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Effect of Sunflower Oil Supplementation on Milk Production, Composition, Fatty Acid Profile and Blood Metabolites of Mehsana Buffaloes

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ABSTRACT

Background: In early post-partum buffaloes, feed intake is generally insufficient to meet the energy demand for milk production; as a consequence, body reserves are mobilized which can result in metabolic disorders and poor production performance. In order to enhance energy intake and reduce the detrimental effects of a negative energy balance, buffalo diets may be supplemented with lipids/vegetable oil. Some reported benefits of vegetable oil supplementation include increased energy concentration in the diet, reduced supply of rapidly fermentable carbohydrates, and better productive performance. Sunflower (*Helianthus annuus* L.) is an important oilseed crop in India. Sunflower seed contains about 48-53 percent of edible oil. Recent studies demonstrated that sunflower oil supplementation improved the milk production and nutritional quality of milk fat which is beneficial to human health. Therefore, the present study was undertaken to investigate the effect of sunflower (*Helianthus annuus* L.) oil supplementation on production performance of lactating Mehsana buffalo.

Methods: Three experimental groups were as, CON: fed on basal diet (Control), SO125: fed basal diet supplemented with 125 ml of sunflower oil/animal/day and SO250: fed basal diet supplemented with 250 ml of sunflower oil/animal/day for a period of 90 days.

Result: There was no difference (P>0.05) in DM intake of lactating buffaloes among the dietary treatments. Dietary inclusion of sunflower oils improved milk yield by 17.9 to 20.7%, 6% FCM yield by 16.4 to 22.9% and ECM yield by 16.0 to 23.0% in SO125 and SO250 groups, respectively as compared to the CON. There were no differences (P>0.05) in milk composition and yields of milk components. The milk fatty acid percentages of C20:0 and C24:0 were significantly decreased due feeding of sunflower oil as compared to the CON. Feeding of sunflower oil did not influence milk fat short chain, medium chain, saturated, unsaturated and polyunsaturated fatty acids in lactating Mehsana buffaloes. The serum concentrations of glucose, total proteins, albumin, urea, creatinine, triglycerides, ALT and AST were not affected (P>0.05) by supplementation of sunflower oil, however, serum cholesterol concentration was significantly (P<0.01) increased in SO125 and SO250 groups as compared to the CON group.

Key words: Buffalo, Milk fatty acids, Milk yield, Serum biochemistry, Sunflower oil.

INTRODUCTION

In India and many parts of the world buffaloes are the main source of milk, meat, and draft power. The buffalo plays a very important role in the South Asian region economically and culturally. It is reported that 79.74% of Asia and 77.9% of world buffalo populations are inhabitants in South Asian countries (Siddiky and Faruque 2017). In India, the total buffalo population is recorded 109.85 million and about 51% of total milk production is shared by these animals (20th Livestock Census 2019). Among the buffalo breeds of India, Mehsana breed is supposed to have been evolved out of crossbreeding between the Murrah and the Surti. Mehsana is a dairy breed of buffalo found in north-Gujarat area and adjoining Maharashtra state. At the beginning of lactation, due to reduced feed intake there is an energetic deficiency, since there is a sudden increase of the energy requirements because of the high demand for galactopoietic, which increases the mobilization of body reserves to maintain the milk yield (Esposito *et al.,* 2014; Sadrasaniya *et al.,* 2022). In order to enhance energy intake and reduce the detrimental effects of a negative energy balance, dairy animal diets may be supplemented with vegetable oil such as sunflower (*Helianthus annuus* L.) oil. Earlier studies have reported benefits of sunflower oil supplementation in lactating animals include increased energy concentration in the diet, reduced supply of rapidly fermentable carbohydrates and better

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productive performance (Morsy *et al*., 2015; Khalifa *et al*., 2016). Moreover, sunflower oil contains 64.1 and 25.2 percent of linoleic acid (*cis*-9, *cis*-12 C18:2) and oleic acid (*cis*-9 C18:1), respectively (Mwakasege *et al.,* 2021).

Milk fat content and fatty acid composition can be significantly altered through dietary strategies such as inclusion of vegetable oils, offering the opportunity to respond to changes in consumer requirements and provide foods more in line with recommendations for improving human health (Joshi *et al.,* 2021). Recent studies found that supplementation of sunflower oil in dairy animals modified milk fatty acid profile towards a healthier profile for human consumption (Ferlay and Chilliard 2020; Lopes *et al*., 2020; Lopes *et al*., 2021). However, most of the studies of feeding sunflower oil have been carried out in lactating cows and goats. We hypothesized that supplementation of sunflower oil in early lactating Mehsana buffaloes will to bring improvement milk yield and fatty acid composition through modified ruminal biohydrogenation and mammary lipogenesis. Thus, the objective of this study is to evaluate the effect of sunflower oil supplementation on milk production, composition, fatty acid profile and blood metabolites of Mehsana buffaloes.

MATERIALS AND METHODS

Animals and experimental design

The present study was conducted at Livestock Research Station, Kamdhenu University (Formally under Sardarkrushinagar Dantiwada Agricultural University) Sardarkrushinagar, Gujarat during year 2021. The experimental protocol followed in this study was approved by the Institutional Animal Ethics Committee (VETCOLL/IAEC/2020/16/PROTOCOL-7). Eighteen lactating *Mehsana buffaloes* (21 days in milk, 465 kg avg. BW, 6.89 kg average daily milk yield) were randomly divided into three groups *viz.* CON: fed on basal diet (Control), SO125: fed basal diet supplemented with 125 ml sunflower oil/animal/day and SO250: fed basal diet supplemented with 250 ml/animal/day of sunflower oil for a period of 90 days. Sunflower oil was mixed into a portion of the concentrate mixture thoroughly and provided once daily during the experimental period. The basal diet was formulated to meet nutrient requirements (ICAR, 2013). The chemical composition of feeds and fodders fed to the experimental animals is given in Table 1.

Sampling and chemical analysis

The samples of feeds and fodders were collected, composited and dried at 60°C in a forced air oven for 48 h, ground to pass through a 1-mm screen using a Wiley mill (Star Scientific Instruments, Delhi, India). The feeds samples were analyzed for dry matter (DM, method 934.01), ash (method 942.05), crude protein (method 976.05), crude fibre and ether extract (method 973.18) according to AOAC (2007). Buffaloes were milked twice a day and individual milk yield for each cow was recorded daily by using electronic weighing balance. The 6% fat corrected milk (FCM) was calculated as stated by Rice (1970): milk yield (kg) × 0.4 + fat yield (kg) × 15/1.3. Energy corrected milk (ECM) was determined according to Davidson *et al.* (2008): 0.327 × milk yield (kg/d) + 12.86 × fat yield (kg/d) + 7.65 × protein yield (kg/d). Milk samples were collected at fortnightly interval for analysis of milk composition (fat, solids-not fat (SNF), protein and lactose) using EKOMILK Ultra Pro Milk Analyzer (Everest Instruments Pvt. Ltd.).

Determination of milk fatty acid profile

For the determination of milk fatty acids, samples of milk were collected at fortnightly interval and pooled. Individual fatty acid in milk was determined as described earlier (Pawar *et al*., 2021). Briefly, milk fatty acids were analysed by isolating milk fat by centrifugation and methylation using sodium methylate. Fatty acid methyl esters were analysed using gas chromatography mass spectrometer (GCMS- QP 2010 Plus) equipped with an auto sampler injector, flame ionization detector and capillary column (60m × 0.25mm × 0.20 mm). The initial oven temperature was 120°C, held for 5 min, subsequently increased to 240°C at a rate of 2°C min^{-1}, and then held for 60 min. Helium at a flow rate of 1 ml/min was used as the carrier gas. Both the injector and the detector were set at 260°C. As an internal standard fatty acid heptadecanoic acid C17:0 (Catalogue number H3500, Sigma-Aldrich, Bangalore, India) was used and a mix of FAME standards (Supelco 37 Component FAME Mix, Sigma Aldrich, Bangalore, India) was used to generate a calibration curve. Fatty acids were identified by comparing their retention times with the fatty acid methyl standards and were expressed as percentage of total fatty acids.

Blood sampling and analysis

At the end (on 90th day) of experimental feeding, blood samples from external jugular vein were collected from each

Table 1: Chemical composition (% DM basis) of feeds and fodder fed to experimental animals.

Composition	Concentrate mixture	Green oat fodder	Jowar hay
Dry matter	94.76	17.44	11.83
Crude protein	20.56	8.92	6.39
Crude fibre	6.38	27.67	32.25
Ether extract	4.15	3.12	1.34
Total ash	12.21	7.34	9.07
NFE	56.70	23.81	21.79

experimental animal in sterilized vials without EDTA. The serum was separated and samples were analyzed for concentrations of glucose, total proteins, albumin, urea, triglycerides, alanine transaminase (ALT), aspartate amino transferase (AST) and cholesterol using Randox Monaco Analyser (Randox Laboratories Ltd., UK).

Statistical analysis

The data obtained were analyzed by one way ANOVA for a randomized complete block design using the SPSS v.16.0 (SPSS Inc., Chicago IL). Significant differences between means of treatments were assessed by the Duncan's test and the differences among treatments were declared significant at $P<0.05$.

RESULTS AND DISCUSSION

Feed intake and production performance

The mean DM intake was 13.32, 12.84 and 13.38 kg/d in CON, SO125 and SO250 groups, respectively. There was no difference ($P>0.05$) in DM intake of lactating buffaloes among the dietary treatments. As free vegetable oils are rapidly released in the rumen and have subsequent potential deleterious effects on the resident microbiota, fibre digestion and DM intake. Therefore, to avoid a decrease in DM intake, the NRC (2001) had recommended that rations for dairy cows with a maximum of 6-7% ether extract (on % DM basis). In the present study, the ether extract concentrations in the ration of lactating buffaloes were below the recommended level. Hence, there was no adverse effect of sunflower oil supplementation (125 or 250 ml/h/d) on DM intake. In agreement with the present findings, no changes in DM intake were reported in dairy cows supplemented with sunflower oil at 1.5, 3.0 and 4.5% of DM (De Souza *et al.,* 2019; Lopes *et al*., 2020). On the contrary to the present findings, Kairenius *et al.,* (2018) observed that DM intake was decreased in dairy cows fed grass silage-based diet containing 500 g/d of sunflower oil.

Dietary inclusion of sunflower oil improved milk yield by 17.9 to 20.7% ($P=121$), 6% FCM yield by 16.4 to 22.9% ($P=177$) and ECM yield by 16.0 to 23.0% ($P=166$) in SO125 and SO250 groups, respectively as compared to the CON group (Table 2; Fig 1). There were no differences ($P>0.05$) in yields of milk fat, SNF, protein and lactose due to supplementation of sunflower oil. The observed improvement in milk yield in sunflower oil supplemented groups might be due to the change in ruminal fermentation pattern leading to increased propionate production in rumen. Propionate is the most important substrate for hepatic gluconeogenesis (accounting for 60-74% total substrate), which is highly associate with milk yield in cows (Aschenbach

Table 2: Effect of sunflower oil supplementation on dry matter intake, milk yield and milk composition of lactating Mehsana buffaloes.

Parameters	Dietary groups†			SEM	Significance
	CON	SO125	SO250		
Dry matter intake (DMI)					
DMI (kg/d)	13.32	12.84	13.38	0.14	NS
DMI (% BW)	2.70	2.64	2.72	0.05	NS
DMI (kg/kg $W^{0.75}$)	0.13	0.12	0.13	0.02	NS
Organic matter intake (kg/d)	12.01	11.90	12.04	0.11	NS
Yield (kg/d)					
Milk	7.44	8.77	8.98	0.34	NS
6% FCM	7.64	8.89	9.39	0.39	NS
ECM	10.70	12.41	13.16	0.54	NS
Fat	0.47	0.54	0.58	0.10	NS
Solids not fat	0.65	0.76	0.79	0.04	NS
Protein	0.30	0.36	0.36	0.05	NS
Lactose	0.31	0.35	0.38	0.04	NS
Milk composition (%)					
Fat	6.19	6.21	6.53	0.02	NS
Solids not fat	8.80	8.88	8.91	0.03	NS
Protein	3.95	4.14	4.10	0.01	NS
Lactose	4.15	4.30	4.29	0.01	NS
Feed efficiency					
Milk (kg)/DMI (kg)	0.53	0.63	0.61	0.02	NS
6% FCM (kg)/DMI (kg)	0.70	0.94	0.82	0.04	NS
ECM (kg)/DMI (kg)	0.88	0.93	0.90	0.02	NS

NS: Non-significant.

FCM: Fat corrected milk; ECM: Energy corrected milk.

†CON: Fed on basal diet (Control), SO125: CON + 125 ml/animal/day of sunflower oil; SO250: CON + 250 ml/animal/day of sunflower oil.

et al. 2010). Besides of that, glucose is a precursor of lactose, an osmotic constituent of milk, which increases water secretion and consequently milk volume (Lei and Simoes 2021). Similar to the present findings, Dai *et al*., (2011) reported significant (P<0.05) increase in milk yield (26.4 *vs.* 25.5 kg/d) in dairy cows supplemented with 2% sunflower oil. In contrast, no effects on milk production performance due to feeding of sunflower oil in dairy cows were reported in the earlier studies (Silva *et al*., 2018; De Souza *et al.*, 2019; Ferlay and Chilliard 2020). The milk composition (fat, SNF, protein and lactose) were not affected (P>0.05) among the dietary groups. Similar to the present findings, Silva *et al*. (2018) and Lopes *et al*. (2020) reported no effect on milk composition of dairy cows fed sunflower oil. However, De Souza *et al.* (2019) reported that there was linear decrease (P<0.05) in percentages of milk fat due to sunflower oil (1.5, 3.0 and 4.5%) supplementation. Dietary supplementation of sunflower oil had no effect (P>0.05) on feed efficiency in lactating buffaloes (Table 2). No effect on feed efficiency may be attributed to similar DM intake among the treatment groups. This is in agreement with the results of Beauchemin *et al.* (2009) who found that supplementation of 3.3% added fat (on DM basis) crushed sunflower seeds in lactating cows had no effect on the feed efficiency.

Milk fatty acid profile

The results of supplementation of sunflower oil on milk fatty acids of lactating Mehsana buffaloes are given in Table 3. The short chain fatty acids (C4:0 to C10:0) and medium chain fatty acids (C12:0 to C16:0) of milk did not differed among the different dietary groups. In long chain fatty acids (> C16:0) of milk fatty acid percentages of C20:0 and C24:0 were significantly decreased due feeding of sunflower oil as compared to the CON. Supplementation of sunflower oil (125 or 250 ml/h/d) did not influenced percentages of milk saturated fatty acids, unsaturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids in lactating Mehsana buffaloes. Supplementation of sunflower oil at both the dose rate did not have any detrimental effect on milk fatty acid composition in lactating buffaloes. Milk fatty acids are mainly derived from two major sources, uptake of long-chain fatty acids from peripheral circulation and *de novo* synthesis of short- and medium-chain fatty acids in the mammary gland (He and Armentano 2011). Moreover, during the onset of lactation, the energy requirements for milk production exceed nutrient intake, and animal experience a period of negative energy balance, causing the mobilisation of long-chained fatty acids from adipose tissue and incorporation into milk fat. In agreement with the present findings, Lopes *et al*. (2021) found that with supplementation of sunflower oil in dairy cows significantly reduced milk fat percentages of long chain fatty acids.

Blood metabolites

The findings of supplementation of sunflower oil on blood metabolites of lactating Mehsana buffaloes are given in Table 4. The serum concentrations of glucose, total proteins, albumin, urea, creatinine, triglycerides, ALT and AST were not affected (P>0.05) by the sunflower oil supplementation in lactating buffaloes. Lack of effect on liver enzymes ALT and AST due to feeding sunflower oil in lactating buffaloes indicates that supplementation did not have any adverse effect on liver function.

The serum level of cholesterol was 129.23, 155.10 and 164.23 mg /dL in CON, SO125 and SO250 groups, respectively. There was significant (P<0.01) increase in serum cholesterol concentration due to supplementation of sunflower oil (125 and 250 ml/d) as compared to the CON group. Vegetable oil supplementation in the diet encourages the production of lipoproteins in the intestine which is the major site of *de novo* cholesterol synthesis in ruminants, thus leads to elevated levels of serum cholesterol. Similarly, earlier studies also reported that supplementation of sunflower oil in diet of dairy cows increased serum cholesterol concentration (De Souza *et al.,* 2019; Lopes *et al*., 2020).

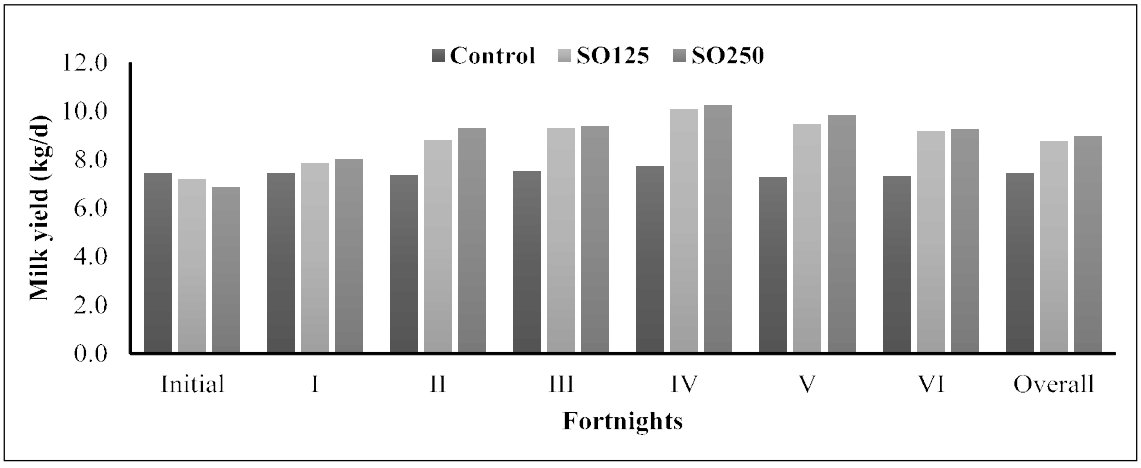

Fig 1: Effect of sunflower oil supplementation on milk yield (kg/d) of lactating Mehsana buffaloes at fortnightly intervals.

Table 3: Effect of supplementation of sunflower oil on milk fatty acid profile (g/100 g FA) of lactating Mehsana buffaloes.

Fatty acid	Dietary groups†			SEM	Significance
	CON	SO125	SO250		
C4:0 (Butyric acid)	2.12	2.07	2.31	0.06	NS
C6:0 (Caproic acid)	2.23	2.10	2.28	0.07	NS
C8:0 (Caprylic acid)	1.53	1.33	1.46	0.07	NS
C10:0 (Capric acid)	3.10	2.86	3.04	0.13	NS
C11:0 (Undecanoic acid)	0.03	0.01	0.01	0.01	NS
C12:0 (Lauric acid)	4.12	4.01	4.10	0.13	NS
C13:0 (Tridecanoic acid)	0.12	0.06	0.04	0.02	NS
C14:0 (Myristic acid)	12.88	13.51	13.05	0.27	NS
C15:0 (Pentadecanoic acid)	2.20	1.97	1.97	0.07	NS
C16:0 (Palmitic acid)	28.62	28.24	27.81	0.41	NS
C16:1 (Palmitoleic acid)	2.79	3.38	3.25	0.26	NS
C17:0 (Heptadecanoic acid)	1.45	1.28	1.36	0.06	NS
C18:0 (Stearic acid)	14.83	14.53	14.92	0.24	NS
C18:1n9t (Elaidic acid)	16.73	17.60	17.10	0.38	NS
C18:1n9c (Oleic acid)	0.83	0.73	0.68	0.08	NS
C18:2n6t (Linolelaidic acid)	0.07	0.07	0.92	0.30	NS
C18:2n6c (Linoleic acid)	3.21	3.69	2.90	0.32	NS
C18:3n3 (gamma-Linolenic acid)	1.02	0.81	0.85	0.06	NS
C20:0 (Arachidic acid)	0.35	0.24	0.26	0.02	*
C20:1 (cis-11-Eicosenoic acid)	0.46	0.58	0.64	0.06	NS
C20:2 (cis-11,14-Eicosenoic acid)	0.06	0.00	0.00	0.01	NS
C20:3n3 (cis-11,14,17-Eicosatrienoic acid)	0.51	0.39	0.43	0.03	NS
C20:4n6 (Arachidonic acid)	0.12	0.11	0.11	0.02	NS
C22:0 (Behenic acid)	0.44	0.35	0.40	0.02	NS
C24:0 (Lignoceric acid)	0.20	0.12	0.12	0.01	*
Saturated fatty acids	75.02	73.26	73.75	0.49	NS
Unsaturated fatty acids	24.99	26.74	26.26	0.50	NS
Monounsaturated fatty acids	20.70	21.95	21.28	0.52	NS
Polyunsaturated fatty acids	4.28	4.80	4.97	0.16	NS

[ab]Means with different superscripts in a row differed significantly (P<0.05).

†CON: fed on basal diet (Control), SO125: CON + 125 ml/animal/day of sunflower oil; SO250: CON + 250 ml/animal/day of sunflower oil.

Table 4: Effect of supplementation of sunflower oil on blood metabolites of lactating Mehsana buffaloes.

Parameters	Dietary groups†			SEM	Significance
	CON	SO125	SO250		
Glucose (mg/dL)	65.66	66.66	67.33	1.37	NS
Total protein (g/dL)	7.32	7.32	7.33	0.10	NS
Albumin (g/dL)	3.62	3.63	3.62	0.07	NS
Urea (mg/dL)	46.94	46.71	48.31	1.03	NS
Creatinine (mg/dL)	1.2	1.22	1.22	0.03	NS
Triglycerides (mg/dL)	6.81	6.93	6.95	0.45	NS
Cholesterol (mg/dL)	129.2	155.1	164.2	4.55	**
ALT (U/L)	44.80	44.25	44.42	1.16	NS
AST (U/L)	136.0	136.2	136.2	3.49	NS

[ab]Means with different superscripts in a row differed significantly (P<0.01).

†CON: Fed on basal diet (Control), SO125: CON + 125 ml/animal/day of sunflower oil; SO250: CON + 250 ml/animal/day of sunflower oil.

CONCLUSION

Based on the results, it may be concluded that sunflower oil supplementation in the diet of lactating Mehsana buffaloes improved milk production performance with beneficial changes in milk fatty acids profile in greater interest of human health.

Conflict of interest: None.

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