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EFFECT OF FEEDING LINSEED AND LINSEED OIL ON THE COMPOSITION OF FATTY ACIDS IN LACTATING MURRAH BUFFALO MILK

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ABSTRACT

The objective of the current study was to determine how supplementing with whole linseed (WLS) and linseed oil (LSO) affected the composition of fatty acids in lactating Murrah buffaloes. Eighteen Murrah buffaloes were separated into three groups (T_0, T_1, and T_2), each consisting of six animals, two weeks after giving birth, according to their body weight (516.50±9.53 kg), amount of milk produced (7.55±0.3 kg), percentage of fat (6.67±0.29%), and lactation stage (2 weeks post-partum). To meet the nutritional needs of Murrah buffaloes, the Control group (T_0) of buffaloes received a basal diet consisting of wheat straw (WS), green fodder, and concentrate mixture (69.03:30.97; roughage: concentrate ratio). In the experimental feeding trial that is being described, the control feed is supplemented with either linseed oil (250 g/d) in T1 and whole linseed (570 g/d) in T_2. The experimental diets are fed to the Murrah buffaloes for a period of two months, beginning at 15 days following parturition. On the other hand, the data was recorded over a six-month period. To determine the nutrient's digestibility, a seven-day digestion trial was carried out following the feeding trial. The results demonstrated that there were no significant effects ($P>0.05$) of linseed oil or whole linseed supplementation on total DMI, live weight change, nutrient digestibility, milk yield, or milk composition. In comparison to the Control group (T_0), diets supplemented with linseed oil and whole linseed diets led to a decrease in C12:0-C16:0 FA ($P<0.05$) and an increase in trans-9,12-C18:2, cis-9,12,15-C18:3, and cis-6,9,12-18:3 ($P<0.05$). Diets rich in linseed produced higher ($P<0.05$) milk fat cis-9,12,15-C18:3, which corresponded to 76.20% and 85.71% in the T_1 and T_2 groups, respectively, when compared to the T_0 group. Milk cis-9,trans-11-CLA significantly increased ($P<0.05$) in T_1 and T_2 (11% and 115%, respectively) compared to the control due to the biohydrogenation of linoleic acid in the buffaloes' rumen. In the T_1 and T_2 groups, there was a significant ($P<0.05$) increase in the concentrations of milk DHA. Consequently, when compared to the control diet, the animals fed linseed oil and whole linseed diets had higher milk unsaturated fatty acids (UFA) (45.06 % and 46.87%) and lower milk saturated fatty acids (27.21% and 30.00%). The UFA to SFA ratio also increased in the LSO and WLS containing groups compared to the Control group ($P<0.05$). In comparison to the Control group, the T_1 (121.21%; 62.61 respectively) and T_2 (124.24%; 61.71% respectively) groups

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showed a significant (P<0.05) improvement in the proportion of total n-3 and n-6 fatty acid. In conclusion, adding linseed oil or whole linseed to Murrah buffaloes did not change the amount of milk produced or its composition; however, it did increase trans-11-C18:1, cis-9, trans-11-CLA, n-3 fatty acid, and unsaturated fatty acid while significantly lowering saturated fatty acid. Thus, adding 250 g/d of linseed oil or 570 g/d of whole linseed to the milk of nursing Murrah buffalo may help to improve the milk's fatty acid profile.

Keywords: *Bubalus bubalis*, buffaloes, fatty acid profile, lactating Murrah buffaloes, linseed, milk production

INTRODUCTION

The world's largest animal populations are found in India. India is home to 20% of the world's cattle and 50% of its buffaloes, the majority of which are milch cows and buffaloes. With 221.06 million tonnes of milk produced in 2021 to 2022, India has led the world in milk production for the previous fifteen years (BAHS, 2022). For millions of Indian rural households, buffaloes are a major source of nutrition and a stable source of income. In India, buffaloes account for 45.06% of all milk produced; the average yield per in-milk buffalo per day was 5.96 kg. India is home to half of the world's buffalo population. With a total livestock population of 40.63 million (Livestock Census, 2019), Madhya Pradesh is the third-largest state in India in terms of total milk production (190.04 lakh MT) in 2021 to 2022. However, Madhya Pradesh only makes up 8.60% of India's total milk production (BAHS, 2022). According to the 2019 Livestock Census, there are approximately 1,01,598 buffaloes in Jabalpur and 10.3 million in Madhya Pradesh overall. In Madhya Pradesh, buffaloes account for 47.68% of the milk produced by species in 2021 to 2022 (BAHS, 2022). To improve energy density and the proportions of desired unsaturated fatty acids in edible products, oilseeds and vegetable oils (fat supplements) are frequently added to ruminant diets (Raes *et al*., 2004). Saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), which make up 69.4% and 25% of the fat acids (FA) in bovine milk, respectively, are normally present in high concentrations. Moreover, bovine milk contains 5% polyunsaturated fatty acids (PUFA), of which 2.3% are cis-PUFA and 2.7% are trans-PUFA. According to Berner (1993), the "ideal" milk fat for human health should contain >82% mono saturated fatty acids (MUFA), <8% saturated fatty acids (SFA), and <10% poly unsaturated fatty acids (PUFA). Although achieving the desired composition of this "ideal" milk fat may not be possible, genetic interventions and alterations in the feeding regimens for dairy cattle and buffaloes may be able to manipulate milk fat.

Over the past 25 years, there has been a lot of focus on nutritional modification of the milk fatty acid (FA) profile. The primary goal has consistently been to raise the levels of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in milk while decreasing the concentration of saturated fatty acids (SFA), with a focus on increasing the content of certain unsaturated fatty acids such as conjugated linoleic acid (CLA) and n-3 fatty acids (Perez and Garnsworthy, 2013). Because these fatty acids have been associated with a lower incidence of cancer, diabetes, obesity, cardiovascular diseases, hypertension, and arthritis, eating a diet high in them may be good for human health (Simopoulos, 1999 and Parodi, 1999). To reduce the incidence

of chronic disease, public health advisories in the majority of developed countries recommend reducing saturated and trans fatty acids area-wise and increasing α-linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) in human food (WHO, 2003).

Oilseeds or oils high in linoleic or linolenic acid appear to be a useful tactic for modifying the ruminant milk fatty acid profile. Indian dairy cows and buffaloes are traditionally fed mustard oil, which has been shown to have a positive impact on milk composition (Dhiman *et al*., 2000) and to contain 46.93% linoleic acid and 9.21% linolenic acid of total fatty acids (Al-Jasass and Al-Jasser, 2012). Based on research conducted by Rubbilar *et al*. (2010); Rabetafika *et al*. (2011); Kajla *et al*. (2015), linseed is a vegetable fat source that is high in polyunsaturated fatty acid (PUFA), accounting for 73% of total fatty acid, moderate in monounsaturated fatty acid (MUFA), amounting to 18% of total fatty acid, and low in saturated fatty acid (9% of total fatty acid). It contains 50% of the total FA and the highest concentration of linolenic acid (C18:3 n3). Supplementing linseed to dairy cattle increased the proportion of cis-9,trans-11-18:2 (c9,t11 CLA) and n-3 FA, particularly linolenic acid, EPA, and DHA in milk. However, increasing the amount of polyunsaturated fatty acids (PUFA) in milk is limited because of the bio-hydrogenation of these FA in the rumen (Glasser *et al*., 2008; Lerch *et al*., 2012; Puppel *et al*., 2013).

There is a lack of information on the effects of whole linseed, mustard oil, and linseed oil on buffaloes, particularly with regard to milk FA profiles. The current study's hypothesis was that feeding lactating buffaloes linseed or linseed oil would improve the amount of n-3 FA and CLA in their milk. Thus, the purpose of this experiment was to ascertain how the composition of milk fatty acids in nursing Murrah buffaloes responded to linseed and linseed oil supplements.

MATERIALS AND METHODS

For the feeding trial, eighteen lactating Murrah buffaloes were specifically chosen, and they were allocated into three experimental groups (T_0, T_1, and T_2), each with six animals. The groups were categorized based on body weight (516.50±9.53 kg), milk production level (7.55±0.3 kg), fat (6.67±0.29%), and lactation stage (2 weeks post-partum). To meet their nutrient needs, the buffaloes in the Control group (T_0) were fed a total basal diet that included wheat straw, fresh greens, and a concentrate mixture (69.03:30.97; roughage: concentrate ratio). The treatments included a base ration supplemented with either 570 g/d of whole linseed (T_2) or 250 g/d of linseed oil (T_1).

The feeding of the experimental diets began at 15 days after parturition and continued for two monthswhile data were gathered for a total of six months. The following proximate principles dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), nitrogen free extract (NFE), and total ash (TA) were further analyzed for samples of different feed materials (wheat straw, concentrate, and berseem) fed to Murrah buffaloes using standard procedures (AOAC, 2012). Following the feeding trial, a seven-day digestion trial was carried out to determine the nutrients' digestibility. Every experimental Murrah buffalo was given individual feed. Feed intake will be recorded on a biweekly basis by difference, and the allotted amount of feed, fodder, and concentrates that were offered and leftovers were picked up from the manger the following morning. Each animal's

body weight was recorded using an electronic weigh bridge at the beginning and the end of the experiment to determine how each animal's body weight changed. Each buffalo's daily milk yield (morning and evening) was documented, and the animal's average milk production was computed. Every month, milk samples were taken from each animal in each treatment and examined using a Lactescent milk analyzer for lactose, fat, SNF, total solids, and milk protein. Thermo Scientific Trace 1300 Series Gas Chromatograph was used to analyze the fatty acid composition of milk samples from individual animals, linseed, and linseed oil at the end of the experiment. The following formulas for the thrombogenic index (TI) and atherogenic index (ArI) were used as described by Ulbricht and Southgate (1991):

$$\text{Atherogenic index (ArI)} = \frac{(C12:0 + 4 \times C14:0 + C16:0)}{[\Sigma MUFA + \Sigma PUFA\ (n\text{-}6 \text{ and } n\text{-}3)]}$$

$$\text{Thrombogenic index (TI)} = \frac{(C14:0 + C16:0 + C18:0)}{[0.5 \times \Sigma MUFA + 0.5 \times \Sigma PUFA\ (n\text{-}6) + 3 \times \Sigma PUFA\ (n\text{-}3) + (n\text{-}3)/(n\text{-}6)]}$$

Using SPSS software (14.1 version), the data collected during the experiment were analyzed using the Randomized Block Design (RBD) method, as outlined by Snedecor and Cochran (1994).

RESULTS AND DISCUSSIONS

The proximate composition (% DM base) of the concentrate mixture, wheat straw, and berseem (Trifolium alexandrinum) provided to the experimental animals were analyzed and are shown in Table 1. When compared to the concentrate utilized in the T_0 (Control) diet, the proximate composition (% DM basis) of the concentrates in the T_1 and T_2 diets did not differ. Tewatia *et al.* (2014); Sharma *et al*. (2006) reported values for the chemical composition of concentrate mixtures in their research that were similar to present findings. Similarly, according to Anonymous (2012), the proximate composition (% DM basis) of wheat straw and berseem matched the nutritional values of commonly available feeds and fodders in India.

Fatty acid profile of linseed and linseed oil

The fatty acid composition of the linseed and linseed oil that were fed to the experimental animals was identified and is shown in Table 2. The percentage of total fatty acid found in different types of linseed and linseed oil was found to be comparable to those of studies conducted by Suksombat *et al*. (2014); Fuentes *et al*. (2008); Foda *et al*. (2012); El-Aziz *et al*. (2012).

Table 3 shows the influence of linseed and linseed oil on dry matter intake, which has been monitored every fortnightly. Throughout the experimental period, no significant ($P>0.05$) difference in DMI (kg/d), DMI (% of BW), or DMI (g/kg MBS) was seen between groups in the current study. In the current experiment, the total fat content of all the diets given to the various groups was approximately 4.50% of DM, falling within the normal range recommended by the NRC (2001). According to NRC (2001), the total amount of dietary lipid in ruminant diets shouldn't be more than 6 to 7% of dietary DM. Feeding an number of oils higher than this could lower the amount of rumen microbial activities, reduce digestibility and cause the animals to consume less dry matter (Shingfield *et al*., 2006). When the total fat content was less than 6% of the DM, the dry matter intake in the current study was consistent with the majority of literature reports that demonstrated smaller effects of concentration and type of fat supplement (Lunsin *et al*., 2012; Mach *et al*., 2013; Suksombat *et al*., 2014). There has been variation

in the effect of unsaturated fatty acids from whole and linseed oils on feed intake in earlier research. Benchaar *et al*. (2012) corroborated this finding by showing that supplementing lactating dairy cows fed a total mixed ration with a 50:50 R:C ratio with linseed oil at 2%, 3%, or 4% dry matter had no effect on dry matter intake. Compared to the current findings, lactating dairy cows fed a diet supplemented with linseed oil at 5.7% dry matter and 3.0% oil (DM basis) from extruded linseed were found to have lower dry matter intake (Martin *et al*., 2008; Lerch *et al*., 2012). Through inhibition of ruminoreticula motility, fatty acid intake may have a direct inhibitory effect on voluntary intake (Chilliard, 1993).

Table 3 shows digestibility of nutrients. The low level of oil supplementation (1.9%) in the Control, Linseed, or Linseed oil treatment groups (measured as a percentage of DM intake) did not change the digestibility of nutrients in the current experiment, nor did it have an impact on the rumen environment. Therefore, when comparing the Linseed and Linseed oil supplemented diets to the Control group, there was no significant ($P>0.05$) difference in the digestibility of DM, CP, EE, CF, NFE, and nutritional density (DCP and TDN%). The fact that the level of oil supplementation (measured as a % of DM consumption) was lower (1.9%) in the Linseed oil and Linseed treatment groups in our study may be the reason why the digestibility of nutrients remained unaffected. Comparable to our results, Benchaar *et al*. (2012); Tewatia *et al*. (2014) found that lactating dairy cows fed a complete mixed ration with a R:C of 50:50 supplementing with linseed oil 2, 3 or 4% DM or linseed and linseed meal had no effect on nutritional digestibility. In contrast to our results, Ueda *et al*. (2003); El-Hamd *et al*. (2015); El-Diahy *et al*. (2016) showed that supplementing dairy cows with linseed oil increased the total-tract digestibility of organic nutrients significantly ($P<0.05$). According to studies by Chung *et al*. (2011); Agustinho *et al*. (2020), supplementing with linseed fat reduced their ability to assimilate nutrients in cattle and buffalo, respectively. The overall digestibility of DM and OM decreased because of the fibrous fraction's decreased digestibility. The high oil content in the diet could be the cause of this reduced digestibility since it could slow down the rumeno-reticulum movement (Chilliard, 1993).

Experimental buffaloes' body weight was measured at the beginning and end of the trial to determine the impact of linseed and linseed oil. The results are shown in Table 3. Because the food in each group of the current experiment met the nutritional needs of the animals and were equally appetizing to them all, the differences in body weight between and within the groups at the beginning and end of the experiment were not statistically significant ($P>0.05$). Moreover, the experimental buffaloes exhibited equal nutrient intake and utilization. Our findings concur with those of Suksombat *et al*. (2014): Petit *et al*. (2001), who similarly reported that the cows' live weight changed during the trial while they were fed linseed oil and whole linseed that had been formaldehyde-treated, regardless of the amount of dietary fat fed to them. The early researchers also corroborated the current findings (Petit, 2003; Sharma *et al*., 2007). Zachut *et al*. (2010), however, found that while the experimental groups' average body weights were similar prepartum, the control cows lost more body weight ($P<0.001$) postpartum than the cows fed extruded flaxseed (1 kg/cow/d). According to Nawaz *et al*. (2007), adding tallow to the diets in increasing amounts (0%, 2%, 4%, and 6% tallow) resulted in body weight gain. Other researchers (Sarwar *et al*., 2003) observed similar

improvements in body weight in lactating cows fed supplemental tallow.

Daily morning and evening measurements of the experimental Murrah buffaloes' milk yield were made, and the average milk production was then calculated (Table 4). In our experiment, supplementing with linseed or linseed oil had no effect on milk yield. The milk yield of buffaloes fed linseed oil/linseed was comparable to that of the control diet in the current study, which may be explained by the fact that supplementing with linseed oil/linseed added about 1.90% extra fat and had no effect on dry matter intake. Supplementation of linseed oil, whole linseed, or linseed meal did not affect animal performance, according to studies by Suksombat *et al.* (2014); Tewatia *et al.* (2014); Benchaar *et al.* (2015) in cows, and Hassan *et al.* (2020) in lactating buffaloes corroborated to our findings. Furthermore, according to Allen (2000), adding oilseeds and hydrogenated fat at a rate of approximately 23 and 30 g/kg DM added fat, respectively, has no effect on the intake of dry matter, which may help to explain why dairy cows fed linseed oil/linseed yield more milk than other cows. Cant *et al.* (1997), in contrast to our results, hypothesized that milk yield would have increased when oil supplementation of more than 500 g/d in lactating cattle. They concluded that the supply of dietary energy through fat in experimental feed may have positively affected the milk yield from linseed oil supplementation. Similarly, Petit (2002); Petit *et al.* (2001, 2007); Zachut *et al.* (2010); El-Aziz *et al.* (2012) all supported an increase in milk production with the feeding of linseed or linseed oil. However, research involving the use of linseed in cow diets has shown that milk production does not always respond favorably to additional fat (Petit, 2003; Gonthier *et al.*, 2005; Rego *et al.*, 2005; Martin e*et al.*, 2008; Brown *et al.*, 2008; Cortes *et al.*, 2010). In fact, rumen function disruptions brought on by high linseed oil intake (i.e. >5% of DMI) were linked to a decline in DMI and nutrient digestibility, which in turn contributed to the decrease in milk production documented in certain earlier studies. Variations in the type of oil, the source of the oil, the amount of supplementation, and the length of the experiment could be the cause of discrepancies in research on the impact of linseed supplementation on dairy cows' milk yield (Caroprese *et al.*, 2010).

The percentage of fat in milk is a crucial indicator of its economic worth. Table 4 shows the monthly estimate of linseed and linseed oil's impact on milk fat percentage. Throughout the trial period, there was no significant difference (P>0.05) in the milk fat percentage between and within the groups in the current study. The average milk fat content recorded in the animals across the various groups in the current study ranged from 6.34 to 6.90% across all groups. Our results are in line with those of Santillo *et al.* (2016); Zachut *et al.* (2010); Petit *et al.* (2004), who discovered that the addition of flaxseed to the buffalo diet had no effect on the amount of fat in the milk. Table 4 shows the monthly estimate of the impact of linseed and linseed oil on milk solid not-fat (SNF) percent. Throughout the experiment, there was no significant (P>0.05) variation in the percentage of milk solid not fat within and between the groups. The milk of Murrah buffaloes had a normal SNF content of 9.65%, according to reports from Verma *et al.* (2017); Behera *et al.* (2018). A monthly estimate of the impact of linseed and linseed oil on milk density (g/cm3) is shown in Table 4. Throughout the experiment, there was no significant (P>0.05) variation in the amount of milk density in each group or between groups. In the current experiment, the Murrrah buffaloes have a milk density (g/cm3) ranging from 1.037

to 1.039. These findings were consistent with those of Kamel *et al*. (2012), who noted that the Murrah buffalo milk's milk density ranged from 1.033 to 1.038 g/cm^3. Table 4 shows the monthly estimate of linseed and linseed oil's impact on milk protein percentage. Throughout the experiment, no significant (P>0.05) variation in the percentage of milk protein was seen between or within the groups in the current study. Buffalo milk's average protein content ranged from 3.81% (Sodi *et al*., 2008) to 3.91% (Dubey *et al*., 1997). The effects of linseed on milk protein content have not been consistently reported in the literature; studies have shown that the effects of linseed on protein content of milk has increased (Petit *et al*., 2001; Cavalieri *et al*., 2005; Fuentes *et al*., 2008), remained unchanged (Benchaar *et al*., 2015; El-Diahy *et al*., 2016), or decreased (Ward *et al*., 2002). Processing of linseed may help to partially explain the results found in the literature. Table 4 shows the monthly estimates of the impact of linseed and linseed oil on the lactose percentage in milk. Throughout the entire trial, there was not a statistically significant (P>0.05) variation in the percentage of lactose in milk between and within groups. According to Sarkar *et al*. (2006), the lactose content of Murrah buffalo milk typically ranges from 5.15 to 5.37%. The range of the lactose content in the current experiment is below normal, ranging from 5.10 to 5.38%. A monthly estimate of the impact of linseed and linseed oil on milk total solids (%) is shown in Table 4. Over the course of the experiment, no significant (P>0.05) variation in the percentage of milk total solids was found between or within the groups in the current study. Buffaloes' total milk solids typically ranged from 16.10 to 16.80% (Mishra *et al*., 2008; Balusami, 2015). The total milk solid in this experiment did not significantly differ (P>0.05) between treatments and fell within the normal range. One possible explanation for this could be that linseed oil or supplementation does not lower the concentration of fat in milk, which in turn does not alter the density of milk or the number of solids in milk. The impact of linseed oil or whole linseed supplementation on the composition of milk in lactating Holstein Friesian crossbred dairy cows was investigated by Suksombat *et al*. (2014). They concluded that there was no significant difference in milk total solids between the groups that received linseed oil or whole linseed feeding and the Control group. Early researchers (Fuentes *et al*., 2008; El-Diahy *et al*., 2016; Hassan *et al*., 2020) who found no significant difference in milk total solids when fed linseed or linseed oil as compared to control also support the results of the current experiment. In contrast to the current research, studies (Cavalieri *et al*., 2005; El-Aziz *et al*., 2012) reported that cows fed flaxseed had significantly higher milk total solids than cows fed control diets. Agustinho *et al*. (2020) recently reported that supplementing milk with flaxseed oil decreased the concentration of milk fat, increasing milk density and tending to lower the total solid concentration in milk.

The fatty acid composition of the milk samples taken from the buffaloes was examined and is shown in Table 5. Diets containing linseed caused noticeable changes in the composition of milk fatty acids in the current study. According to Suksombat *et al*. (2014), linseed oil may have the following effects on milk FA levels:

i) reduce SFA,

ii) increase UFA,

iii) increase the amount of CLA and linolenic acid, and

iv) reduce the ratio of n-6 FA to n-3 FA.

These potential alterations rely on flax seeds' extremely high C18:3n-3 content (40 to 45%).

In the current study, the group fed linseed and linseed oil showed a significant (P<0.05) decrease in milk saturated fatty acid (%), short chain fatty acid (%), medium chain fatty acid (%), and the ratio of omega 6 to omega 3 when compared to the control. In contrast to the Control group, the Treatment group showed a significant (P<0.05) increase in milk unsaturated fatty acid (%), long chain fatty acid (%), milk n-3 fatty acid, and milk n-6 fatty acid in the Linseed and Linseed oil fed group.

In the current study, buffaloes fed linseed oil and linseed diets tended to produce milk with lower levels of saturated fatty acids (27.21% and 30.00%) and higher levels of unsaturated fatty acids (UFA) (45.06% and 46.87%) when compared to controls, with an increase in the ratio of unsaturated to saturated fatty acids in the linseed oil and Linseed fed groups when compared to controls (P<0.05). Similarly, higher (P>0.05) milk cis-9, 12,15-C18:3 concentrations were discovered; these corresponded to 76.20% and 85.71% in the Linseed oil and Linseed supplemented groups, respectively, in comparison to the Control group. Furthermore, the groups supplemented with linseed and those given linseed oil had higher concentrations of milk DHA (P<0.05).

Fuentes *et al*. (2008) in lactating Holstein cows and Kholif *et al*. (2011) in buffalo fed linseed in their diet both supported the decrease in saturated fatty acid and increase in unsaturated fatty acid in buffalo milk in the current study. Comparing the diets of lactating dairy cows supplemented with LSO and WLS diets to the Control group, Suksombat *et al*. (2014) also found that there was a decrease (P<0.05) in C12:0-C16:0 FA and an increase (P<0.05) in trans-9, 12-C18:2, cis-9, 12, 15-C18:3, and cis-6, 9, 12-18:3.

In the current study, milk cis-9, trans-11-CLA significantly (P<0.05) increased in the Linseed oil and Linseed fed groups (110% and 115%, respectively) compared to the Control group because of the bio-hydrogenation process of linoleic acid in the rumen. More conjugated linoleic acid and C18:1 trans isomer was found in milk fat from cows fed extruded flaxseed supplementation, according to a later study (Mustafa *et al*., 2003; Gonthier *et al*., 2005; Chilliard *et al*., 2009). Dhiman *et al*. (2000); El-Aziz *et al*. (2012); Lerch *et al*. (2012); Mach *et al*. (2013); Weisbjerg *et al*. (2013); Suksombat *et al*. (2014); Tewatia *et al*. (2014) also supported the increase in the milk CLA percent in the current study. Previous studies on whole flaxseed supplementation (1,000 g/d) in Simmental and Holstein-Friesian cow milk reported a slight but non-significant increase in CLA compared to the control (Kennelly and Khoransani, 1992; Cattani *et al*., 2014; Santillo *et al*., 2016; Kathirvelan and Tyagi, 2009; Arif *et al*., 2003); however, the administration of 1,200 g/d per cow of whole flaxseed in Italian Friesian cows produced a significant increase in the amount of CLA in milk (Caroprese *et al*., 2010). Stearoyl-CoA desaturase gene expression and activity variations in the mammary gland may be the cause of the varying CLA content behavior in various species and breeds fed whole flaxseed. But the river buffalo stearoyl CoA desaturase has more genetic variability than the homologous genes in sheep, goats and cattle (Pauciullo *et al*., 2010).

Human health has been linked to CLA (Grummer and Carroll, 1991), as human diets containing C12:0, C14:0, and C16:0 have been shown to have hypercholesterolemic effects (Ward *et al*., 2002), while C18:0 and C18:1 have been shown to effectively lower plasma cholesterol levels (Hu *et al*., 2001).

The fatty acids that were affected by

flaxseed supplementation were categorized as short chain, medium chain, and long chain fatty acids (Table 6). In the current study, T_1 and T_2 had lower levels of short chain fatty acid and medium chain fatty acid (49.21 and 53.64%, respectively; 26.63 and 28.77%, respectively) when compared to the Control group. In contrast to the control, the long chain fatty acid level was higher in T_1 and T_2 (24.71% and 24.81%, respectively). Agustinho *et al*. (2020) found that adding flaxseed oil to the diet of water buffaloes decreased ($P<0.05$) the amounts of medium-chain FA (C14:0, C14:1n-9, C15:0, C16:0, C16:1n-7, and C17:0) and SFA (C8:0, C10:0, and C12:0) and increased (C18:2n-6) the amounts of long-chain fatty acids (C18:2n-6) in milk fat. Santilo *et al*. (2016) also found that milk from buffalo fed a diet supplemented with flaxseed had lower levels of medium-chain and short-chain fatty acids (C8:0 and 10:0). According to Nawaz *et al*. (2007), increased uptake of long-chain fatty acid by mammary glands may inhibit the synthesis of short- and medium-chain fatty acids due to negative feedback inhibitions on acetyl coenzyme A carboxylase and increased incorporation of long-chain fatty acid into milk fat. Dietary fat supplementation provides additional long-chain fatty acid for milk fat synthesis. The creation of trans isomers as a result of long-chain fatty acid biohydrogenation in the rumen may be the cause of this inhibition.

The group Fed linseed and Linseed oil exhibited lower levels of both the thrombogenic index (TI) and atherogenic index (AI) compared to the Control group ($P<0.05$). According to Valeille *et al*. (2006), milk fat has a high concentration of pro-atherogenic saturated fatty acids, primarily lauric, myristic, and palmitic acid. The degree and interaction of fatty acid atherogenic characteristics are determined by the atherogenic index. The current studies show an increase in C18:2 c9 t11 CLA concentration, which is thought to have anti-atherogenic effects. According to Valeille *et al*. (2006), particular feeding techniques should be used to raise the concentration of rumenic acid in milk to enhance its anti-atherogenic qualities.

According to Ulbricht and Southgate (1991), fatty acids with carbon chains C14:0, C16:0, and C18:0 have thrombogenic properties. When linseed or linseed oil is added to a buffalo's diet, the amount of saturated fatty acids (C14:0, C16:0, and C18:0) is reduced, which in turn lowers the thrombogenic index value. Huang *et al*. (2008) demonstrated the advantageous impact of dietary vegetable oil supplementation and found that the thrombogenic and atherogenic index of dairy milk was lowered ($P<0.05$) by supplementing soy oil, which is a source of C18:1 C9 and C18:2 C9 C12.

In the current experiment, T_1 (121.21%; 62.61%, respectively) and T_2 (124.24%; 61.71%, respectively) groups showed a significantly ($P<0.05$) higher proportion of total n-3 FA and n-6 FA compared to T_0 group. In contrast to the T_0 group, the ratio of n-6 to n-3 FA in their milk fat was lower ($P<0.05$) in the T_1 and T_2 groups. Petit and Cortes (2010) discovered, in line with the current findings, that feeding linseed raises the amount of linolenic acid in milk fat when compared to a control diet. Additionally, Petit (2002) found that feeding whole linseed to cows instead of calcium soaps made from palm oil resulted in a lower n-6 to n-3 FA ratio. When buffalo are fed flaxseed, Santillo *et al*. (2016) also observed a decrease in the n-6/n-3 ratio in their milk. Agustinho *et al*. (2020) have also reported a 74% decrease in the milk n6/n3 ratio when water buffaloes are fed flaxseed oil.

According to Simopoulos (1999), a lower n-6/n-3 ratio may lower the risk of coronary heart disease. The n-6/n-3 ratio is crucial because it

Table 1. Chemical composition of concentrate mixtures, berseem and wheat straw on dry matter basis (%).

Particulars	Concentrate mixture			Berseem	Wheat straw
	T_0	T_1	T_2		
Moisture	6.72	6.76	6.87	13.35	90.86
OM	90.35	90.60	90.79	88.35	91.11
CP	19.89	19.78	20.02	15.90	3.29
EE	4.53	4.59	4.57	2.20	1.39
CF	6.82	6.56	6.71	20.55	35.79
Ash	9.65	9.40	9.21	11.65	8.89
NFE	59.11	59.67	59.49	49.70	51.64

Table 2. Fatty acid profile of linseed and linseed oil (% of total fatty acid).

Fatty acids	Common name	Scientific name	Linseed	Linseed oil
C12:0	Lauric acid	Dodecanoic acid	0.032	0.07
C14:0	Myristic acid	Tetradecanoic acid	0.38	0.05
C16:0	Palmitic acid	Hexadecanoic acid	4.96	3.47
C18:0	Stearic acid	Octadecanoic acid	4.48	7.10
cis-9-C18:1	Oleic acid	Octadecenoic acid	19.80	19.89
cis-9,12-C18:2	Linoleic acid	Octadecadienoic acid	14.46	19.07
cis-9,12,15-C18:3	γ–Linolenic acid	All-*cis*-6,9,12-Octadecatrienoic acid	50.78	48.89
SFA	-	-	9.82	10.69
UFA	-	-	85.04	87.85

Table 3. Effect of linseed and linseed oil on digestibility (%) of organic nutrients, body weight and dry matter intake (kg/d) and in Murrah buffaloes.

Attributes	T_0	T_1	T_2
Digestibility of organic nutrients (%)			
DM	67.98±2.81	66.30±1.38	68.43±1.63
CP	67.76±1.87	68.25±1.56	68.39±0.63
EE	78.44±1.51	79.42±0.67	78.96±1.10
CF	58.69±1.21	60.05±1.20	59.07±0.96
NFE	71.85±0.54	70.01±0.92	71.27±0.47
Body weight			
0 Day (initial), kg	516.01±11.72	525.83±12.48	507.67±22.47
180^{th} Day (final), kg	518.67±16.61	532.50±11.48	509.17±21.04
Average BW, kg	517.33±14.01	529.17±11.80	508.42±21.75
% Change in BW (+/-)	+0.52	+1.26	+0.29
Dry matter intake			
Average DMI (kg)	12.69±0.27	13.15±0.21	12.75±0.25
Average DMI (kg/100 kg BW)	2.46±0.07	2.50±0.09	2.51±0.14
Average DMI (g /kg $W^{0.75}$)	117.21±2.66	119.75±2.72	119.21±4.85

Table 4. Effect of linseed and linseed oil on milk yield (kg/d) and milk composition of Murrah buffaloes.

Months	T_0	T_1	T_2
Milk yield (kg)	7.78±0.81	7.92±0.77	7.89±0.78
Milk fat (%)	6.58±0.19	6.72±0.34	6.73±0.38
Solid not fat (%)	9.59±0.09	9.70±0.10	9.62±0.22
Milk density (g/cm^3)	1038.30±0.25	1038.49±0.24	1038.55±0.16
Milk protein (%)	3.84±0.05	3.85±0.06	3.87±0.05
Milk lactose (%)	5.27±0.08	5.26±0.06	5.28±0.07
Milk total solids (%)	16.16±0.22	16.42±0.44	16.34±0.42

Table 5. Effect of linseed and linseed oil on milk fatty acid composition (% of total fatty acid) of Murrah buffaloes.

Fatty acids	Common Name	Scientific Name	T_0	T_1	T_2
C4:0	Butyric acid	Butanoic acid	2.68^{a}±0.40	1.26^{b}±0.11	1.39^{b}±0.05
C6:0	Caproic acid	Hehanoic acid	2.28^{a}±0.34	0.99^{b}±0.13	1.18^{b}±0.17
C8:0	Caprylic acid	Octanoic acid	2.12^{a}±0.36	0.95^{b}±0.26	0.63^{b}±0.12
C10:0	Capric acid	Decanoic acid	2.67^{a}±0.50	1.76^{b}±0.33	1.32^{b}±0.27
C12:0	Lauric acid	Dodecanoic acid	3.37^{a}±0.48	2.21^{b}±0.13	2.07^{b}±0.25
C14:0	Myristic acid	Tetradecanoic acid	12.04^{a}±2.02	7.16^{b}±1.15	7.22^{b}±0.27
C16:0	Palmitic acid	Hexadecanoic acid	21.15^{a}±2.23	16.55^{b}±3.55	15.82^{b}±2.90
C17:0	Margaric acid	Heptadecanoic acid	1.69^{a}±0.40	0.70^{b}±0.11	0.64^{b}±0.12
C18:0	Stearic acid	Octadecanoic acid	15.77±1.53	14.84±2.30	14.37±2.04
C14:1	Myristoleic acid	*cis*-9-Tetradecenoic acid	0.61^{b}±0.03	0.70^{a}±0.04	0.66^{ab}±0.08
C15:1	-	*cis*-10-Pentadecenoic acid	0.10^{b}±0.02	0.16^{a}±0.02	0.17^{a}±0.02
C16:1	Palmitoleic acid	*cis*-9-Hexadecenoic acid	1.06^{b}±0.17	1.78^{a}±0.12	1.82^{a}±0.07
C17:1	-	*cis*-10-Heptadecenoic acid	0.23±0.04	0.27±0.03	0.27±0.05
C18:1	Vaccenic acid	*trans*-11-Octadecenoic acid	21.05^{b}±1.35	29.74^{a}±2.90	30.18^{a}±1.74
C20:1	Paullinic acid	*cis*-13-Eicosenoic acid	0.21^{b}±0.01	0.32^{a}±0.03	0.34^{a}±0.03
C18:2n6	Linolelaidic acid	all-*trans*-9,12-Octadecadienoic acid	1.79^{b}±0.53	2.99^{a}±0.13	3.01^{a}±0.33
C18:2 (CLA)	Linoleic acid	Mixture of *cis*-9,*trans*-11 *and trans*-10,*cis*-9-Octadecadienoic acid	0.20^{b}±0.07	0.42^{a}±0.04	0.43^{a}±0.04
C18:3n6	γ–Linolenic acid	All-*cis*-6,9,12-Octadecatrienoic acid	0.07^{b}±0.01	0.14^{a}±0.03	0.13^{a}±0.06
C18:3n3	α–Linolenic acid	All-*cis*-9,12,15-Octadecatrienoic acid	0.21^{b}±0.01	0.37^{a}±0.02	0.39^{a}±0.03
C20:3n6	Dihomo- γ – linolenic acid	All-*cis*-6,9,12-Eicosatrienoic acid	0.07^{b}±0.02	0.11^{a}±0.02	0.15^{a}±0.02
C20:4n6	Arachidonic acid	All-*cis*-5,8,11,14-Eicosatetraenoic acid	0.29±0.22	0.32±0.06	0.30±0.05
C22:6n3	Cervonic acid	All-*cis*-4,7,10,13,16,19-Docosahexaenoic acid	0.12^{b}±0.03	0.36^{a}±0.02	0.35^{a}±0.09
Unknown	-	-	10.22±1.68	15.90±2.10	17.16±1.94

Means bearing different superscripts [a, b] (row) differs significantly ($P<0.05$).

Table 6. Saturated fatty acids, unsaturated fatty acids, nutritional indexes of milk from Murrah buffaloes.

Item	T_0	T_1	T_2
Saturated fatty acid (SFA)	$63.77^{a}\pm7.31$	$46.42^{b}\pm6.30$	$44.64^{b}\pm 6.10$
Unsaturated fatty acid (UFA)	$26.01^{b}\pm5.74$	$37.68^{a}\pm8.10$	$38.20^{a}\pm8.22$
UFA/SFA	$0.41^{b}\pm0.03$	$0.81^{a}\pm0.04$	$0.85^{a}\pm0.03$
Short chain fatty acid	$9.75^{a}\pm0.95$	$4.96^{b}\pm0.65$	$4.52^{b}\pm0.59$
Medium chain fatty acid	$40.25^{a}\pm1.93$	$29.53^{b}\pm1.14$	$28.67^{b}\pm1.06$
long chain fatty acid	$39.78^{b}\pm2.03$	$49.61^{a}\pm2.83$	$49.65^{a}\pm1.96$
Atherogenic index (AI)	$2.79^{a}\pm0.25$	$1.26^{b}\pm0.19$	$1.22^{b}\pm0.13$
Thrombogenic index (TI)	$3.38^{a}\pm0.29$	$1.76^{b}\pm0.20$	$1.68^{b}\pm0.10$
n-6 Fatty acid	$2.22^{b}\pm0.83$	$3.56^{a}\pm1.39$	$3.59^{a}\pm1.41$
n-3 Fatty acid	$0.33^{b}\pm0.06$	$0.73^{a}\pm0.007$	$0.74^{a}\pm0.03$
n-6/n-3	$6.73^{a}\pm0.35$	$4.88^{b}\pm0.29$	$4.85^{b}\pm0.35$

increases inflammation by a factor of 15-20:1. This is because, according to Marventano *et al*. (2015), fatty acid n-6 and fatty acid n-3 compete for the enzymes that cause desaturation and elongation. This can lead to a decrease in the synthesis of eicosatetraenoic and docosahexaenoic acids while raising the concentration of arachidonic acid, which serves as a substrate for the synthesis of compounds that are pro-aggregating, pro-inflammatory, and vasoconstricting (Korotkova and Lundberg, 2014).

CONCLUSION

In conclusion, adding linseed and linseed oil to a buffalo's diet based on its total mixed ration did not alter the amount or composition of milk produced; nevertheless, it did increase n-3 FA, unsaturated fatty acids, cis-9, trans-11, and trans-10, cis-9-CLA, and other fats. However, the addition of linseed and linseed oil reduced total cholesterol and saturated fat. In order to improve the milk fatty acid profile, it is advised that buffalo diets be supplemented with 250 g/d of linseed oil or 570 g/d of whole linseed.

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