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Quality of milk fat obtained from cows and buffaloes fed a diet supplemented with flaxseed or soybean oils

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ABSTRACT. The experiment was carried out to evaluate the quality of anhydrous milk fat (AMF) of cows and buffaloes supplemented with flaxseed oil (FO), soybean oil (SO), or their mixture (FSO). Lactating crossbred cows and buffaloes were fed with control diet or with one of three supplements: 2% FO, 2% SO, and 2% FSO according to a double 4 *x* 4 Latin Square Design. The diets with FO, SO, or FSO reduced saturated FA, mainly C4:0, C14:0 and C16:0, while increased the unsaturated FA C18:1 and C18:2 in milk from cows and buffaloes. Cholesterol content decreased in cow's AMF while increased in buffalo's AMF when a diet supplemented with FO, SO, or FSO. The diet with SO or FSO increased the content of vitamin E in AMF obtained from cows (25.06 and 17.89 mg 100 g^{-1}) and buffaloes (28.48 and 30.32 mg 100 g^{-1}) compared with the control diet (11.02 and 15.68 mg 100 g^{-1}), respectively, which correlated positively with scavenging activity for DPPH• (r^2 = 0.66) and ABTS• (r^2 = 0.67) radicals. Solid fat content (SFC) was high for cow's AMF, with 58.12-60.37% at 5°C compared to that of buffalo's AMF, with 52.37-56.98%, but was low for cow's AMF at >15°C. Finally, supplementing a diet with vegetable oils, particularly SO, improves the quality of AMF; increases USFA/SFA ratio, vitamin E content, and antioxidant activities.

Keywords: anhydrous milk fat; flaxseed and soybean oils; fatty acid profile; vitamin E; radical scavenging activities; solid fat content.

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Introduction

The nutrient values of milk and their product, such as the composition of the different fatty acids (FA) and vitamins, are affected mainly by species, breed, season, stage of lactation, and diet (González-Martín, Palacios, Revilla, Vivar-Quintana, Hernández-Hierro, 2017). The quality of milk fat is influenced by numerous interacting dietary factors, which including quality and composition of forage, the amount and quality of fiber, the site and rate of degradability of starch, the FA composition of dietary lipids, and digestibility of fat supplements (Conte, Serra, & Mele, 2017; Kholif & Olafadehan, 2022). Based on the potential benefits on human health as to prevent cancer and cardiovascular diseases (Wales, Kolver, Egan, & Roche, 2009), there has been substantial interest in increase the content of polyunsaturated FA (PUFA) in ruminant products, especially conjugated linoleic acid (CLA), linoleic and linolenic acids, and others bioactive compounds (Wales et al., 2009; Lerch, Shingfield, Ferlay, Vanhatalo, & Chilliard, 2012). Numerous experiments have shown that oilseeds and oils rich in PUFA had been efficient to improve the milk FA profile, what also can be affected by to the nature and form of forage (Morsy et al., 2015; Santillo, Caroprese, Marino, Sevi, & Albenzio, 2016; Kholif, Morsy, Abd El Tawab, Anele, & Galyean, 2016, Kholif, Morsy, & Abdo, 2018). Considering the different species, cows, buffaloes, and goat fed the diets containing oilseed has been shown improving n-3 PUFA content in milk (Cattani, Mantovani, Schiavon, Bittante, & Bailoni, 2014; Caroprese et al., 2016). Similar to FA, the fat-soluble vitamins content in milk (A, E, and β-carotene) are depending on the amounts consumed by the cows (Jensen, Johannsen, & Hermansen, 1999). Increased intake of α-tocopherol by cows increases the α-tocopherol content in milk and has attracted greater interest because they reduce oxidation of milk fat (Weiss & Wyatt, 2003).

Flaxseed and soybean oils are excellent sources of n-9 FA, n-6 FA, n-3 FA, carotene, tocopherols, and phytochemicals (Onetti, Shaver, McGuire, & Grummer, 2001). Flaxseed is naturally high in beneficial fatty acids, especially n-3 fatty acids such as α-linolenic acid (45-52%) and high in antioxidants nutrients such as lignans, phenolic compounds, flavonoids, and α-, β-, γ-, δ-tocopherols (Pouzo, Descalzo, Zaritzky, Rossetti, & Pavan, 2016). A portion of supplemental linoleic acid enhances the trans11-18:1 and cis9, trans11-18:2

(CLA) content of lipid fraction in plasma and milk fat. Production of these isomers in the rumen is enhanced as linoleic acid intake increases, indicating the ability of microbes to hydrogenate can be overcome by high levels of unsaturated fatty acids (Loor, Quinlan, Bandara, & Herbein, 2002). Antonacci, Bussetti, Rodriguez, Cano, & Gagliostro (2018) reported that the soybean-linseed oil blend at 50% resulted in the highest number of favorable nutritional changes in ewe's milk taking into account the decrease in the hypercholesterolemic fraction of milk, the simultaneous increase in vaccenic, rumenic and linolenic acids, the n-6/n-3 ratio lower than 4 and a low atherogenic index. Therefore, it can be expected that a diet with vegetable oils rich in unsaturated fatty acids and secondary fatty components could not only improve the nutritional properties (fatty acid profile) but could also improve the physical properties of milk. Thus, in the present study the goal was to evaluate the effect of diets containing flaxseed oil (FO), soybean oil (SO), or their mixture (FSO) on the compositional (fatty acids profile, sterols content and vitamin E), physical (solids fat content), and pro-health (acid value, peroxide value, antioxidant activities and oxidative stability) properties of anhydrous milk fat (AMF) from crossbred cows and buffaloes.

Material and methods

Materials

Crude flaxseed oil (*Linum usitatissimum*) was obtained from Shubra Meless Village, Gharbia, Egypt. Crude soybean oil (*Glycine max*) was obtained from Al-Majd Company for the extraction and refining of vegetable oils, Sadat City, Egypt. Cholesterol (~99%), stigmasterol (~95%), β-sitosterol grade I (~99%), 1-Diphenyl-2-picrylhydrazyl (DPPH•), and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•) were purchased from Sigma-Aldrich, USA. All chemicals and reagents from different suppliers were of analytical grade.

Methods

Experimental design–animals and feeding

Two experiments on cows and buffaloes were conducted individually at a private experimental farm in Om Dinar, Embaba, Giza, Egypt. Care and handling of animals were as outlined in the Guide for the Care and use of Agricultural Animals in Agricultural Research and Teaching (Federation of Animal Science Societies, Champaign, IL, USA) and approved by the National Research Centre, Egypt. Eight early lactating buffaloes and eight early lactating crossbred cows, with 3-4 years, average 515 ± 26 and 420 ± 20 Kg BW, respectively; each species was assigned randomly into 4 groups (2 animals/group) using 4 *x* 4 Latin Square Design with 28 days interval periods. All animals were housed in a tie-stall barn and individually fed according to National Research Council (NRC, 2001) recommendations (plus 10% margin) with free access to water. The basal diet was feed to the animals which contained per kg, as dry matter basis, 600g of berseem clover (*Trifolium alexandrinum*) and 400g of concentrates feed mixture (Table 1). As recommended by the results of previous research (Ye et al., 2009; Hassan, Shazly, Kholif, Sayed, & Abd El-Aziz, 2020) and to prevent negative effect of feeding high levels of plant oil (e.g., decreased feed intake and fiber digestion), animals were fed the basal diet (control) or the basal diet supplemented at 2% of total daily dry matter (DM) intake with crude soybean oil (SO), crude flaxseed oil (FO), or their mixture (FSO, 1:1 v/v). The oils were stored at room temperature and mixed manually with the diets once daily and fed individually two times a day at 7 and 18h in two equal portions.

Table 1. Ingredient and chemical composition of total mixed ration of experimental lactating buffaloes and cows (DM basis)[1].

	Berseem	CFM
Roughage: Concentrate ratio	0.6	0.4
Chemical composition (g $100g^{-1}$ DM)		
Dry matter	91.2	91.4
Ash	31.6	14.9
Organic matter	88.4	85.2
Crude protein	12.3	15.9
Ether extract	2.89	5.04
Crude fiber	2.66	18.12
Nitrogen free extract	39.0	46.1

[1]previosuly reported by Hassan et al. (2020); CFM, concentrate feed mixture consisted of crushed yellow corn, cotton seed meal, wheat bran, calcium carbonate, minerals and vitamins and common salt at rate of 50:25:20:2:2:1, respectively.

Sampling and anhydrous milk fat preparation

Buffaloes and cows were milked by hand twice a day at 7 and 18h during the last three days of each experimental period. Milk samples were collected during morning and evening times from each animal. The sample of each animal represented mixed samples of constant percentage of the evening and morning yield. Milk was de-creamed, and anhydrous milk fat (AMF) was isolated according to the method described by Amer, Kupranyez and Baker (1985) and frozen at -20°C until analysis

Determination of fatty acids profile

The fatty acid methyl ester of AMF was prepared according to the method of Association of Official Analytical Chemists (AOAC, 2007). Fatty acid methyl esters were injected into (HP 6890 series GC) apparatus provided with a DB-23 column (60 m x 0.32 mm x 25 µm). Carrier gas was N_2 with flow rate 2.2 mL/min, splitting ratio of 1:50. The injector temperature was 250°C and that of Flame Ionization Detector (FID) was 300°C. The temperature setting was as follows: 50 to 210°C $min.^{-1}$ and then held at 210°C for 25 min. peaks were identified by comparing the retention times obtained with standard methyl esters.

Determination of sterols fractions

The AMF samples were prepared and dissolved in methanol before HPLC analysis (Borkovcová, Janoušková, Dračková, Janštová, & Vorlová, 2009). Analyses were determined by the reverse phase HPLC on Gemini-Nx 5u, C_{18}, 250 × 4.6 mm column was used. Analyses were performed on the liquid chromatograph HPLC Knauer, Germany, UV detector at 250 nm. Isocratic elution with mobile phase of methanol and water (95:5) mixture at flow rate 0.7 ml/min was used. Column temperature was set up at 35°C; injection volume was 20 µL. Data were collected and evaluated by software clarity chrome (knauer, Germany) according to cholesterol, stigmasterol and β-sitosterol as external standard.

Determination of vitamin E

Vitamin E (α-tocopherol) of the AMF samples was prepared according to the method described by Abd El-Aziz, Mahran, Asker, Sayed, and El-Hadad (2013) and was determined by HPLC (Knauer, Germany) equipped with UV detector at 250 nm. Gemini-Nx 5u, C18, 250 × 4.6 mm column was used. The mobile phase was a mixture of hexane and isopropanol (99:1 v v^{-1}) at a flow rate 1.5 mL $min.^{-1}$. The concentration of α-tocopherol in the samples was obtained by comparing their peak areas with the peak area of standard in a relation to concentration.

Determination of solid fat content profile

The solid fat content (SFC) profile of the AMF samples was determined by Nuclear Magnetic Resonance (NMR, Model: MARAN–SFC, Company: Resonance Instruments Ltd) according to the method described in International Union of Pure and Applied Chemistry (IUPAC, 1987). The AMF samples were measured at 5, 15, 25, and 35°C. The sample in NMR tube was melted at 70°C for 30 min. and then chilled 0°C for 90 min. and held at the measuring temperature for 60 min prior to measurement.

Determination of peroxide and acid values

Acid value of AMF samples was determined according to Method 969.17, AOAC (2007). Peroxide value (mEq Kg^{-1} fat) was determined according to the method described by Egan, Kirk and Sawyer (1981).

Determination of antiradical activities

The DPPH$^{\cdot}$ and ABTS$^{\cdot}$ radical-scavenging activities of the AMF was estimated according to the methods of Brand-Williams, Cuvelier and Berset (1995) and Re et al. (1999) with some modifications, respectively. To 3.8 mL of working solution (25 mg DPPH L^{-1} methanol or 7 mM ABTS solution with 2.45 mM $K_2S_2O_8$,) was mixed with 200 µL of AMF samples. The degree of de-colorization was measured at 517 nm for the DPPH$^{\cdot}$ and at 700 nm for ABTS$^{\cdot}$ radical-scavenging activity assays, in a spectrophotometer (Shimadzu spectrophotometer, UV–Vis. 1201, Japan) after incubation for 20 min. in the dark at room temperature (25 ± 2°C) and centrifugation at 3000 xg for 5 min. Both ABTS• and DPPH• scavenging activities were calculated using the following formula:

(%) Antiradical activity = $[(A_0 - A_S) / A_0] \times 100$

where: A_0 is the absorbance of the control (DPPH), and A_S is the absorbance of the sample.

Determination of oxidative stability

Rancimat Metrohm instrument (Ud.CH-9100 Herisau, Switzerland, Model 679) was used to estimate the oxidative stability of the AMF samples as the induction period (h) under accelerated conditions (110ºC, air flow at 20 L h^{-1}) according to the method described by Coppin and Pike (2001). Oxidative stability was defined as the point of maximum change of the rate of oxidation (induction period).

Statistical analysis

Data of the two experiments (i.e. on buffaloes and cows) were analysed together using a duplicate 4 × 4 Latin square design with four periods and four treatments. The PROC MIXED of SAS 9.4 (SAS, 2008) was used. Individual cows/buffaloes were the experimental units. The statistical model was: $Y_{ijkl}=\mu + S_i + T_j + P_k + A_l(S_i) + E_{ijkl}$, where Y_{ijkl} is each individual observation for a given variable, μ is the overall mean, S_i is the square effect, T_j is the treatment effect, P_k is the period effect, $A_l(S_i)$ is the effect of animal (cow/buffaloes) within the square and E_{ijkl} is the residual error. When *F*-test was significant at $p < 0.05$, values of means were compared using the difference probability option of the least squares mean statement.

Results and discussion

Fatty acid profile of AMF

Supplementing the diet of cows and buffaloes with FO, SO, or FSO reduced total saturated FA ($p = 0.024$) mainly, C4:0, C14:0 and C16:0, even though an increase in C18:0 (Table 2). The unsaturated FA (USFA) were increased mainly, C18:1 ($p = 0.007$) and C18:2 as well as C18:1t ($p > 0.05$). This means that supplementation of the lactating animal's diet with a source rich in the USFA improves the fatty acid profile (USFA/SFA ratio) and reducing the entry of SFA into dairy foods. Similar observations were found by Smet et al. (2010) and Livingstone, Lovegrove and Givens (2012) in milk fat obtained from lactating animals fed a diet rich in the USFA. Adding USFA-rich oils can reduce the lipogenesis in the mammary gland, thus, reducing the milk fat and SFA content. That coincides with the observed changes in the fatty acid profile of AMF, particularly the increase in USFA in rations containing SO and FO (Castro, Martinez, Isabel, Cabezas, & Jimeno, 2019). Vargas-Bello-Pérez & Garnsworthy (2013) reported that milk fatty acids are entirely related to the ruminant's diet, including the feed, intake levels, and accumulation of USFA, especially oil plants. Additionally, the increase in *Trans*-fatty acids is due to the bio-hydrogenation of monounsaturated FA and polyunsaturated FA in the rumen (Vargas-Bello-Pérez & Garnsworthy, 2013). The C18:1 concentration was highest in AMF obtained from SO or FSO-fed buffaloes when compared to buffaloes fed a control diet ($p = 0.009$). However, AMF obtained from SO fed-cows had the highest content of C18:3 compared to buffalo's AMF ($p = 0.048$). On the other hand, buffalo's AMF displayed higher contents of C4:0, C18:0 ($p = 0.02$) and C18:1, while lower contents of C14, C18:2 and C18:3 compared to cow's AMF. The difference between buffalo's and cow's AMF in C16:0, as a long SFA, was not clear. The difference in milk fat content between buffaloes and cows can be attributed to the difference in mammary gland metabolism (Franzolin & Alves, 2010) and the difference in rumen bacteria between species, which influence physiological responses to dietary changes (González-Martín et al., 2017). Other nutritional and physiological factors such as lactation stage, parity, and rumen microorganisms, have been discovered to account for some of the variation in milk fatty acid profile in buffaloes and cows (Penchev, Ilieva, Ivanova, & Kalev, 2016). Furthermore, unsaturated fatty acid concentrations in milk depend mainly of the amount in the small intestine as a result of ruminal bio-hydrogenation escape, allowing them to be incorporated into milk fat (Kholif & Olafadehan, 2022). Nutritional factors account for approximately 50% of the variation in milk fat composition and more than 60% of milk fatty acids originate from plasma uptake, whereas the rest are synthesized in the mammary gland (Chilliard & Ferlay, 2004). Furthermore, unsaturated fatty acid concentrations in milk depend mainly of the amount in the small intestine as a result of ruminal biohydrogenation escape, allowing them to be incorporated into milk fat (Kholif & Olafadehan, 2022). These findings are consistent with those of Penchev et al. (2016), who found that buffalo milk contains significantly less C8:0 to C14:0 and significantly more C18:1, as well as lower total SFA than cow's milk. Other researchers found that buffalo milk had higher levels of C18, Trans-fatty acids, C18:2 and CLA than cow milk (Ménard et al., 2010).

Table 2. Fatty acids profile of anhydrous milk fat of lactating buffaloes and cows fed a diet supplemented with flaxseed oil, soybean oil, or their mixture.

Fatty acids	Cow's AMF				Buffalo's AMF			
	CD	DFO	DSO	DFSO	CD	DFO	DSO	DFSO
Saturated fatty acids (%)								
C_4	2.45	2.34	2.38	1.69	3.79	3.54	2.26	2.00
C_6	1.57	1.44	1.54	1.60	1.77	1.48	1.03	0.92
C_8	0.97	0.92	0.96	0.96	0.76	0.58	0.44	0.41
C_{10}	2.66	2.48	2.62	2.52	1.26	0.97	0.83	0.80
C_{12}	2.43	2.22	2.36	2.21	1.80	1.46	1.33	1.31
C_{13}	0.06	0.06	0.06	0.06	0.07	0.06	0.05	0.06
C_{14}	9.28	8.60	8.95	8.56	8.78	7.11	7.14	6.99
C_{15}	1.51	1.30	1.29	1.36	1.16	1.05	1.10	1.09
C_{16}	25.0^{ab}	20.9^{b}	22.2^{b}	22.0^{b}	27.9^{a}	22.4^{b}	21.9^{b}	21.9^{b}
C_{17}	1.11	1.08	0.98	0.94	0.82	0.75	0.77	0.82
C_{18}	14.7^{d}	16.5^{bcd}	16.1^{cd}	15.8^{cd}	15.4^{d}	19.5^{a}	18.0^{abc}	19.2^{ab}
C_{20}	0.51	0.71	0.50	0.63	0.32	0.44	0.41	0.54
C_{22}	0.20	0.21	0.19	0.21	0.16	0.16	0.18	0.22
Unknown	2.21	1.49	1.47	1.61	1.94	2.43	2.28	1.77
Total	64.8^{ab}	60.3^{cd}	61.6^{bc}	60.2^{cd}	65.9^{a}	61.9^{bc}	57.7^{cd}	58.1^{cd}
Unsaturated fatty acids (%)								
$C_{14:1}$	0.49	0.41	0.46	0.43	0.38	0.23	0.29	0.27
$C_{15:1}$	0.40	0.36	0.32	0.36	0.30	0.28	0.28	0.28
$C_{16:1}$	1.21	0.95	0.98	1.05	1.26	0.93	0.92	0.89
$C_{17:1}$	0.34	0.36	0.25	0.28	0.25	0.19	0.22	0.22
$C_{18:1}$	27.0^{c}	29.4^{bc}	29.4^{bc}	30.3^{bc}	27.7^{c}	31.4^{ab}	34.5^{a}	34.2^{a}
$C_{18:1T}$	0.52	0.41	0.65	0.74	0.81	1.15	1.27	1.43
$C_{20:1}$	0.09	0.22	0.27	0.15	0.21	0.27	0.28	0.12
$C_{18:2}$	3.85	5.60	4.22	4.68	1.91	1.85	2.17	2.41
$C_{18:3}$	0.79	1.29	0.86	0.98	0.29	0.53	0.41	0.51
$C_{18:4}$	0.54	0.69	0.98	0.89	0.99	1.21	1.98	1.62
Total	35.2	39.7	38.4	39.8	34.1	38.1	42.3	41.9
PUFA	5.18bc	7.58a	6.06a	6.55a	3.19c	3.59c	4.56bc	4.54bc
USFA/SFA	0.54^{b}	0.66^{a}	0.62^{ab}	0.66^{a}	0.52^{b}	0.62^{ab}	0.73^{a}	0.72^{2}a

AMF, anhydrous milk fat; SFA, saturated fatty acids; USFA, unsaturated fatty acids; PUFA, poly unsaturated fatty acids; CD, control diet; DFO, diet supplemented with flaxseed oil; DSO, diet supplemented with soybean oil; DFSO, diet supplemented with mixture of flaxseed and soybean oils (1:1).

Sterols fractions of AMF

The cholesterol concentration in cow's AMF was higher than that in buffalo's milk, as shown in Figure 1; the difference was not statistically significant ($p > 0.05$). The cholesterol content of cow's AMF was varied between 273 to 306.5 mg 100 g^{-1}, while varied between 248.6 to 293.6 mg 100 g^{-1} in buffalo's AMF. Similar findings were previously published by Barłowska, Szwajkowska, Litwinczuk and Krol (2011) and Abd El-Salam & El-Shibiny (2011), who reported that although buffalo milk has a higher fat content than cow milk, it has less cholesterol content. Low cholesterol content may be related to the larger size of fat globules in the buffalo's milk; 5 vs. 3.5 µm in cows milk (Ménard et al., 2010). Because cholesterol is present in the milk fat globule membrane, the small fat globules, which characterized by a larger surface area of fat globule membrane, are connected with a relatively higher concentration of cholesterol in milk (Ceballos et al., 2009). Nevertheless, neither stigmasterol nor β-sitosterol could be detected in both buffalo's and cow's AMF. Regarding the type of feeding, supplementing the diet with FO, SO, or FSO had an appositive effect (reduced) on the cholesterol content of cow's AMF. Cholesterol content decreases from 306.5 mg 100 g^{-1} AMF of cows received control diet to 291.6, 286.5, and 273 mg 100 g^{-1} of AMF obtained from FO, SO, or FSO fed-cows, respectively ($p > 0.05$). Inversely, high cholesterol content was found in AMF obtained from lactating buffaloes fed a diet supplemented with FO, SO, or FSO. This means that the cholesterol content of AMF depends more on the type of animal than a diet. Pietrzak-Fiecko and Kamelska-Sadowska (2020) reported that the concentrations of cholesterol and fatty acids in milk, as well as the overall fat content, differ depending on mammalian species, genetic, physiological, nutritional factors and environmental conditions.

Vitamins E concentration in AMF

In general, the diets supplemented with FO, SO, or FSO showed a positive effect on the concentration of vitamin E in AMF, resulting in 1.14, 2.27, and 1.62 times increases in cow's AMF and 1.02, 1.82, and 1.93 times

increases in buffalo's AMF, when compared to cows received the control diet, respectively (Figure 2). However, the concentration of vitamin E in AMF obtained from SO fed-animals was higher than that obtained from FO fed-animals ($p = 0.008$). The crude SO is characterized by the highest content of total tocopherols (1090-1328 mg kg^{-1}) compared to crude FO, 425 mg kg^{-1} (Matthäus & Özcan, 2015; Kiczorowska et al., 2019). A similar, Calderón et al. (2007) found a rapid increase in vitamin E in both plasma and milk after the first week of feeding diets high in carotenoids and vitamin E. At the end of the experimental period (6 weeks), vitamin E concentrations in plasma and in milk fat were linearly related to the proportion of vitamin E in the diet (25, 125, or 250 IU kg^{-1} diets). Weiss & Wyatt (2003) found that increased α-tocopherol intake resulted in a linear increase in plasma α-tocopherol concentration, thus increased α-tocopherol concentration in milk. The α-tocopherol increased from 11.4 to 31.8 mg kg^{-1} fat when α-tocopherol concentration increased from 25 to 250 U kg^{-1} DM. This study also showed that buffalo's milk fat was higher in vitamin E content than cow's milk fat. Milk composition depends mainly on the genetics differences between breeds within each species of animals; however, nutrition can dramatically change it (Kapadiya et al., 2016).

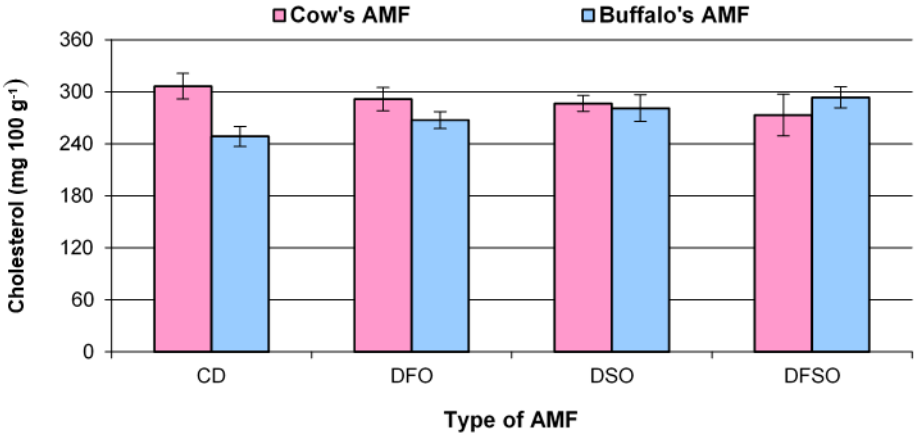

Figure 1. Cholesterol content of anhydrous milk fat of lactating cows and buffaloes fed a diet supplemented with flaxseed oil, soybean oil, or their mixture.

AMF, anhydrous milk fat; CD, control diet; DFO, diet supplemented with flaxseed oil; DSO, diet supplemented with soybean oil; DFSO, diet supplemented with mixture of flaxseed and soybean oils (1:1).

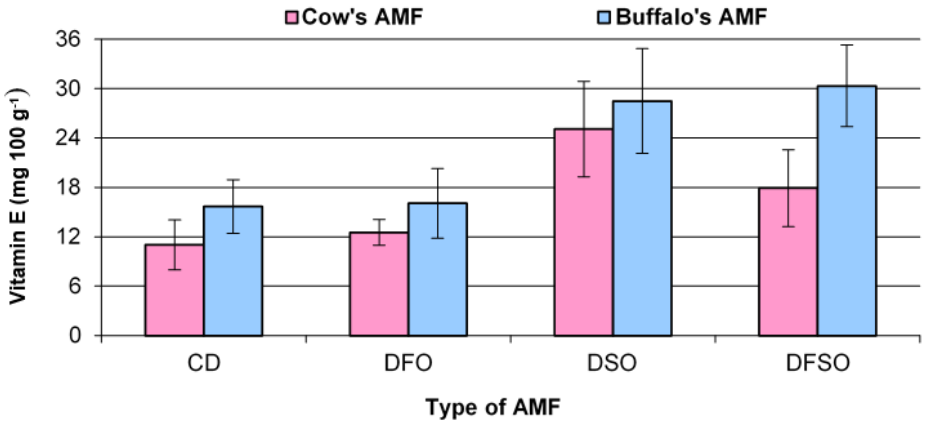

Figure 2. Vitamin E (α-tocopherol) concentration of anhydrous milk fat of lactating cows and buffaloes fed a diet supplemented with flaxseed oil, soybean oil, or their mixture.

AMF, anhydrous milk fat; CD, control diet; DFO, diet supplemented with flaxseed oil; DSO, diet supplemented with soybean oil; DFSO, diet supplemented with mixture of flaxseed and soybean oils (1:1).

Solid fat content profile of AMF

Solid fat content (SFC) profile is an important characteristic for prophesying the fat functionality at different stages of manufacturing such as rolling, baking of dough and butter spreadability (Kaylegian, 1999). At temperature ⩽ 15°C, the SFC of cow's AMF fed a diet supplemented with FO, SO, or FSO was slightly lower than cows received the control diet (Table 3). When the temperature increased, the difference in SFC among all experimental AMF decreased. These results agree with Smet et al. (2010), who reported that a higher

content of USFA in AMF resulted in an increased proportion of low melting triglycerides, which lowered the solid, particularly at refrigerator temperatures. Inversely, SCF for buffalo's AMF, which had high USFA content, was higher at temperature ⩽ 15°C than control AMF; the difference was not significant. These results may be related to cholesterol and fat-soluble vitamins content; higher contents of cholesterol and fat-soluble vitamins may affect the crystallization behavior of milk fat, which decreases the SFC of AMF at temperature < 15°C. Abd El-Aziz et al. (2013) found that adding palm oil (up to 60%) to AMF reduced cholesterol content, as well as reduced SFC at 0 and 10°C. A similar trend, but less marked, was observed in buffalo's AMF at 25 and 35°C. This difference could be attributed to a higher content of stearic acid in experimental buffalo's AMF (Table 2) than control AMF.

Table 3. Solids fat content of anhydrous milk fat of lactating cows and buffaloes fed a diet supplemented flaxseed oil, soybean oil, or their mixture.

Temperature (°C)	Cow's AMF				Buffaloes' AMF			
	CD	DFO	DSO	DFSO	CD	DFO	DSO	DFSO
5	60.37^{a}±3.32	58.12^{ab}±1.2	58.31^{ab}±0.71	58.85^{ab}±1.34	52.37^{b}±3.53	55.98^{ab}±4.38	54.58^{ab}±0.99	56.98^{ab}±4.24
15	41.21^{a}±3.18	39.53^{ab}±0.78	39.58^{ab}±2.05	40.95^{ab}±1.26	36.35^{b}±3.39	39.11^{ab}±1.06	38.07^{ab}±0.92	40.12^{ab}±2.61
25	14.99^{a}±1.41	15.00^{a}±2.12	15.00^{a}±0.84	15.51^{a} ±1.55	16.18^{a}±1.20	19.97^{a}±1.22	17.00^{a}±0.85	19.41^{a} ±0.94
35	1.99^{a}±0.56	1.88^{a}±0.42	2.10^{a}±0.37	2.00^{a} ±0.42	4.03^{a}±0.49	6.11^{a}±1.27	4.17^{a}±0.35	5.44^{a} ±0.77

Means (n = 4, ±SD) with the same letters in the same row are not significantly different ($p < 0.05$); AMF, anhydrous milk fat; CD, control diet; DFO, diet supplemented with flaxseed oil; DSO, diet supplemented with soybean oil; DFSO, diet supplemented with mixture of flaxseed and soybean oils (1:1).

Acid and peroxide values and antiradical activities of AMF

The acid value is used to quantify acidic constituents, whereas the peroxide value is used to detect peroxide in unsaturated fats or oils, which is one of the first signs of rancidity. As shown in Table 4, there is no clear effect of feeding FO and SO on the concentration of free fatty acids (FFA) and the peroxide value (PV) of the resulting milk fat, except for a slight increase in the concentration of FFA ($p > 0.05$) and a slight decrease in the PV ($p > 0.05$). Similar findings suggest that increasing energy intake and energy balance during the first 4 months of lactation does not reduce FFA concentration in goats' milk (Dønnem, Randby, & Ekn, 2011). To evaluate the antioxidant activities on AMF the scavenging activity of DPPH$^{\bullet}$ and ABTS$^{\bullet}$ radicals was estimated (Table 4). The AMF from SO and FSO fed-cows and buffaloes showed a high scavenging activity for ABTS$^{\bullet}$ radicals compared to that of animals received FO or control diet ($p = 0.001$). There was no significant difference in the ABTS$^{\bullet}$ radical scavenging activity on AMF from FO fed-animals and control fed-animals. A similar, but less marked, slight improvement was observed in the DPPH$^{\bullet}$ radical scavenging activity on AMF from SO and FSO fed-cows but significantly higher in AMF of buffaloes fed a diet supplemented with FSO resulting increases 1.53 times compared to a control diet. The increase in both ABTS$^{\bullet}$ and DPPH$^{\bullet}$ radical scavenging activities may be due to an increase in some bioactive lipid components such as USFA (Table 1), and vitamin E (Fig 2) in AMF of animals fed a diet supplemented with FO, SO or FSO. The DPPH• and ABTS• radical-scavenging activities were highly positively correlated with vitamin E concentration, $r^2 = 0.66$ and 0.67, respectively. Khan et al. (2019) reported that carotenoids, and vitamin E, lipid-soluble antioxidants, can directly scavenge the free radicals and quencher of singlet oxygen in milk fat.

Table 4. Radical scavenging activity, peroxide value and acid value of anhydrous milk fat of lactating cows and buffaloes fed a diet supplemented with flaxseed oil, soybean oil, or their mixture.

Items	Cow's AMF				Buffalo's AMF			
	CD	DFO	DSO	DFSO	CD	DFO	DSO	DFSO
Acid value (mg KOH g^{-1})	0.31^{b} ±0.08	0.34^{b} ±0.01	0.35^{b}±0.06	0.33^{b} ±0.06	0.40^{ab}±0.01	0.48^{a}±0.08	0.39^{ab}±0.04	0.49^{a} ±0.05
Peroxide value (mEq Kg^{-1})	0.63^{a} ±0.12	0.44^{ab}±0.11	0.41^{ab}±015	0.45^{ab}±0.06	0.58^{a} ±0.11	0.43^{ab}±0.07	065^{a} ±0.14	0.25^{b} ±0.04
Radical-scavenging activities (%)								
• DPPH$^{\bullet}$ radicals	15.32^{b}±1.0	17.54^{ab}±1.5	20.11^{ab}±2.6	18.15^{ab}±3.2	14.54^{b}±3.5	15.60^{ab}±3.2	16.25^{b}±1.6	22.27^{a}±3.0
• ABTS$^{\bullet}$ radicals	23.20^{c}±2.7	25.28^{c}±2.0	45.82^{a}±1.8	42.07^{a}±3.6	16.92^{c}±2.0	18.53^{c}±2.8	47.45^{a}±3.4	32.08^{bc}±1.7

Means (n = 4, ±SD) with the same letters in the same row are not significantly different ($p < 0.05$); AMF, anhydrous milk fat; CD, control diet; DFO, diet supplemented with flaxseed oil; DSO, diet supplemented with soybean oil; DFSO, diet supplemented with mixture of flaxseed and soybean oils (1:1); DPPH$^{\bullet}$, 1-Diphenyl-2-picrylhydrazyl; ABTS, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid.

Oxidative stability of AMF

The effect of supplementing the diet of both buffaloes and cows with FO, SO, or FSO on the oxidative stability of AMF, measured as induction period (IP) at 110°C, is presented in Figure 3. There was no difference in the IP between both cow's and buffalo's AMF that was feed with control diet and also with oil addition.

Regarding the type of feeding, a slight decrease was observed in the IP of both cow's and buffalo's AMF obtained from diets containing oils compared to the control diet ($p = 0.17$), which may be related to a rise in USFA (Scott, Duncant, Sumnert, & Watermant, 2003). This suggests that the increase in α-tocopherol, as shown in Figure 2, does not increase the stability of milk fat against oxidation, but may acts as pro-oxidants at high concentrations. Redondo-Cuevas, Castellano, Torrens and Raikos (2018) found that the USFA and total tocopherols were the main individual factors that correlated negatively with oxidative stability ($r^2 = 0.304$, $r^2 = 0.223$, respectively). Zhao et al. (2013) indicated that cows fed long-chain FA exhibit positive effects on milk FA composition, but may decrease oxidative stability of milk fat. Additionally, the decrease in oxidative stability of AMF obtained from diets with oils at 110°C may correlate with FFA content, as shown in Table 4. Free fatty acids which, act as pro-oxidants, decrease the surface tension of oil and increase the diffusion rate of oxygen from the headspace into the oil to accelerate oil oxidation (Mistry & Min, 1988).

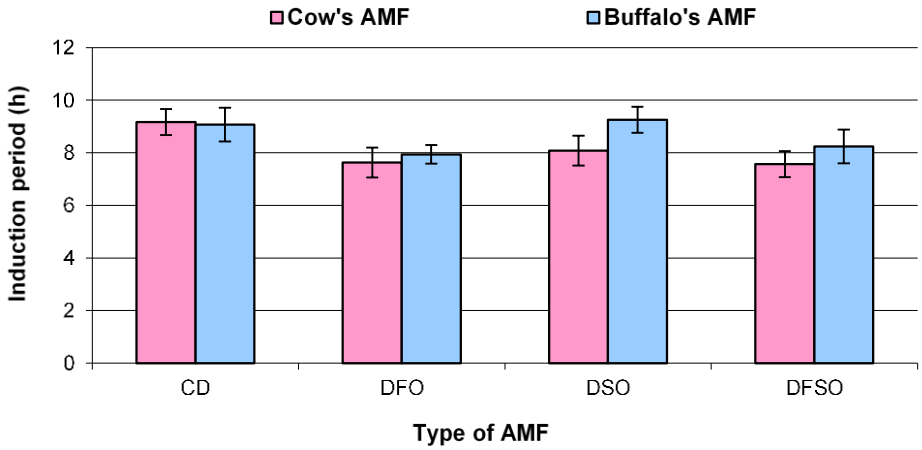

Figure 3. Oxidative stability of anhydrous milk fat of lactating cows fed a diet supplemented with flaxseed oil, soybean oil, or their mixture. AMF, anhydrous milk fat; CD, control diet; DFO, diet supplemented with flaxseed oil; DSO, diet supplemented with soybean oil; DFSO, diet supplemented with mixture of flaxseed and soybean oils (1:1).

Conclusion

The addition of 2% of FO, SO and SFO (especially SO) based on DMI to the diet of cows and buffaloes improves the health and nutritional properties of milk fat by reducing SFA and increasing the proportion of USFA/SFA, antioxidant activity and vitamin E. The highest vitamin E content was observed in SO diets. The AMF obtained from animals fed a diet supplemented with SO or FSO also exhibits a high scavenging activity for DPPH• and ABTS• radicals, which correlated positively with vitamin E concentration. This study did not give a clear answer whether the cholesterol content of AMF was more affected by diet or by animal type; the cholesterol content in cow's AMF decreased with all experimental diets, and vice versa for buffalo's AMF. Additionally, the physical properties of AMF, such as SFC, are affected not only by its content of USFA but also by its contents of lipid-minor components such as cholesterol and vitamin E.

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