

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/315272267>

# Modulation of Milk Fatty Acid Profile, Milk Yield and Composition through Supplementation of Omega-3 Fatty Acid in Transition Cow's Diet

Article · January 2015

CITATIONS

0

READS

192

7 authors, including:



**Girgo Galmessa Ulfina**

Ethiopian Institute of Agricultural Research

65 PUBLICATIONS 172 CITATIONS

[SEE PROFILE](#)



**Shiv Prasad**

G. B. Pant University of Agriculture and Technology, Pantnagar

108 PUBLICATIONS 583 CITATIONS

[SEE PROFILE](#)



**Arumugam Kumaresan**

Southern Regional Station of ICAR - National Dairy Research Institute

223 PUBLICATIONS 2,118 CITATIONS

[SEE PROFILE](#)



**Parvinder Singh Oberoi**

National Dairy Research Institute

21 PUBLICATIONS 64 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Duckweed innovation project as Dairy animal feed: MILK QUALITY AND YIELD EVALUATION OF CROSSBRED DAIRY COWS FED WITH OR WITHOUT DUCKWEED REPLACED COMMERCIAL DAIRY RATION IN ETHIOPIA [View project](#)



Unusual interplay of contrasting selective pressures on  $\beta$ -defensin genes implicated in male fertility of the Buffalo (*Bubalus bubalis*) [View project](#)

# Modulation of Milk Fatty Acid Profile, Milk Yield and Composition through Supplementation of Omega-3 Fatty Acid in Transition Cow's Diet

Ulfina Galmessa<sup>1</sup>, Shiv Prasad<sup>2</sup>, A. Kumaresan<sup>3</sup>, P. S. Oberoi<sup>4</sup>,  
R. K. Baithalu<sup>3</sup>, U. Neelam<sup>5</sup>, and A. K. Dang<sup>6</sup>

<sup>1</sup>Animal Sciences Department, College of Agriculture and Veterinary Sciences, Ambo University,  
P. O. Box 19, Ambo, Ethiopia. Corresponding author, E-mail: r.ulfinaa@yahoo.com

<sup>2</sup> Indian Council for Agricultural Research-DARE, New Delhi - India

<sup>3</sup> Livestock Production and Management, National Dairy Research Institute (NDRI), Karnal-Haryana, India

<sup>4</sup> Livestock Production and Management, National Dairy Research Institute (NDRI), Karnal-Haryana, India

<sup>5</sup>Dairy Chemistry Division, National Dairy Research Institute (NDRI), Karnal-Haryana, India

<sup>6</sup>Dairy Cattle Physiology Division, National Dairy Research Institute (NDRI), Karnal-Haryana, India

## Abstract

Multiparous crossbred cows (Holstein Frisian \* Tharparkar) (n = 16) were used to study the effect of omega-3 fatty acid (Flaxseed) supplementation on milk yield, milk composition and modulation of fatty acid profile of milk fat. The selected cows were randomly allotted to one of the 2 treatment groups, 8 cows each based on their parity, weight and their EPA (Expected producing ability), under Completely Randomized Design (CBD). Cows in control group were fed the routine NDRI ration, whereas the treatment group received 0.75 kg crushed flaxseed (Omega-3 fatty acid) per day per head over and above the routine feeding of the institute. Supplements were fed during transition period (3 weeks before expected calving and until 3 weeks after parturition). Milk samples for milk composition and fatty acids analysis were collected at weekly interval starting second day of parturition. Milk composition was estimated using Lacto Scan milk analyzer. Milk yield was immediately recorded during milking using sensitive balance. Fat corrected milk, solid corrected milk and the energy density of milk were calculated as per their formulae. For milk fat acid profile analysis, fatty acid methyl esters (FAME) were separated and measured in packed glass column using gas chromatograph (NETEL Chromatograph, Michro-9100), equipped with flame ionization detector and temperature control modules. Data were analysed using analysis of variance (ANOVA) procedures of SAS. Results indicated that treatment had significant effect on milk yield, peak milk yield, and milk protein concentration and milk fat profiles. Saturated fatty acids (SFA) in milk fat were significantly reduced, whereas poly-unsaturated fatty acids (PUFA) were significantly increased in omega-3 fatty acid (Flaxseed) supplemented groups. The most remarkable result was the highly significant increase of  $\alpha$ -Linolenic acid (C18:n3) (by 174.6%) in omega-3 fatty acid supplemented cows' milk fat compared with control cows. Moreover, short chain fatty acids were depressed while long chain fatty acids were significantly ( $P < 0.05$ ) enhanced by inclusion of 6.5% crushed flaxseed in the ration of dairy cows. Thus, it can be concluded from the present study that inclusion of crushed flaxseed (Omega-3 fatty acid) in the ration of milking animals during transition period increase milk yield and modulate the fatty acid profile of milk fat which would ultimately improve the therapeutic properties of the milk for consumers.

**Key words:** Omega-3 fatty acid, fatty acid profile, PUFA, milk yield, milk composition, dairy cows

---

## Introduction

The potential health benefits of polyunsaturated fatty acids (PUFA) such as n-3 family are tremendous. In present scenario, people have become more aware and health-conscious and are interested in including products healthier and safer in their diets. Milk and milk products are rich in fat which contains more saturated fatty acids rather than unsaturated fatty acids. These saturated fatty acids are not safe for human health. For example, Bobe (2013) described that diets high in saturated fat like myristic (C14:0) and palmitic (C16:0) acids have been found to have undesirable hypercholesterolemic effects which increases the risk of coronary heart disease. On the other hand, long chain polyunsaturated fatty acids (PUFA), especially Linoleic (C18:2n-6), and  $\alpha$ -Linolenic (C18:3n-3) have anti-carcinogenic (Parodi, 1997; Bobe, 2013) and potentially cardio-protective roles in humans (Massaro *et al.*, 1999). As a result, there is growing interest to manipulate dairy cow diets to increase concentrations of PUFA in milk fat. To this effect, scientists all over the world are trying to increase both monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) in milk by dietary manipulation.

Traditional cattle feed mixtures of maize, grains, alfalfa hay and other grass silages results in dairy products with low concentrations of Omega-3 fatty acids and other PUFAs (Bobe, 2013) which indicate the need to introduce feed supplement high in

essential fatty acid like omega-3 to the ration of dairy animals. Flaxseed is the richest source of  $\alpha$ -Linolenic acid (ALA, 18:3n-3) among all oilseeds and is known to increase the concentration of PUFA in milk in dairy animals (Kennelly, 1996). Flaxseed contains a high oil level (40% of total seed weight), with  $\alpha$ -Linolenic acid constituting about 58% of oils total fatty acids (Mustafa *et al.*, 2002, Petit, 2002, 2003). Omega-3 fatty acids (including  $\alpha$ -linolenic acid) have several health benefits to humans including a decrease in the incidence of different types of cancer (skin, breast, colon, uterus), cardiovascular diseases, hypertension, reduction in the risk of mental disorders (postpartum depression), manic-depressive psychosis, dementias such as alzheimeris disease, arthritis and an improvement in visual acuity (Simpolous, 1996; Weight *et al.*, 1998; Freemantle *et al.*, 2006; Harris *et al.*, 2008). Moreover, diets rich in omega-3 fatty acids (including- Linolenic acid) reduces blood platelet aggregation, blood triglycerides and cholesterol levels and the occurrence of blood clots and show both antithrombolytic and anti-inflammatory effects ( Nash *et al.*, 1995; Leeson and Caston, 1996; Simpoulos, 1996).

It is anticipated that flaxseed having high oil content may be used as a fat source in dairy cows ration. Feeding flaxseed does not only support higher milk production but also improves reproductive efficiency (better conception rate and lower pregnancy losses (Petit *et al.*, 2001; Petit and Twagramungu, 2006) and the immunity

status of dairy cows (Yaqoob and Calder, 1995; Miles and Calder, 1998; Petit and Twagiramungu, 2006). It is also known to have environmental importance since it reduces intrinsic methane production from ruminants (Martin *et al.*, 2008). Therefore, the present study was designed to increase milk production and change milk fatty acid profile and milk composition through supplementation of flaxseed as source of omega-3 fatty acids in dairy cows during transition period.

## Materials and Methods

### Description of the study

#### area

The present study was conducted at Livestock Research Centre of National Dairy Research Institute (N.D.R.I.), Karnal - Haryana, India. The NDRI, Karnal is located on 29° 43' N latitude and 76° 58' E longitudes at an altitude of 250 meters above the mean sea level in the bed of Indo-Gangetic alluvial plain. The area has four major seasons in the year viz. winter (December to March), summer (April to June), rainy (July to September) and autumn (October and November). The minimum ambient temperature falls to near freezing point in winter and maximum goes approximately up to 45°C in May/June months of summer. The average annual rainfall is 700 mm, most of which is received from July to September. A subtropical climate prevails in the area. The land in the area is very productive with sufficient irrigation facilities for year round growing green fodder for the animals.

### Animals and feeding management

Karan Fries (KF) crossbred cows (Tharparkar \* Holstein) maintained at Livestock Research Centre of National Dairy Research Institute, Karnal-Haryana state, India were utilized for the study. Sixteen multiparous pregnant cows entering their 2<sup>nd</sup> parity and above were selected for transition period feeding experiment (3 weeks before and 3 weeks after parturition). The selected cows were randomly allotted on the basis of their parity, weight and their EPA (Excepted producing ability) value to one treatment group and one control group of 8 cows each under Completely Randomized Design (CRD). Cows in control group were fed the routine NDRI ration for pregnant and lactating cows (Table 1), whereas the treatment group received 750 g crushed flaxseed (Omega-3) per day per head over and above the routine feeding of the institute. All cows were fed on iso-energetic diet formulated as per the recommendation of NDRI to meet or exceeded the predicted requirements of National Research Council (NRC, 2001). All cows were fed *ad libitum* with fresh feed provided each morning after milking. The concentrate was fed at the rate of 2.0 kg per animal for body maintenance in general. Milking cows were given additional concentrate at the rate of 1.0 kg for every 2.5 kg milk production, above 5.0 kg milk yield. Water was available free choice.

Table 1. Proximate composition and fibre fractions of feeds used at NDRI farm

Feedstuffs	DM%	CP%	EE	NDF	ADF	CF	Total Ash
Maize ( <i>Zea mays</i> )	17.31	8.82	2.52	57.22	37.64	28.8	9.43
Oats ( <i>Avena sativa</i> )	15.00	9.00	1.92	64.34	40.94	26.29	9.53
Jowar ( <i>Sorghum bicolor</i> )	23.00	7.64	1.76	27.81	38.42	31.42	9.25
Berseem ( <i>Trifolium alexandrinum</i> )	14.92	14.69	2.57	47.89	38.89	26.96	15.7
Wheat straw	90.00	3.54	0.87	87.01	50.20	52	10.80
Concentrate	90.00	22.01	5.33	30.82	16.10	14.63	7.90

### Milk sampling and analysis

Milk samples for milk composition and fatty acids analysis were obtained from 8 cows of each dietary group at weekly interval starting from the second day of parturition. Samples were collected from 3 consecutive milkings (morning, noon, evening) mixed in proportion to obtain a composite sample. The milk composition (Fat %, protein %, SNF %, Lactose %) was estimated using Lacto Scan milk analyzer. The milk samples were maintained at 28-32°C at the time of analysis, which is the calibration temperature of analyzer. Milk yield was immediately recorded during milking using sensitive balance.

**Fat corrected milk (FCM):** Milk production can be converted to a fat corrected basis to evaluate the energy output in milk. The formula for kg of 4% fat corrected milk (FCM) used is:  $(0.4 \times \text{milk}) + [15 \times (\% \text{fat}/100) \times \text{milk}]$ .

**Solid corrected milk (SCM):** Solid corrected milk is usually calculated for the subsequent determination of milk energy. SCM was calculated by the

following equation given by Tyrrel and Reid (1965).

$$\text{SCM (kg)} = 12.3 (\text{F}) + 6.56 (\text{SNF}) + 0.0752 (\text{M})$$

Where, F, SNF and M were expressed as kg of fat, kg of solid non fat and kg of milk yield respectively.

### Milk fatty acid profile

A separate 2 ml of milk samples from each cow were collected every 7 days starting from the 2<sup>nd</sup> day of parturition up to 4<sup>th</sup> week of DIM. The aliquot was stored at -20°C for Fatty acid analysis. Milk fat was extracted according to the modified procedures of O'Fallon *et al.* (2007). Fatty acid methyl esters (FAME) were separated and measured in packed glass column using gas chromatograph (NETEL Chromatograph, Michro-9100), equipped with flame ionization detector and temperature control modules.

A programme of total 36 min was standardized. The sample was injected at an initial temperature of 50°C maintained for 3 min and then raised

after the emergence of butyric acid peak to 150°C by holding for 3 min till the emergence of myristic acid (C<sub>14:0</sub>) peak with a ramp rate of 5°C/min, then again raised up to 200°C with same ramp rate till the emergence of oleic acid (C<sub>18:1</sub>) and then maintained at 200°C for 5 min until emergence of the peak of Linolenic acid on chromatograph.

The reference standards of different saturated and unsaturated fatty acids were also run under similar conditions of the gas liquid chromatography for identifying the particular fatty acid by comparing their retention time with that of obtained fatty acids profile.

### Specification for running the Gas liquid chromatograph

- Glass column: Length 1.80 cm x ¼ inch internal diameter, packed with 10 per cent diethylene glycol succinate (DEGS)
- Detector: Flame Ionization Detector
- Carrier gas flow rate: 30ml/min.
- Carrier gas: Nitrogen
- Air pressure: 1psi
- Detector temperature: 240 °C.
- Injector temperature: 230 °C.
- Sample volume: 5µl.
- Nitrogen pressure: 3psi
- Hydrogen pressure: 2.5psi

By using the above specifications, chromatographs of different fatty acids profile were obtained and the percentage of each fatty acid was calculated automatically by software provided by the GLC Company.

Significance was declared at  $P \leq 0.05$  and a trend at  $0.05 < P \leq 0.1$ , unless otherwise stated. When a significant F-test was detected, multiple comparisons were made to discriminate among the means using Tukey's honestly significant difference (HSD) procedure.

### Statistical analysis

All data were subjected to ANOVA for Completely Randomized Design using the Procedure of General Linear Model of SAS (SAS institute, 2002) for repeat measures. GraphPad PRISM was used to analyze both in group and/or in Column wise for lines and graph comparisons. The following model was used for most of the parameters:

$$Y_{ijkl} = \mu + T_i + P_j + (T*P)_k + e_{ijkl}$$

Where,  $y_{ijkl}$  = mean of individual observation,

$T_i$  = the effect of treatment,

$P$  = effect of DIM (days in milk),

$(T*P)_k$  = interaction between treatment effect and DIM, if any,

$e_{ijkl}$  = random error effect

## Results and Discussion

### Milk yield (MY), Fat corrected milk yield (FCMY) and Solid corrected milk yield (SCMY)

The least square means of weekly milk yield in KF crossbred cows fed on diets supplemented with omega-3 fatty acid (Flaxseed) and control diet are presented in Table 2. The milk yield for the first week for omega-3 fatty acid supplemented ( $13.72 \pm 1.379$ ) and control group of cows ( $12.36 \pm 1.621$ ) were statistically similar. However, higher milk yield at the second week of lactation for cows fed on diets

supplemented with omega-3 Fatty acid ( $18.72 \pm 0.684$ ) as compared to control cows ( $16.29 \pm 1.097$ ) was significant ( $P < 0.05$ ). At week 3 ( $P = 0.0642$ ) and week 4 ( $P = 0.0597$ ) cows from the flaxseed group showed a tendency to yield higher milk than the other group of cows. Thereafter, the difference in milk yield was not significant up to 7 weeks or around 60 DIM (days in milk). It was observed that cows from control group reached their peak yield by 4 weeks earlier than the cows fed on diets supplemented with omega-3 fatty acid. By the 8<sup>th</sup> week, cows supplemented with omega-3 fatty acid (flaxseed) outsmarted the other group up to 11<sup>th</sup> weeks of lactation where they reached their peak milk production. Consequently, highly significant difference ( $P < 0.01$ ) was observed in milk production between omega-3 fatty acid supplemented and control cows at 10<sup>th</sup> and 11<sup>th</sup> weeks of lactation.

From the present study it was clearly observed that omega-3 fatty acid (flaxseed) supplementation to cows increased their milk yield and lactation peak which would have positive implication in the total milk production. These results are in line with the findings from Petit *et al.* (2004) who observed higher milk yield in cows fed flaxseed compared with those fed control diet (sunflower seed). Petit *et al.* (2007) also reported higher milk yield and lower fat percent for cows fed whole flaxseed as compared to those fed on supplement rich in SFA which corroborates the present finding. Similarly, the study on the effect of prill fat supplementation on milk production

and composition during mid-lactation in crossbred cows (Mahendra *et al.*, 2014) revealed that milk yield was significantly ( $P < 0.05$ ) higher in supplemented group as compared to control cows. Moreover, da Silva *et al.* (2009) observed that milk production tended to be greater when cows were fed with ground flaxseed (22.8 kg/d) as compared to those fed whole flaxseed (21.4 kg/d). But there are contradictory literatures to the present findings on the effect of flaxseed on milk yield (Petit, 2002; Ambrose *et al.*, 2006). The discrepancy might be the processing method (crushed seed) we used instead whole flaxseed that most study used, even though da Silva *et al.* (2009) observed that processing of flaxseed had no effect on 4% fat-corrected milk yield.

In agreement to the present findings, Petit *et al.* (2003) reported that there is an increase in the milk production by 2.65 kg/d in formaldehyde treated flaxseed as compared to whole flaxseed supplemented cows. They also observed increased milk production efficiency (1.31 vs. 1.21) expressed in kg of 4% FCM per kg of DMI. In contrary, Martin *et al.* (2008) and Cortes *et al.* (2010) reported statistically similar milk yield in cows fed crushed flaxseed in DM compared with those fed no flaxseed. Inclusion of rolled flaxseed from 9% to 15% on DM basis did not affect milk yield (Ambrose *et al.*, 2006). Petit (2002), who fed either whole untreated or whole formaldehyde treated flaxseed or sunflower seed, also found no difference in milk yield among treatment groups.

Table 2. The least squares means ( $\pm$  SE) of weekly MY of KF cows fed on diets supplemented with omega-3 FA (flaxseed) during Transition Period

Milk yield (kg)	Treatments		Significance level
	Omega-3 FA	Control	
Week 1	13.72 $\pm$ 1.379	12.36 $\pm$ 1.621	ns
Week 2	18.72 $\pm$ 0.684	15.29 $\pm$ 1.097	*
Week 3	20.92 $\pm$ 0.730	19.15 $\pm$ 0.943	ns
Week 4	21.94 $\pm$ 0.896	20.06 $\pm$ 1.085	ns
Week 5	22.14 $\pm$ 0.770	20.14 $\pm$ 1.283	ns
Week 6	22.58 $\pm$ 0.773	20.58 $\pm$ 1.340	ns
Week 7	23.09 $\pm$ 0.789	21.11 $\pm$ 1.393	ns
Week 8	23.55 <sup>a</sup> $\pm$ 0.712	19.73 <sup>b</sup> $\pm$ 1.377	*
Week 9	23.61 <sup>a</sup> $\pm$ 0.684	18.83 <sup>b</sup> $\pm$ 1.497	*
Week 10	23.75 <sup>a</sup> $\pm$ 0.579	18.54 <sup>b</sup> $\pm$ 1.286	**
Week 11	23.11 <sup>a</sup> $\pm$ 0.701	17.91 <sup>b</sup> $\pm$ 1.249	**
Week 12	22.17 $\pm$ 0.486	18.54 $\pm$ 1.077	ns
Week 13	22.13 $\pm$ 0.917	19.40 $\pm$ 1.274	ns
Week 14	21.34 $\pm$ 1.014	18.09 $\pm$ 0.987	ns
Week 15	20.58 $\pm$ 1.022	17.25 $\pm$ 0.945	ns

The yield of 4% fat-corrected milk (FCMY, kg/d), solid corrected milk yield (SCMY, kg/d) and milk energy (Mcal) are presented in Table 3. The mean differences in FCMY in the two feeding groups was not significant ( $P > 0.05$ ), whereas the mean differences in SCMY and milk energy were significant between treatments.

### Milk composition

Milk fat is the most variable of milk composition. The least squares means of fat percent in non-supplemented and supplemented cows are presented in Table 3 and Figure 1. The slightly lower milk fat content in cows fed flaxseed ( $4.385 \pm 0.2010$ ) was statistically similar to those cows fed with control diet ( $4.680 \pm 0.1243$ ). The current finding was corroborated by Petit *et al.* (2007) who reported that fat supplementation reduce fibre intake which had increase

fat percent of milk even though there was no significant differences in feed intake. They also reported higher milk yield and lower fat percent for cows fed whole flaxseed as compared to those fed on supplement rich in SFA. Decreased milk fat percentage was also observed (Mustefa *et al.*, 2002) in cows fed 7% raw flaxseed, and in cows fed a supplement containing 70% extruded flaxseed at 21.2% of the diet (Chilliard *et al.*, 2009). The type of processing used in the present study (crushing) might play significant role to enhance the passage of flaxseed to the lower tract and hence less oil being released in the rumen, which would limit the negative effect of oil on fibre digestion and thus on DMI, although, da Silva *et al.* (2009) observed no effect of processing of flaxseed on milk fat concentration.



The least squares means of protein percent in milk of cows supplemented with or without omega-3 fatty acid are depicted in Table 3 and Figure 1. There was significant ( $P<0.05$ ) variation in protein concentration between the two groups of cows. The high mean protein content in flaxseed supplemented cows ( $3.419 \pm 0.0316$ ) was significantly ( $P<0.0001$ ) different from control cows ( $3.218 \pm 0.0157$ ). Similarly, Petit *et al.* (2003) reported higher concentration of protein in milk for cows fed flaxseed (3.38%) compared with sunflower seed (3.21%) although inclusion of flaxseed in the diet did not change concentration or

yield of protein in other studies (Kennelly and Khorasani, 1993; Petit *et al.*, 2004).

The two way analysis of variance for Solid not fat (SNF) percent showed highly significant difference for treatments and DIM ( $P<0.01$ ). Results indicated that cows from flaxseed group yielded ( $9.854 \pm 0.433$ ) higher ( $P<0.01$ ) SNF compared with control ones ( $8.979 \pm 0.14$ ) (Table 3). The present result is at par with the findings of Petit (2003) who reported similar SNF concentrations among cows fed on flaxseed and control diet during mid-lactation.

Table 3: Milk composition, FCMY, SCMY and milk energy of crossbred cows supplemented with omega- 3 fatty acid source flax seed DTP

Milk constituents	Treatment		Significance level
	Omega-3 FA	Control	
Milk yield, kg/d	$19.87 \pm 0.449$	$17.47 \pm 0.449$	*
FCMY	$23.14 \pm 0.805$	$22.26 \pm 1.418$	ns
SCMY	$27.82^a \pm 1.290$	$22.83^b \pm 6.010$	**
Milk energy (Mcal)	$20.8614^a \pm 0.968$	$17.125^b \pm 0.968$	**
Milk composition			
Fat (%)	$4.385 \pm 0.2010$	$4.680 \pm 0.1243$	ns
Protein (%)	$3.419^a \pm 0.0316$	$3.218^b \pm 0.0157$	**
SNF (%)	$9.854^a \pm 0.433$	$8.979^b \pm 0.141$	**
Lactose (%)	$4.875 \pm 0.144$	$5.144 \pm 0.144$	ns

\* Means which bears different superscripts in a row differ significant at  $P<0.05$

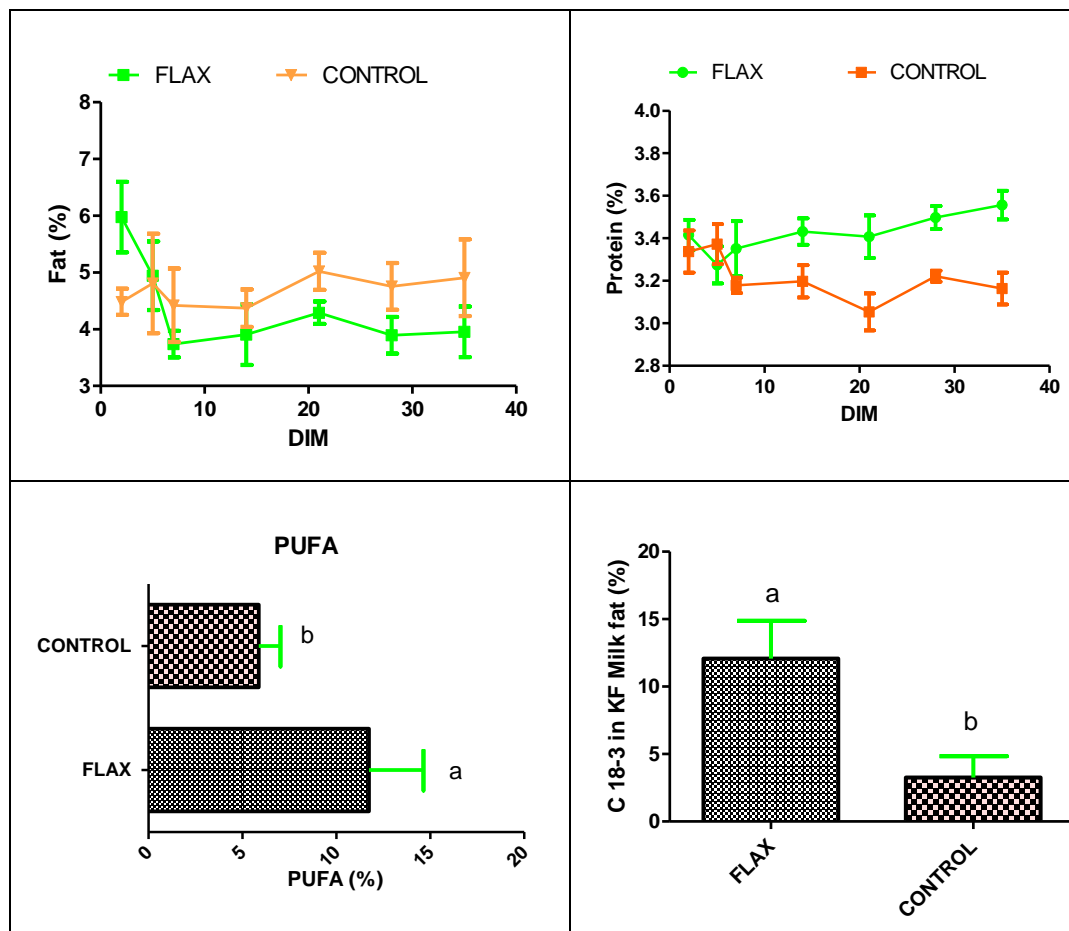


Figure 1. Milk fat (%), Protein (%), PUFA (%) and C18-n3 (%) of KF cows supplemented with omega-3 fatty acid during transition period

Lactose, the most stable constituents of milk composition did not vary also in the present study. The present result is corroborated by Petit (2003) who reported similar lactose concentrations between supplemented and control group of cows.

**Milk fat acid profile**

The effects of the experimental diet on fatty acid profiles of milk fat are presented in Tables 4 & 5. Supplementation with omega-3 (flaxseed) during transition period has

significantly ( $P < 0.05$ ) affected milk fatty acids profile. Results indicated that the concentration of C4 ( $2.69 \pm 0.05$ ) was lower ( $P < 0.05$ ) in the milk fat of flaxseed supplemented cows when compared with those fed on control diets ( $4.27 \pm 0.06$ ). Concentrations of C6, C7, C8, C10, C14, and C15 were similar among treatments. But feeding flaxseed significantly ( $P < 0.05$ ) increased the concentration of all C18 fatty acids in milk fat compared with control cow's milk fat.

Dietary treatments had a significant impact on milk fatty acid profile where short chain fatty acids were increased in the milk fat of cows from control group, whereas, long chain fatty acids increased in the flaxseed supplemented group (Table 4 & 5). The most remarkable aspect of these results is the very high level of  $\alpha$ -Linolenic acid, ALA (C18:3) (6.37% of total FA) in milk from cows receiving the crushed flaxseed, demonstrating the large potential to increase omega-3 FA in milk (Figure 1). In the present study we provided fat in the form of crushed flaxseed which probably increased the passage of flaxseed and reduce the probability of bio-hydrogenation in the rumen. As a

result, the content of ALA in milk fat was increased by 174.6% and that of Linoleic acid (C18:2) content increased by 83.5% with flaxseed supplementation as compared to the control group. Short chain fatty acids were depressed while long chain fatty acids were significantly ( $P < 0.05$ ) enhanced by inclusion of 6.5% crushed flaxseed in the ration of dairy cows. However, higher concentration of  $\alpha$ -Linolenic acid C18:3 and Linoleic acid C18:2 than the present study were also reported by Ambrose *et al.* (2006), who observed depressed medium chain fatty acids but increased long chain fatty acids when cows were subjected to flaxseed supplementation.

Table 4: Least Squares Means of Fatty acid profile in milk fat of KF cows supplemented

Fatty acid % of total fatty acids	Treatment		Significance level
	Omega-3 FA	Control	
Butyric; C4:0	2.69 <sup>b</sup> ± 0.05	4.27 <sup>a</sup> ± 0.96	*
Caproic; C6:0	1.63 <sup>b</sup> ± 0.03	3.04 <sup>a</sup> ± 0.05	*
Caprylic; C8:0	0.77 ± 0.03	1.95 ± 0.04	Ns
Decenoic; C10:1	2.18 ± 0.06	2.73 ± 0.08	Ns
Dodecenoic; C12:1	2.33 <sup>b</sup> ± 0.05	3.37 <sup>a</sup> ± 0.05	*
Myristic; C14:0	7.91 <sup>b</sup> ± 0.09	11.98 <sup>a</sup> ± 0.1	*
Myristoleic; C14:1	1.86 ± 0.05	1.21 ± 0.05	Ns
Pentadecylic; C15:1	0.99 ± 0.04	0.91 ± 0.05	Ns
Palmitic; C16:0	25.74 <sup>b</sup> ± 0.43	27.19 <sup>a</sup> ± 0.45	**
Palmitoleic; C16:1	1.62 ± 0.05	1.55 ± 0.06	Ns
Margaric acid; C17:0	0.41 ± 0.03	0.41 ± 0.06	Ns
Stearic; C18:0	12.23 ± 0.19	11.64 ± 0.14	Ns
Oleic acid; C18:1	24.57 <sup>a</sup> ± 0.76	21.58 <sup>b</sup> ± 0.52	**
Linoleic acid; C18:2	5.37 ± 0.09	3.57 ± 0.08	Ns
Arachidic; C20:0	3.36 <sup>a</sup> ± 0.07	2.76 <sup>b</sup> ± 0.06	*
$\alpha$ -Linolenic acid; C18-3	6.37 <sup>a</sup> ± 0.06	2.32 <sup>b</sup> ± 0.06	**

\*means with different superscripts in a row differ significant at  $P < 0.05$ .

Table 5. Least Squares Means  $\pm$  SE concentrations of different fatty acids in milk of cows fed n diets supplemented with omega – 3 fatty acid (flaxseed) DTP

Type of FA	Treatments		
	Omega-3 FA	Control	Significance level
LCFA	80.66 <sup>a</sup> $\pm$ 1.424	69.61 <sup>b</sup> $\pm$ 2.511	**
SCFA	19.37 <sup>b</sup> $\pm$ 1.232	28.55 <sup>a</sup> $\pm$ 1.131	**
SFA	54.74 <sup>b</sup> $\pm$ 2.860	63.24 <sup>a</sup> $\pm$ 1.526	**
USFA	45.29 <sup>a</sup> $\pm$ 2.807	34.92 <sup>b</sup> $\pm$ 1.771	**
PUFA	11.74 <sup>a</sup> $\pm$ 1.807	5.87 <sup>b</sup> $\pm$ 1.009	**
MUFA	33.55 $\pm$ 1.445	29.03 $\pm$ 2.483	Ns

\*Means with different subscripts in a row differ significantly, LCFA=long chain fatty acid; SCFA=short chain fatty acid; SFA=saturated fatty acid; USFA=unsaturated fatty acid; PUFA=polyunsaturated fatty acid; MUFA=monounsaturated fatty acid; Flax=flaxseed with omega-3 fatty acid (Flaxseed) DTP

The proportion of Saturated fatty acids (SFA) in milk fat was 8.5% higher ( $P < 0.001$ ) in the control group than in the flax group, whereas, those of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in milk fat were about 9.4% higher ( $P < 0.001$ ) in the flaxseed supplemented cows than the control group. The values observed in the present study were higher than those reported by Zachut *et al.* (2010) for extruded flaxseed, Gointher *et al.* (2005) for raw, micronized and extruded flaxseed feed (12.7% of DM) and Chilliard *et al.* (2009), who fed cows on a diet containing 21.2% of a supplement containing 70% extruded flaxseed. Discrepancies between studies could be attributed to differences in the type of processing, intakes, and production level. In the present study the processing method used (crushed instead of whole flaxseed) might have contributed to significant variation in saturated and unsaturated fatty acids. Similarly, in the feeding of ground compared with whole flaxseed, da Silva *et al.* (2009) observed decreased concentrations of

C16:0, C17:0, and *cis*6-20:4 and increased those of *cis* 6 - C18:2, *cis* 9, *trans* 11- C18:2, and *cis* 3 - C18:3 in milk fat. In corroboration to the present findings, they observed a decrease in concentrations of medium-chain and saturated fatty acids and a trend for higher concentrations of long-chain fatty acids in milk fat by feeding ground compared with whole flaxseed.

## Conclusion

The present study was conducted to assess changes occurring in milk fatty acid profiles, milk yield and composition as a result of omega-3 source flaxseed supplementation to ration of crossbred cows. The results clearly show that supplementation of omega-3 fatty acid in the diet of dairy cows significantly increase milk yield, milk protein percent; alters the chain of fatty acids and increase unsaturated fatty acids in milk fat, especially that of C18-n3 ( $\alpha$ -Linolenic acid). Therefore, it can be concluded that supplementation of crushed (processed) flaxseed successfully modulate the fatty acid

composition of milk fat that might improve the nutritional value of milk from human health point of view.

## Acknowledgement

The authors would like to acknowledge the Indo-African fellowship programme for fund grant. The technical and material help rendered by Dairy Chemistry and Animal Biochemistry Departments were highly acknowledged. Special thanks go to Dr. Kamal Gandhi of dairy chemistry and Dr. Rav Kant Saini of Animal Biochemistry who rendered help during FAME preparation as well as Fatty acid analysis using GC.

## References

- Ambrose, J. D., J. P. Kastelic R. Corbett, P. A. Pitney, H. V. Petit, J. A. Small, P. Zalkovic. 2006. Lower pregnancy losses in lactating dairy cows fed a diet enriched in  $\alpha$ -Linolenic acid. *J. Dairy Science*, **89**: 3066–3074.
- Biplob, K. R. 2011. Body condition score in relation to certain physiological and performance parameters of Sahiwal and Karan Fries cows. Ph.D. Thesis submitted to National Dairy Research Institute, NDRI, Karnal (Haryana)-India.
- Bobé, G. 2013. Cows fed flaxseed produce more nutritious dairy products. *J. Dairy Sci.*, Online: retrieved on 31<sup>st</sup> June, 2014.
- Chilliard, Y., C. Martin, J. Rouel & M. Doreau. 2009. Milk fatty acids in dairy cows fed whole crued linseed, extruded linseed, or linseed oil, and their relationship with methane output. *J. dairy Sci.*, **92**: 5199-5211.
- Chouinard, P. Y., V. Girard & G. J. Brisson. 1998. Fatty acid profile and physical properties of milk fat from cows fed calcium salts of fatty acids with varying unsaturation. *J. Dairy Sci.*, **81**: 471-481.
- Cortes, C., D. C. da Silva-Kazama, R. Kazama, R. Gagnon, C. Bencheur, G. T. D. Santos, L. M. Zeoula & H. V. Petit. 2010. Milk composition, milk fatty acid profile, digestion, and ruminal fermentation in dairy cows fed whole flaxseed and calcium salts of flaxseed oil. *J. Dairy Sci.*, **93** (7-8):3146-3157.
- da Silva, D. C., G.T.D. Santos, A.F. Branco, J.C. Damasceno, R. Kazama, M. Matsushita, J. A. Horst, W.B.R. dos Santos, H.V. Petit. 2009. Production performance and milk composition of dairy cows fed whole or ground flaxseed with or without monensin. *J. Dairy Sci.*, **90**: 2928-2936.
- Freemantle, E., M. Vandal, J. Trymbaly\_Mercier, S. Trymbaly, J. C. Blachere and M. E. Begin. 2006. Omega-3 fatty acids, energy substrates and brain function during aging. *Prostaglndins, Leukotrienes and Essential fatty acids*, **75**(3): 213-220.
- Gonhier, C., A. F. Mustafa, D. R. Ouellet, P.Y. Chouinard, R. Berthiaume H.V. Petit. 2005. Feeding micronized and extruded flaxseed to dairy cattle: effect on blood parameters and milk fatty acid composition. *J. Dairy Sci.* **88**: 748-756.
- Harris, W. S., M. Miller, A. P. Tighe, M. H. Davidson and E. J. Schaefer. 2008. Omega-3 fatty acids and coronary heart disease risk: Clinical and mechanistic perspectives. *Atherosclerosis*, **197**(1): 12-24.
- Kennelly, J. J. & G. R. Khorasani. 1993. Influence of flaxseed feeding on fatty acid composition on cow's milk. In proceedings of 54<sup>th</sup> Flaxseed Inst.

- Conf., Fargo, ND. J. F. Carter, ed. North Dakota state Univ., Fargo.
- Kennelly, J. J. 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. *Anim. Feed Sci. Technol.*, **60**:137-152.
- Leeson, S. and L. Caston. 1996. Getting the Omega into the egg. Feeding flaxseed to hens boosts human health. *Agri. Food Res.*, **19**(3): 6-8.
- Martin, C., J. Rouel, J. P. Jouany, M. Doreau and Y. Chillyard. 2008. Methane output and diet digestibility in response to feeding dairy cow's crude linseed, extruded linseed, or linseed oil. *J. Ani. Sci.*, **86**: 2642-2650.
- Massaro, M., Carluccio, M. A. and de Caterina, R. 1999. Direct vascular antiatherogenic effects of oleic acid: a clue to the cardiprotective effects of the Mediterranean diet. *Cardiologia*, **44**: 507-513.
- Miles, E. A. and Calder, P. C. 1998. Modulation of immune function by dietary fatty acids. *Proceedings of Nutritional Society*, **57**:277-292.
- Mustafa, A. F., Mckinnon, J. J., Christensen, D. A. And He, T. 2002. Effect of feeding micronization of flaxseed on nutrient disappearance in the gastrointestinal tract of steers. *Anim. Feed Sci. Technol.*, **49**:123-132.
- Nash, D. M., R. M.G. Hamilton and H. W. Hulan. 1995. The effect of dietary herring meal on the omega-3 fatty acid content of plasma and egg yolk lipids of laying hens. *Can. J. Anim. Sci.*, **75**: 247-253.
- O' Fallon, J. R., Nelson, M. L. & C. T. Gaskins. 2007. A direct method for fatty acid methyl ester synthesis: application to wet meat, tissues, oils, and feedstuffs. *J. Anim. Sci.*, **85**(6):1511-1521.
- Parodi, P. W. 1997. Cow's milk fat components as potential anti-carcinogenic agents. *J. Nutr.*, **127**:1055-1060.
- Petit, H. V. 2002. Digestion, milk production, milk composition, and blood composition of dairy cows fed whole flaxseed. *J. Dairy Science*, **85**:1482-1490.
- Petit, H. V. 2003. Digestion, milk production, milk composition, and blood composition of dairy cows fed formaldehyde treated flaxseed or sunflower seed. *J. Dairy Sci.*, **86**: 2637-2646.
- Petit, H. V. and H. Twagaramungu. 2006. Conception rate and productive function of dairy cows fed different fat sources. *Theriogenology*, **66**: 1316-1324.
- Petit, H. V., C. Germiquet, & D. Lebel. 2004. Effect of feeding whole, unprocessed sunflower seeds and flaxseed on milk production, milk composition and prostaglandin secretion in dairy cows. *J. Dairy Science*, **87**: 3889-3898.
- Petit, H. V., Palin, M. F. & L. Doepel. 2007. Hepatic lipid metabolism in transition dairy cows fed flaxseed. *J. Dairy Science*, **90**: 4780-4792.
- Petit, H. V., R. J. Dewhurst, J. G. Proulx, M. Khalid, W. Haresign & H. Twagiramungu. 2001. Milk production, milk composition, and reproductive function of dairy cows fed different fats. *Canadian J. of Anim. Science*, **81**: 263-271.
- SAS Institute. 2002. SAS Software User's Guide. Version 9.3. SAS Inst., Inc., Cary, NC.
- Simpolous, G. W. 1996. Omega-3 fatty acids and public health. *Proc. Flax Council of Canada Conf. Flax, the next decade*, 5-28, Winnipeg, MB.
- Tyrrel, H. F. and Reid, J. T. 1965. Prediction of the energy values of cow's milk. *J. Dairy Sci.*, **48**: 1215-1223.

- Weight, T., McBride, B. and Holub, B. 1998. Decosahexaenoic acid-enriched milk. *World Rev. Nutr. Diet.*, **85**:160-165.
- Yaqoob, P. and P. C. Calder. 1995. The effect of dietary lipid manipulation on the production of murine T-cell-derived cytokines. *Cytokines*, **7**: 540-553.
- Zachut, M., A. Arieli, H. Lehrer, L. Livshitz, S. Yakoby & U. Moallem. 2010. Effects of increased supplementation of n-3 fatty acids to transition dairy cows on performance and fatty acid profile in plasma, adipose tissue, and milk fat. *J. Dairy Science*, **93**:5877-5889.