provided by “ShafashirToos industrial dairy farmers’ production and distribution Cooperative” and Ferdowsi University of Mashhad.

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