RESEARCH ARTICLE

# Effects of Feeding Flaxseeds and Rapeseeds on Milk Fatty Acid Composition in Crossbred Dairy Cattle

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## Abstract

Effects of feeding oil seeds on fatty acid profile of milk fat was studied on fifteen multiparous HF × Kankrej dairy cows (15 DIM) fed with three experimental diets, *viz*., T1: control, Total Mixed Ration (TMR) without oil seeds; T2: TMR with ground flaxseeds @ 4.5% and T3: TMR with ground rapeseed @ 4.5%. The experimental TMRs were offered along with 15 kg green cereal fodder as per the guidelines of ICAR standards for 120 days. The milk samples were collected at the end of the experiment for fatty acid analysis. The results revealed significant (p<0.05) increase in the concentration of total unsaturated fatty acids by 21.5 and 29.5% and significant decrease in the saturated fatty acids by 8.0 and 11.0 % in T2 and T3, respectively, as compared to T1. Medium Chain Fatty Acids decreased significantly (p<0.05) in T2 and T3, while Short Chain Fatty Acids remained at par among all treatments. The Long Chain Fatty Acids increased significantly (p<0.05) in T2 and T3 groups, respectively, as compared to T1. The concentration of Omega 3 was found higher by 16.3 and 69.0% in T2 and T3 than T1. The significantly higher Omega 9 fatty acids concentration was found in both T2 and T3 by 32.57 and 30.0%, respectively, as compared to T1. Treatments had no effects on DMI, milk yield and milk composition. It is concluded that feeding flaxseeds and rapeseeds to dairy cows @ 4.5% in TMR altered milk fatty acid composition in beneficial way by increasing healthy fatty acids and decreasing non-desirable fatty acids.

**Key words:** Dairy cows, Fatty acid composition, Milk, Omega3 fatty acids, Unsaturated fatty acids.

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## Introduction

Food is one of the most important and modifiable lifestyle determinants of human health. In present scenario the functional and nutraceutical food are more preferred and consumed by the health-conscious people. Omega-3 fatty acids are beneficial over omega-6 fatty acid as omega-3 has anti-inflammatory effect, while omega-6 has pro-inflammatory effect. Several research have shown health benefits of omega-3 fatty acids to humans including a decrease in the incidence of cancer, cardiovascular diseases, hypertension, and arthritis and an improvement of visual acuity (Simopoulos, 1999). Milk and milk products are considered as complete food containing most of the nutrients required for healthy life and that's why enormous part of anthropological diet comprises of milk and milk products throughout the world. Milk fat contains low concentrations of n-3 fatty acids and high levels of saturated fatty acids (Kennelly, 1996). Unsaturated fats are important to include as part of a healthy diet because essential fatty acids are not synthesised in the body by its own and must be included in the diet. Oil seeds are one of the main sources of unsaturated fatty acids among which flaxseeds and rapeseeds are rich sources of omega-3 fatty acids. Flaxseed has high linolenic acid constituting approximately 55% of total fatty acids (Mustafa *et al*., 2002). Rapeseed contains 41-43 % oil, of which about 12 % is α-linoleic acid. Rapeseeds are considered as a good source of cis-9 18:1 and cis-13 22:1 as well as protein (20-25 %) (Sakhno, 2010). This study was conducted with the aim of manipulating fatty acids ratio in milk fat through dietary interventions in lactating crossbred dairy cows.

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## Materials and Methods

### Animals, Housing and Diets

Fifteen multiparous lactating (15 days in milk, DIM) HF × Kankrej (50:50) dairy cows were assigned to one of the three dietary treatments in a complete randomised design. Each treatment group was comprised of five animals, and they were fed with the experimental diets. The composition of the experimental diets is given in Table 1. The experimental period was of 120 days from 15 days of parturition. The experimental TMR were offered along with 15 kg green cereal fodder as per ICAR (2013) standards. Throughout the experimental period, the animals were maintained in an open asbestos shed with cemented floor, having arrangement for individual feeding. The three feeding treatments were control (T1): TMR without oil seeds; T2: TMR with ground flaxseeds @ 4.5 % and T3: TMR with ground rapeseed @ 4.5%. Measured quantity of TMR was offered to the animals in two parts, during morning and afternoon.

### Milk and Milk Fat Composition

Daily milk yield was recorded based on records available, while 50 mL of representative milk sample of each experimental animal was collected in a clean plastic bottle through full hand milking at fortnightly interval for milk composition study. The milk composition analysis was carried out on automatic milk analyzer (Lactoscan, Milkotronic Ltd) for total solids (%), Fat (%), solids not fat (%), protein (%), lactose (%) and salt (%). 4% fat corrected milk (FCM) yield (kg) was calculated from the values of milk yield and fat percentage of milk using standard formula. The milk fatty acid estimation was carried out at the end of experiment by extracting and preparing milk (AOAC, 2001) followed by GC-FID program (Agilent-7890B with open lab software) at CALF Lab of NDDB, followed by quantification of fatty acids as follows:

Inlet-Split (15:1) at $250^0$ C, 1 µL injection was allowed to pass through the Column- HP-88 100 m x 0.250 µm x 0.2 µm at the column flow of 1.8 mL/min (Nitrogen-constant flow mode). The oven protocol was Oven- 100° C 5 min hold, 4°C/min to 120 °C, 25°/min to 175 °C, 2°C/min to 198 °C, hold for 3 min, 2° C/min to 240 °C, hold for 3 min. Detector- Flame Ionisation Detector - Temperature 260 °C, air flow-400 mL/min, Hydrogen Flow-40 mL/min and Makeup Nitrogen flow-25 mL/min was used. Quantification was done by obtaining individual peaks for individual fatty acid methyl esters and was compared with certified reference standard containing Fatty Acid Methyl Esters of 37 fatty acids (Purchased from Sigma Aldrich-FAME standard). A FAME standard was injected in every batch of samples.

### Statistical Analysis

Data were statistically analysed by Completely Randomized Design (Factorial) as described by wasp software. Some of

**Table 1:** Ingredients and chemical composition of the TMRs (%)

| Ingredients | TMR Treatments | | |
|---|---|---|---|
| | Control (T1) | Flaxseed (T2) | Rapeseed (T3) |
| Wheat straw | 45 | 45 | 45 |
| Soyabean meal | 17 | 15.5 | 15.5 |
| Maize | 13 | 11.5 | 11.5 |
| Rice | 13 | 11.5 | 11.5 |
| Flaxseed | - | 4.5 | - |
| Rapeseed | - | - | 4.5 |
| Molasses | 10 | 10 | 10 |
| Mineral mixture | 2 | 2 | 2 |
| **Chemical composition of TMR (% on DM basis)** | | | |
| Crude protein (CP) | 12.4 | 12.56 | 12.55 |
| Ether extract (EE) | 2.67 | 2.99 | 4.43 |
| Crude fiber (CF) | 29.22 | 24.94 | 22.35 |
| Total Ash (TA) | 10.74 | 10.96 | 10.53 |
| Nitrogen fee extract (NFE) | 44.97 | 48.53 | 50.12 |
| Acid Insoluble Ash (AIA) | 5.7 | 5.59 | 5.27 |
| Neutral detergent fibre (NDF) | 58.44 | 55 | 55.4 |
| Acid detergent fibre (ADF) | 30.07 | 31.76 | 31.89 |
| Cellulose (C) | 23.82 | 22.73 | 22.55 |
| Hemi-cellulose (HC) | 28.37 | 23.24 | 23.51 |
| Acid detergent lignin (ADL) | 14.30 | 10.78 | 9.56 |

the data were also analysed by one-way ANOVA using SPSS software 20.00 version.

## Results and Discussion

The ingredients and chemical composition of three treatments are presented in Table: 1. The chemical composition of the TMRs was generally similar among diets, except for EE, which was lowest for the control diet. The FA composition of oilseeds used during whole experiment is presented in Table 2. Both oilseeds were found rich source of n-3, n-6, poly unsaturated fatty acid, long chain fatty acid, while saturated fatty acids concentration was found lower.

**Table 2:** Fatty acid composition of flaxseed and rapeseed (g/100 g of fat)

| Fatty Acid | Flaxseed | Rapeseed |
|---|---|---|
| Oleic acid (C18:1n9c) | 27.61 | 9.29 |
| Linoleic acid (C18:2n6c) | 11.74 | 14.36 |
| Linolenic acid (C18:3n3) | 46.63 | 5.28 |
| SCFA | 0.05 | 0.01 |
| MCFA | 6.74 | 2.43 |
| LCFA | 92.75 | 97.56 |
| n3 | 11.70 | 6.82 |
| n6 | 11.74 | 62.51 |
| n9 | 27.64 | 9.56 |
| MUFA | 27.99 | 24.72 |
| PUFA | 58.54 | 70.95 |
| Total Saturated FA | 13.01 | 4.33 |
| Total Unsaturated FA | 86.52 | 95.67 |

The DMI was not found intercalated with dietary treatments (Table 3). The lack of difference in DM intake among treatments indicates that linseeds and rapeseeds were readily accepted by dairy cows. Similarly, no adverse effect of flaxseed on DMI in dairy cows has been reported by earlier workers (Gonthier *et al*., 2005; Fuentes *et al*., 2008; Brossillon *et al*., 2018). Likewise, for rapeseed, Loor *et al*. (2002) observed decrease, whereas Johansson *et al*. (2015) reported increase in DMI in dairy cattle, respectively, while others (Givens *et al*., 2009; Jacobs *et al*., 2011; Kliem *et al*., 2019) reported at par results among the groups for DMI.

The milk yield and composition are presented in Table 3. The average milk yield was 10.43, 9.93 and 9.08 kg/d in T1, T2 and T3, respectively. It decreased by 13% in T3 as compared to control group, though the difference was non-significant. However, the 4% FCM was more or less similar in all the groups which revealed no adverse effect of oil seeds on production performance of animals. Earlier researchers using flaxseed (Velez, 2012; Schossow, 2019) and rapeseed (Collomb *et al.,* 2004; Egger *et al*., 2007; Givens *et al*., 2009; Johansson *et al.,* 2015) did not reveal any significant effect on milk yield and 4% FCM in dairy cows. However, Fuentes *et al*. ( 2008 ) and Velez (2012) observed lower milk yield and 4% FCM yield in dairy cows offered flaxseed based ration. Reynolds *et al*., (2002) revealed that oilseeds did not release fatty acids in the rumen fluid which may responsible for unaffected rumen functioning.

The composition of milk was more or less similar in all treatments which suggest no adverse effect of feeding flaxseed and rapeseed to dairy cattle on milk composition. The amount of dietary fat transferred directly to milk fat is influenced by ruminal biohydrogenation, absorption, and deposition in adipose tissue (Palmquist *et al*., 1993) and reduction in de novo synthesis of fatty acids. The extent to which dietary lipid is protected from microbial attack in the rumen may be an important factor influencing the extent of change in milk fat percent and milk fat yield. The absence of milk fat depression in present study would suggest that the reduction in de novo synthesis of fatty acids is equal to increase in concentration of polyunsaturated fatty acids (Gonthier *et al*., 2005).

Non-significant effect of flaxseed on milk fat (Petit, 2015; Schossow, 2019), milk lactose (Ferlay *et al*., 2013; Petit, 2015; Schossow, 2019), milk protein (Côrtes *et al*., 2010; Petit, 2015) and milk SNF (Schossow, 2019) has been reported, which is in agreement with our findings. However, increase in milk fat has been reported by some workers (Flowers *et al*., 2008; Caroprese *et al*., 2010) while increase in milk protein was reported by others (Flowers *et al*., 2008; Moallem, 2009; Caroprese *et al*., 2010) due to inclusion of different forms of flaxseed in the dairy cattle ration. Contrary to present study, marked reduction in milk fat is reported in plant oil fed dairy animals by Shingfield *et al*. (2006) and Rego *et al*. (2009) .

Similarly for rapeseed also non-significant effects have been found by some workers for milk fat (Dai *et al*., 2011; Jacobs *et al*., 2011; Johansson *et al*., 2015), for milk lactose (Egger *et al*., 2007; Givens *et al*., 2009; Johansson *et al*., 2015), and for milk protein (Collomb *et al*., 2004; Dai *et al*., 2011; Jacobs *et al*., 2011) . In contrast to the current study, significant lower milk fat production was found in rapeseed and sunflower oil fed animals as compared to flaxseed oil fed animals (Rego *et al*., 2009).

In the present study the total unsaturated fatty acids were found ($P<0.05$) higher in T2 (flaxseed) and T3 (rapeseed) groups by 21.5 and 29.5%, respectively, as compared to control (T1). Similarly Petit *et al*. (2004) and Dai *et al*. (2011) observed 11.14 and 23.00% higher unsaturated fatty acid in flaxseed and rapeseed fed dairy cows, respectively, than that of control group.

Saturated fatty acids were found ($p<0.05$) lower by 8 and 11% in T2 and T3 groups respectively than T1 which agrees with earlier studies for flaxseed (Petit *et al*., 2004; Chilliard *et al*., 2007; Moallem, 2009) and for rapeseed ( Egger *et al*., 2007; Givens *et al*., 2009; Dai *et al*., 2011) fed dairy cows. Givens *et al*. (2009) found reduction in total SCFA in response to replacing calcium salts of palm oil distillate (CPO) with rapeseed oil or milled rapeseeds which was mainly due to reductions in short and medium chain fatty acids. However, in some studies (Fuentes *et al*., 2008; Côrtes *et al*., 2010; Jacobs *et al*., 2011) non-significant decrease in saturated fatty acids in dairy cattle fed flaxseed and rapeseed at different levels has been reported. The non-significant changes in fatty acids in such studies may be due to the transfer efficiency, as the transfer efficiency of fatty acids from feed to milk are low and reduce as the degree of unsaturation of the fatty acids increase (Gonthier *et al*., 2005).

The milk omega-3 fatty acids concentration was found higher by 16.3 and 69.0% in T2 and T3, respectively. There was decrease in omega-6 fatty acids by 1.74% in T2, while increased concentration was found by 84.70% in T3. The results are supported by other studies (Givens *et al*., 2009; Côrtes *et al*., 2010; Dai *et al*., 2011) for flaxseed and rapeseed feeding at different levels of inclusion rate in diets of dairy cows. The non-significant changes for C18:3 among the groups could also be due to formation of EPA and DHA from it, as C18:3 is the precursor of EPA and DHA both.

The omega-9 fatty acids concentration was found higher ($p<0.05$) in both the T2 and T3 groups as compared to T1 by 32.58 and 30.06%, respectively, which agrees with the earlier study results (Gonthier *et al*., 2005; Moallem, 2009; Rego *et al*., 2009) for flaxseed, and (Egger *et al*., 2007; Givens *et al*., 2009; Dai *et al*., 2011) for rapeseed fed dairy cows. The higher concentration of omega-9 fatty acid in milk fat may be due to lower total bio-hydrogenation intermediates, on account of less 18:1 isomer (Rego *et al*., 2009) or greater duodenal flow of 18:0 and plasma availability for desaturation in the mammary gland.

The concentration of total SCFA was 13.02, 10.52 and 10.23 in T1, T2 and T3 respectively. Though the difference was not significant, 19 and 21% less SCFA was observed in T2 and T3, respectively. Since the SCFA are saturated FA, decrease in their concentration in T2 and T3 indicated desirable modification, due to flaxseeds and rapeseeds. The present findings are in agreement with results of Gonthier *et al*. (2005) and Côrtes *et al*. (2010) for dairy cows at different levels of inclusion rate since the partial biohydrogenation increases the synthesis of trans-isomers of fatty acids and their concentrations in milk fat, which may then inhibit de novo synthesis of SCFA. In the present study the reduction in C14:0 and C16:0 in treatment groups was accomplished by an increase in the concentration of oleic acid and, at a lower stage of stearic acid.

The oilseeds supplementation decreased ($p<0.05$) MCFA and increased ($p<0.05$) LCFA in milk fat as compared to control diet. The MCFA lowered by 26.08 and 28.43% in T2 and T3

**Table 3:** Dry matter intake (DMI), milk production and milk composition of experimental animals fed with control, flaxseed and rapeseed containing diets

| Item | Dietary treatments | | | SEM | P value |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | | |
| DMI (kg/d) | 12.88 | 12.82 | 12.48 | 0.35 | >0.05 |
| Milk yield (kg/d) | 10.43 | 9.93 | 9.08 | 1.25 | >0.05 |
| 4% FCM yield (kg/d) | 9.1 | 9.65 | 8.82 | 1.06 | >0.05 |
| FAT% | 3.82 | 3.86 | 4.11 | 0.30 | >0.05 |
| Protein% | 3.16 | 3.17 | 3.13 | 0.05 | >0.05 |
| Lactose% | 4.84 | 4.85 | 4.79 | 0.07 | >0.05 |
| SNF% | 8.82 | 8.87 | 8.63 | 0.09 | >0.05 |

**Table 4:** Milk fatty acid profile of lactating cows (g/100g of milk fat)

| Fatty Acid | T1 | T2 | T3 | Level of Significance |
|---|---|---|---|---|
| Oleic acid (C18:1n9c) | 19.63 | 25.97 | 25.31 | **<0.05** |
| Linoleic acid (C18:2n6c) | 0.94 | 0.97 | 0.95 | >0.05 |
| Linolenic acid (C18:3n3) | 0.47 | 0.51 | 0.77 | >0.05 |
| SCFA | 13.02 | 10.52 | 10.23 | >0.05 |
| MCFA | $52.69^a$ | $38.95^b$ | $37.71^b$ | **<0.05** |
| LCFA | $34.08^b$ | $50.42^a$ | $51.72^a$ | **<0.05** |
| n3 | 0.54 | 0.64 | 0.93 | >0.05 |
| n6 | 1.27 | 1.25 | 2.34 | >0.05 |
| n9 | $21.28^b$ | $28.21^a$ | $27.67^a$ | **<0.05** |
| MUFA | 5.79 | 5.14 | 5.70 | >0.05 |
| PUFA | $1.98^b$ | $2.16^b$ | $4.46^a$ | **<0.05** |
| Total Saturated Fatty Acids | $72.46^a$ | $66.67^b$ | $64.36^b$ | **<0.05** |
| Total Unsaturated Fatty Acids | $27.39^b$ | $33.26^a$ | $35.48^a$ | **<0.05** |

$^{a,b,c}$Means within a row with no common superscript differ significantly at $P < 0.05$.

groups, respectively, than that of T1. The LCFA was found higher by 48 and 52% in T2 and T3, respectively, as compared to T1. The increase in LCFA is related with the apparent reduction in de novo synthesis of FA (≤ 16:0) in the mammary gland (Grummer, 1991). The present findings agree with other studies (Gonthier *et al*., 2005; Petit and Côrtes, 2010).

The results of average concentration of PUFA and MUFA in different treatments are given in Table 4. The MUFA concentration in all groups were at par which agrees with study of Petit and Côrtes ( 2010) for whole and ground flaxseeds @ 36 and 72 g/kg DM to dairy cows. However, in some studies (Gonthier *et al*., 2005; Moallem, 2009; Rego *et al*., 2009) MUFA was found higher in flaxseed fed groups than that of control group.

The PUFA content increased by 9% in flaxseed group while it significantly increased by 125% in rapeseed group than control group. The significantly higher PUFA in treatment groups as compared to control group may be due to higher ($p<0.05$) concentration of long chain fatty acids in both oilseeds groups as they are rich sources of unsaturated fatty acids. Since PUFA are good fat, increase in their content due to flaxseed and rapeseed is as per the objective. The present findings are supported by other studies (Gonthier *et al*., 2005; Fuentes *et al*., 2008; Moallem, 2009; Ferlay *et al*., 2013) in which flaxseed was offered to dairy cows at different levels. Similarly, in rapeseed fed dairy cows higher PUFA was observed (Dai *et al*., 2011; Altenhofer *et al*., 2014) than of control group which agrees with the current study.

## Conclusions

Addition of ground flaxseed and rapeseed in TMR @ 4.5% did not modify voluntary feed intake and nutrient intake. The at par results of milk production and milk composition were suggesting that both oilseeds are acceptable fat sources and can be substitute to each other in the diet of dairy cows. In comparison to the control diet the rapeseed and flaxseed diets had stronger effect on milk fatty acid composition. The increase in PUFA, TUFA, omega-3, omega-9, while lower amount of SCFA and saturated fatty acid were observed in oilseeds treatments. However, rapeseed feeding seems to be more effective in increasing PUFA and TUFA by 125 and 30%, respectively, while a reduction of omega-6 FA was achieved by feeding flaxseed. In general, inclusion of oilseed in ration of dairy cattle brings valuable changes in milk fatty acid which may improve the health of consumers as well as there was no any adverse effect was found in animals.

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