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Effect of feeding calcium salts of soybean oil fatty acids on physical and chemical characteristics of milk in cows

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ABSTRACT

The present study was conducted to determine the effect of feeding calcium salts of soybean oil on composition of milk fat and characterize the modified milk fat. Sahiwal cows (18) of first and early lactation were stratified into 3 groups in a completely randomized design. Calcium salts of soybean oil fatty acids (CSSFA) were fed at 2 different levels i.e. 150 and 300 g CSSFA/cow/day with third group kept as control. Feeding CSSFA significantly decreased the content of short chain fatty acids and increased the concentration of unsaturated fatty acids in both the treated groups. The milk yield and fat increased with no effect on cholesterol content. Melting point and oxidative stability was also less in the treated groups. Sensory evaluation indicated no effect on color, taste and smell of milk with CSSFA feeding.

Key words: Calcium salts, Chemical characteristics, Cows, Soybean oil, Milk, Physical characteristics

Milk fat is criticized for having higher content of saturated fatty acids and lower content of polyunsaturated fatty acids that are atherogenic, and raise the risk of cardiovascular disease by increasing plasma cholesterol and low density lipoproteins (LDL). The amount and composition of milk fat can be influenced by means of feeding. Most strategies involve supplementing diet with plant oils or oil seeds (Abu Ghazaleh and Holmes 2009, Baer *et al.* 2001). Calcium salt of fatty acids is a rumen bypass fat; the objective of bypassing rumen is to save the beneficial unsaturated fatty acids from microbial biohydrogenation and formation of transisomers (Parodi 1999). Sahiwal cattle is the most important bread of dairy cows in Pakistan, naturally resistant against ticks and many diseases, possess the capability of adjusting in hot and humid climate, and existing quite successfully in almost 29 countries of the world. Modification of fatty acid profile of milk of Holstein Friesian cows was achieved by Dhiman *et al.* (2000). The present investigation was planned to study the effect of feeding calcium salts of soybean oil on composition of milk fat and characterize the modified milk fat on the basis of certain physico-chemical and sensory characteristics.

MATERIALS AND METHODS

Experimental station and raw materials: Sahiwal cows (18) of first and early lactation were randomly selected and assigned 2 different treatments of CSSFA i.e. T_1 (150-CSSFA/cow/day) and T_2 (300-CSSFA/cow/day) both treatments were compared with a control without any addition of CSSFA (T_0) for 8-weeks. Green fodder (sorghum) *ad lib.*, wheat straw 3 kg/cow/day, free excess to water, equal number of installers, same barn conditions, 3 kg concentrate (inclusion of CSSFA for T_1 and T_2) was given to each cow per day. Isocaloric and isonitrogenous feed was formulated at LPRI according to the Table 1.

Table 1. Detail of feed

Ingredients	T_0 (Control)	T_1	T_2
Cotton seed cake %	25	43	51
CSSFA grams	–	150	300
Maize gluten 30%	15	5	–
Maize grains ground %	10	15	25
Wheat bran %	8	15	12
Rice polishing %	15	10	–
Molasses %	10	5	–
Sunflower meal %	15	–	–
Mineral mixture %	2	2	2
Total	100	100	100
Crude protein %	17.6	17.54	17.97
Metabolizable energy	2.76	2.74	2.83

CSSFA: Calcium salts of soybean oil fatty acids.

Collection and analysis of milk samples: Proportionate milk samples were collected after adjustment period of 14-

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days, milk of morning and evening was proportionately mixed (50-mL/liter), triplicate samples were collected for each group, immediately cooled down to 4°C and analyzed for chemical composition by a kit as per instructions of the manufacturer.

Fatty acid profile: Fatty acids profile was determined as fatty acid methyl esters, 300-μL melted and well mixed sample was taken into 11-mL screw capped test tube, dissolved in 3-mL isooctane and 2-mL 1.5 N sodium methoxide was added vortexed at higher speed for exactly 3-min, allowed to separate for 5 min and the supernatant was injected into gas chromatograph using flame ionization detector as per standard IUPA C method (1987) 2301.

Characterization of modified milk fat: Milk fat was extracted by thermo centrifuge, at 10,000 RPM for 10 min in 250 mL screw capped centrifuge tubes, stored at –60 °C in the biomedical freezer and characterized for iodine value, refractive index, unsaponifiable matter, free fatty acids, color, oxidative stability by following the respective methods of AOCS (1990). The concentration of cholesterol was measured as per Rudel and Morris (1973). Color was determined with tintometer as per the manufacturer. Refractive index was measured at 40°C by a digital refractometer according to the method of AOCS (1990).

Oxidative stability of milk fat with modified fatty acid profile: Schaal oven test was performed by keeping 20±0.1 g samples in triplicate beakers at 63±2 °C in an oven for 5 days; peroxide value was measured as an indication of oxidative stability after 5 days. Oxidation products in the form of conjugated dienes were measured by dissolving 1 g sample in isooctane and absorbance was measured at 232 nm in the ultraviolet region on a spectrophotometer (Paquot 1979). Induction period was measured as per Metrohm (1993).

Sensory evaluation: Milk was pasteurized and evaluated for color, smell, taste and overall acceptability by a panel of 75-Judes on Hedonic scale (Larmond 1987).

Statistical analysis: All determinations were carried out in triplicate and the data were expressed as mean±SD. The data were analyzed by using completely randomized design and significant difference (P<0.05) among the treatments was calculated by using Duncan Multiple Range Test (Steel *et al.* 1997).

RESULTS AND DISCUSSION

Fatty acid profile: The concentration of short chain fatty acids (Table 2) in both the treatment groups was significantly lesser than the control, while long chain fatty acids were considerably higher over the control group; oleic acid was the dominant fatty acid in T_2 which was 37.8% higher than the control. The lauric acid which is considered to be the most detrimental fatty acid (Mc Sweeney and Fox 2003) from the point of view of atherosclerosis decreased from 2.71 to 1.39 which was 48.7% lower than the control. CSSFA contain approximately rumen protected 45% oleic acid and 9.2% linoleic acid, which may be the major reason for the marked changes in the fatty acid composition of milk. The fatty acid composition of Friesian cows was tailored by Elliott *et al.* (1996). Cows fed on CSSFA produced milk with significantly different fatty acid profile from the control group with appreciably higher concentrations of long chain fatty unsaturated fatty acids. Rumen protected unsaturated fatty acids included in the diet of Holstein Friesian cows @ 300 g/cow/day increased the concentration of C18: 1 and C18: 2 by 27.9 and 32.1%, respectively (Palmquist *et al.* 1993). Similar results were reported by Ashes *et al.* (1992) *and* Whitlock *et al.* (2002).

Table 2. Fatty acid profile and cholesterol content of milk of cows

Fatty Acid	T_0	T_1	T_2
C4: 0	3.84±0.09a	3.35±0.03b	2.74±0.15c
C6: 0	2.39±0.07a	2.17±0.11b	1.720.34±c
C8: 0	1.37±0.12a	1.25±0.02b	0.82±0.28c
C10: 0	2.55±0.10a	2.18±0.05b	1.760.05±c
C12: 0	2.71±0.04a	1.84±0.03b	1.39±0.08c
C14: 0	9.87±022a	8.93±0.15b	6.15±0.18c
C16: 0	24.96±0.34c	35.24±0.57b	37.22±0.79a
C18: 0	14.27±0.45a	12.77±0.44b	11.85±0.65c
C18: 1	27.49±0.67c	32.37±0.35b	38.22±0.62a
C18: 2	2.24±0.06c	2.66±0.11b	4.01±0.12a

Nonsignificant (P>0.05), significant (P<0.05). Means of triplicate experiments; means sharing same letter in a row are statistically nonsignificant at 0.05 level of significance. T_1: 150 g CSSFA/cow/day, T_2: 300 g CSSFA/cow/day, T_0: Control, Refer Table–1 for the detail of treatments

Cholesterol: Feeding CSO at both the levels slightly decreased the concentration of cholesterol from milk but it was statistically nonsignificant (P>0.05). In the other studies related to the feeding of calcium salts of fatty acids and copper to red and white cows (Brzoska and Sala 2001) reported that the cholesterol content of the milk from the experimental group and control was not significantly different from each other.

Milk production and composition: Feeding CSSFA progressively increased the milk production capability of cows. The average milk production in control, T_1 and T_2 was 6.89, 8.16 and 10.05 liter/day. The increase in milk production at T_1 and T_2 level was 15.6 and 31.3%. The increasing trend in milk production was also reported by Ashes *et al.* (1997) who studied the impact of CSSFA on milk production of first parity cows. Feeding CSSFA significantly increased fat content of the milk at both levels of inclusion (150 and 300 g/cow/day). Fat content increased from 4.35 to 4.95%, protein, lactose, ash content and SNF were not affected.

Characterization of fat: Feeding CSSFA did not show any

Table 3. Characterization of milk fat with modified fatty acid composition

Treatments	FFA (%)	PV (meq/kg)	IV	USM (%)	Color R+Y	RI (40°C)	MP (°C)
To	0.08±0.022a	0.17±0.02a	38.17±0.12c	0.66±0.05a	24±0.12a	1.451±0.001c	34.5±0.25a
T_1	0.08±.0.031a	0.18±0.01a	41.14±0.18b	0.68±0.04a	22±0.29a	1.458±0.002b	33.2±0.41b
T_2	0.09±0.015a	0.21±0.01a	45.55±0.34a	0.72±0.07a	25±0.45a	1.469±0.001a	31.0±0.34c

Means with similar letter in a column are not different; Refer Table 1 for the detail of treatments; FFA: free fatty acids, PV: peroxide value; IV: iodine value, USM: unsaponifiable matter, RI: refractive index, MP: melting point, R+Y: red plus yellow (Lovibond Tintometer Scale)

adverse effect on free fatty acids content and it rather improved the degree of unsaturation in the experimental samples (Table 3). Peroxide value did not differ in the control and experimental groups. Peroxides are the oxidation product generated during autoxidation of fats and oils and higher levels of peroxides are generally associated with poor keeping quality. Iodine value increased linearly with increased addition level of CSSFA. Iodine value is related to degree of unsaturation; fats having higher iodine value are generally recognized as more suitable for human health (Fox and Mc Sweeney 1998). Refractive index is considered a good measure to estimate the unsaturation of fats and oils. Feeding CSSFA significantly increased the refractive index. The melting point of both the treatments was significantly less than the control ($P<0.05$). Melting point of fats is related to the extent of occurring saturated/unsaturated fatty acids; fats having higher proportions of saturated fatty acids have a higher solid fat index and higher melting points and vice versa (Erickson 1999). Fats having lower melting characteristics are considered more healthful than those who have higher melting points (Ghatak and Bandyopadhyay 2007).

Oxidative stability: Determination of conjugated dienes (Table 4) gives a good indication of oxidative deterioration in terms of oxidation products produced in the lipid substrates (Pritchard 1991). Feeding CSSFA to cows did not have any significant effect (Table 5) on the extent of oxidation products in the form of conjugated dienes (fresh milk). The value of this parameter increased throughout the storage period of 6 days, the maximum increase was observed in T_2 after 6 days. Unsaturated fatty acids are susceptible to autoxidation which results in the onset of free radical mechanism and formation of oxidation products (Erickson 1999). Gonzalez *et al.* (2001) while studying the effect of feeding CSSFA on the oxidation behavior of modified fat reported that that modified fats were more susceptible to autoxidation than the unmodified fats.

Table 4. Conjugated dienes of milk with altered fatty acid profile (ε1CM [λ232])

Parameters	Storage period (days)	T_0	Treatments	
			T_1	T_2
Conjugated dienes	0	0.32±0.02a	0.32±0.01a	0.34±0.02a
	2	0.38±0.04c	0.45±0.12b	0.61±0.01a
	4	0.49±0.03c	0.67±0.04b	0.86±0.05a
	6	0.52±0.02c	0.83±0.03b	1.12±0.09a

Means with similar letter in a row are not different ($P>0.05$). Refer Table 1 for the detail of treatments.

Table 5. oxidative stability of milk fat with altered fatty acid profile

Parameters	T_0	Treatments	
		T_1	T_2
Induction Time (Hours)	6.22±0.11a	5.82±0.08b	4.65±0.19c
PV (After Five Days)	6.68±0.15c	8.93±0.32b	11.15±0.22a

Means with different letter in a row are different. Refer Table 1 for the detail of treatments.

Determination of induction period may serve as a good parameter to forecast the expected shelf life/keeping quality of fats and oils; higher induction time is associated with prolonged keeping quality and vice versa (Mahuya *et al.* 2008). Induction period progressively decreased with increasing level of inclusion of CSSFA in the feed of cows. Induction period is associated with the presence of pro-oxidants and the fatty acid make up of fats. Fats containing higher proportions unsaturated fatty acids offer less resistance towards accelerated autoxidation and possess low induction period than the fats containing higher concentrations of saturated fatty acids (Fereidoon 2005). Gonzalez *et al.* (2001) studied the oxidation resistance of milk fat with modified acid profile achieved by feeding calcium salts of fatty acid and reported that modified fat (containing a higher concentration of unsaturated fatty acids) showed less resistance towards autoxidation.

Sensory evaluation: The results of sensory evaluation of freshly pasteurized milk did not show any discernable difference in taste, smell, color and overall acceptability of milk from CSSFA supplemented cows. Gonzalez *et al.* (2001) studied the sensory characteristics of milk fat with modified fatty acid profile and reported that sensory score of milk fat with modified fatty acid profile was comparable to the control when fresh. However, sensory score decreased during storage due to the development of oxidized flavor. The milk fatty acid profile may affect the sensory characteristics of the dairy products made from it (Hillbrick and Augustin 2002). Oxidation of unsaturated fatty acids particularly the unsaturated fatty acids may develop fishy, nutty, grassy, oxidized and stale flavor in milk after long term storage or having exposure to light and pro-oxidants (Ghatak and Bandyopadhyay 2007).

Our results suggested that CSSFA up to 300 g/cow/day may be included in the feed of Sahiwal cows to produce milk with higher content of unsaturated fatty acids and improved physical characteristics for better health with acceptable sensory characteristics.

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