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MILK PRODUCTION AND COMPOSITION, MILK FATTY ACID PROFILE, NUTRIENTS DIGESTIBILITY AND BLOOD COMPOSITION OF DAIRY BUFFALOES FED CRUSHED FLAXSEED IN EARLY LACTATION.

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SUMMARY

A total of 21 multiparous lactating buffaloes averaging 561±10 kg body weight (BW) were allotted at calving to three groups of seven buffaloes blocked for similar calving dates to determine effects of feeding crushed flaxseed on dry matter (DM) intake. milk production, milk composition. milk fatty acid profile. nutrients digestibility and concentration of some blood metabolites. Buffaloes within each block were assigned to one of three iso-net energy for lactation total mixed rations containing no flaxseed (control diet). 17 g/kg DM crushed flaxseed (Low flaxseed) and 34 g/kg DM crushed flaxseed (High flaxseed). Diets were fed for *ad-libitum* intake from calving to week 16 of lactation. Crushed flaxseed supplementation had no effect on body weight (BW) and dry matter intake. Low flaxseed increased ($P<0.05$) milk production compared with other treatments. While. the tow levels of crushed flaxseed increased 4% fat corrected milk (FCM) compared with control. Milk fat was increased with increasing level of flaxseed while. other milk components were not affected by flaxseed supplementation. Feed efficiency was improved by flaxseed supplementation to ration. Flaxseed supplementation was not affect nutrients digestibility. Blood serum total lipids and cholesterol were decreased by crushed flaxseed supplementation compared with control. The main difference in milk fatty acid profile determined was conjugated linolenic acid (CLA). n-3 fatty acid proportions. total and poly unsaturated fatty acids which were higher in milk fat of animals fed low flaxseed compared to animals fed high flaxseed and control. Buffaloes fed the control diet had higher proportions of C18:0. C20.0 and total saturated fatty acids. and lower proportions of C18.1 trans9 and C18:1 cis9. in milk fat than those fed flaxseed.

Keywords: *flaxseed; digestibility; milk production; milk fatty acids; blood parameters; buffaloes.*

INTRODUCTION

Research has shown several health benefits of n-3 fatty acids to humans including a decrease in the incidence of cancer, cardiovascular diseases, hypertension, and arthritis and an improvement of visual acuity (Wright et al., 1998). Milk fat contains low concentrations of n-3 fatty acids and high levels of saturated fatty acids (SFA), particularly C16: 0, which has hypercholesterolemic properties (Kennelly, 1996). Increasing the level of α-linolenic acid, an n-3 fatty acid, and other polyunsaturated long-chain fatty acids (LCFA) with reducing the proportion of C16:0 can therefore be considered an attractive way to modify milk composition, which would increase human consumption of milk and dairy products. Flaxseed contains a high oil level (40% of total seed weight), with α-linolenic acid constituting approximately 55% of total fatty acids of the oil (Mustafa et al., 2002; Petit, 2002, 2003). Feeding flaxseed to dairy cows reduces the concentrations of short-chain fatty acids (SCFA) and medium chain fatty acids (MCFA) and increases that of LCFA in milk fat (Mustafa *et al.*, 2003; Petit, 2003).

Feeding flaxseed generally results in the lowest omega 6 to omega 3 FA ratio in milk fat (Petit, 2002), which may improve milk FA profile and result in better human health. In general, untreated whole flaxseed is readily eaten by dairy cows, and feeding up to 150 g/kg DM as flaxseed had no effect on DM intake of early (Petit, 2002) and late lactation (Martin et al., 2008) dairy cows. However, feeding 142 g/kg versus 93 g/kg DM whole flaxseed decreased milk fat concentration and tended to decrease digestibility of the diet (Petit and Gagnon, 2009).

Physical breakdown of the flaxseeds in the diet could contribute to increased milk production by dairy cows. Indeed, in a short-term experiment (i.e., 3 week periods): dairy cows fed 100 g/kg DM ground flaxseed had a 6.5% increase (1.2 kg/d) in milk production compared to those fed 100 g/kg DM whole flaxseed (da Silva *et al.*, 2007). Similarly, feeding rolled, compared to whole, untreated flaxseed at 100 g/kg DM increased milk production of early lactation cows by 10% (Kennelly, 1996). Processing flaxseed may also improve milk FA profile from a human health perspective due to increased proportions of conjugated linoleic acid and omega 3 FA and decreased proportions of medium-chain and saturated FA in milk fat of cows fed ground compared to those fed whole flaxseed (da Silva *et al.*, 2007). However, most comparisons of effects of flaxseed processing on productivity of dairy cows have used short-term experiments of less than 2 months. There is no information on effects of feeding crushed flaxseed used long-term from calving through the first half of lactation of lactating dairy buffaloes.

The objective of this study was to determine effects of crushed flaxseed supplementation to diets in tow levels on the productive performance (i.e., feed intake, nutrients digestibility, milk production and milk composition), milk FA profile and blood parameters in early lactation dairy buffaloes.

MATERIALS AND METHODS

Flaxseed was obtained from El Harraz Market, Cairo, Egypt and crushed in the Experimental Farm in Shalakan, Faculty of Agriculture, Ain Shams University.

Buffaloes, experimental design and diets:

This study was conducted at the Experimental Farm in Shalakan, Faculty of Agriculture, Ain Shams University and Dairy Science Department, National Research Center, Dokki, Cairo, Egypt during November 2009 to April 2010 using 21 lactating buffaloes (body weight (BW): 561±10 kg). Buffaloes were blocked for similar expected calving dates. The experiment was extended from calving to 16th week of lactation. Buffaloes were housed in tie stalls and fed individually. Buffaloes within groups were assigned randomly to one of three total mixed rations (TMR; Table 1). There were three groups of seven buffaloes. The three TMR consisted of a control TMR with no crushed flaxseed (Control), a TMR with 17 g/kg DM (DM basis) crushed flaxseed (Low flaxseed), and a TMR with 34 g/kg DM crushed flaxseed (High flaxseed). All diets were designed to have similar concentrations of crude protein (CP) and net energy for lactation (NEL). Diet was formulated to meet the animal's requirements (A.R.C., 1983). Diets were fed twice daily at 08:00 and 16:00h at ad libitum. Animals were milked twice daily at 06:00 and 15:00 h. Milk production was recorded at every milking. The BW of each buffalo was determined every 4 weeks through experimental period. Dry matter intake (DMI) was recorded every four weeks by weighing feeds offered and refused by the animals. Fresh water was available to the animals all time.

Apparent digestibility:

Three digestibility trials were applied during the last three days every month using three animals from each group. Silica was used as an internal marker for determining the digestibility (Ferret et al. 1999). At 4 h after the morning feeding, fecal samples (approximately 100 g wet weight) were collected from the rectum during the last three days every month and pooled by buffalo for each period, dried at 55 °C for 48 h. and then ground to pass a 1 mm sieve in a feed mill (FZ102, Shanghai Hong Ji instrument Co., Ltd., Shanghai, China) for chemical analysis. Dry matter excreted in feces was calculated by dividing silica input in the feeds (grams of silica per day) by silica output in the feces (grams of silica per day). The digestibility coefficient of certain nutrient was calculated according to the following formula (Ferret et al. 1999):

$$Digestibility = 100 - \left[100\ x\ \frac{\%\ indicator\ in\ feed}{\%\ indicator\ in\ feces}\ x\ \frac{\%\ nutrient\ in\ feces}{\%\ nutrient\ in\ feed} \right]$$

Sample collection and analyzes:

Samples of TMR were collected and composed monthly, composited samples were mixed thoroughly and subsampled for chemical analyzes. Milk samples were obtained once every two weeks from each buffalo for two consecutive milkings and pooled within buffalo relative to production to obtain one composite milk sample per buffalo for determine milk composition. Milk samples were stored at +4 °C until analyzed for fat, protein, lactose, total solids, urea N and ash. Milk samples were pooled every month within treatment relative to production to obtain one composite milk sample per treatment and frozen at −20 °C until analyzed for milk FA profile. Blood was collected from all buffalo on week 4, 8, 12 and 16 postpartum 4 h after the morning feeding to determine some blood parameters concentrations. Blood was withdrawn from the jugular vein into vacutainer tubes (Becton Dickinson and Cie, Rutherford, NJ, USA). Tubes were immediately placed on ice and centrifuged within 1 h at 4 °C for 30 min at 3000×g. b Blood serum were separated and frozen at −20 °C for subsequent analysis.

Table (1): Ingredient and chemical composition of total mixed diets of lactating buffaloes fed no flaxseed (control), 17 or 34 g/kg DM crushed flaxseed.

Ingredient (g/kg)	Control	Low Flaxseed	High Flaxseed
Berseem hay	330	330	330
Rice straw	275	275	275
Yellow corn	61	51	41
Soybean meal	83	76	69
Wheat bran	200	200	200
Sunflower meal	20	20	20
Urea	5	5	5
Crushed flaxseed	0	17	34
Calcium carbonate	3	3	3
Minerals and Vitamins[a]	23	23	23
Chemical composition (g/kg DM)			
Dry matter	901.1	900.5	900.6
Organic matter	896.6	896.9	897.1
Crude protein	162	161	162
Ether extract	37.2	39.1	41.0
Crude fiber	235.9	235.8	235.7
Neutral detergent fiber (NDF)	387	386	384
Acid detergent fiber (ADF)	231	230	229
NE_L (Mj/kgDM)[b]	6.3	6.3	6.3
Fatty acids (g/kg total fatty acids)[c]			
14.0	10	10	10
16.0	331	329	330
18.0	50	46	41
18.1	230	233	233
18.2n-6	242	244	245
18.3n-3	117	119	122
20.0	8	8	8
22.0	11	10	10
24.0	1	1	1

[a] *Contained 141 g/kg of Ca, 27 g/kg of P, 65 g/kg of Mg, 14 g/kg of S, 120 g/kg of Na, 6 g/kg of K, 944 mg/kg of Fe, 1613 mg/kg of Zn, 484 mg/kg of Cu, 1748mg of Mn, 58mg/kg of I, 51 mg/kg of Co, 13 mg/kg of Se, 248,000 U/kg of vitamin A, 74,000 UI/kg of vitamin D3 and 1656 IU/kg of vitamin E.*

[b] *Calculated using published values of feed ingredients (NRC, 2001).*

[c] *Mean of pool sample from three samples prepared by compositing pool samples from three daily samples collected from six buffaloes per diet during week 8 of lactation.*

Dry matter of TMR was determined by drying at 105 °C for 48 h (AOAC, 1990; Method 930.15). The TMR were dried at 55 °C and ground to pass a 1mm screen in a Wiley mill before analyzes of ether extract (EE), crude fiber (CF) and ash (AOAC, 1990). Organic matter was determined by difference. The concentration of a NDF in TMR was determined with sodium sulfite and heat stable α-amylase and expressed exclusive of residual ash (Van Soest *et al.*, 1991). The ADF content of TMR was determined according to AOAC (1990; Method 973.18). The nitrogen in TMR was determined by a Kjeldahl method (955.04; AOAC, 1990). Blood serum samples were analyzed for concentrations of serum total protein was determined as described by Armstrong and Carr (1964), albumin (Doumas *et al.* 1971), total lipids (Postma and Stroes, 1968) cholesterol (Raltiff and Hall 1973) and serum GOT and GPT (Reitman and Frankel, 1957). Globulin was calculated. pH of milk was determined using a digital pH-meter. Milk samples were analyzed for total solids, fat, true protein, urea nitrogen and lactose by infrared spectrophotometry (Foss 120 Milko-Scan, Foss Electric, Hillerød, Denmark) according to A.O.A.C. (1997) procedures. Solids-not-fat (SNF) was calculated. Fat corrected milk (4% fat) was calculated by using the following equation according to Gaines (1928):

FCM = 0.4 milk yield (gm) + 15 fat yield (gm).

Fatty acids in milk and TMR were extracted and methylated according to method 996.06 of AOAC (1998). Tritridecanoin (Sigma, Oakville, ON, Canada) was used as the internal standard. A GLC reference standard (GLC-569; Nu-Chek Prep Inc., Elysian, MN, USA) was used to estimate correction factors for short-chain FA (4:0–10:0). Individual FA was identified by comparison of gas chromatography peaks with peaks of known standards (GLC-463; Nu-Chek Prep Inc.). Fatty acid methyl esters were separated on an Agilent 6890 GLC fitted with an Agilent auto sampler (model #7683, Agilent injector, Agilent Ltd., Mississauga, ON, Canada) and a flame ionization detector. Agilent Chemstation Rev. B.01.03 (204) software was used for chromatogram integration and analysis. Samples were introduced onto a 100m Supelco (Oakville, ON, Canada) SP-2560 column (part number 24056) via 1µl splitless injections. The temperature program was: level one, 45 °C held for 4min; level two, 45–150 °C at 13.0 °/min increments, then held for 47 min; level three, 150–215 °C at 4°C/min increments, then held for 35 min. Injector temperature was set at 250 °C and the detector was set at 260 °C. Column head pressure was set at 30 psi. A 4mm i.d. splitless injection liner (Agilent Ltd., Mississauga, ON, Canada) was used for all injections. Gas flow rates were: helium (carrier) 1.1 ml/min, helium (make up) 25 ml/min, compressed 350 ml/min and hydrogen 35 ml/min.

Statistical analysis:

All results were analyzed using the MIXED procedure of SAS (1998). Data on milk fatty acid profile were analyzed as a complete random design where treatment was the main source of variation. Data on feed intake, nutrients digestibility, blood parameters, milk production and milk composition were analyzed as a randomized block design using PROC MIXED of SAS. Data were expressed as mean values when there was no interaction between week and treatment (i.e., $P>0.10$). When a significant F-test was detected (i.e., $P<0.05$), treatment means were separated using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Feed Intake, body weight and Diet Digestibility:

There was no difference ($P>0.05$) among treatments for dry matter intake (DMI), expressed in kilograms per day and live body weight (Table 2). The daily DMI of each group was recorded, and the average DMI in the control, low and high crushed flaxseed groups were 15.98, 15.99 and 15.63 kg/d, respectively. The proportions of whole flaxseed added to the dietary dry matter of the crushed flaxseed diets were 1.7 and 3.5%, respectively. Crushed flaxseed was not significantly affected on all nutrients digestibility as dry matter, organic matter, ether extract, crude fiber and nitrogen free extract (Table 2). In the current study, dry matter intake was not significantly affected by flaxseed supplementation. Crushed flaxseed is readily accepted by dairy cows, and feeding up to 15% of the total dry matter as flaxseed has no effect on DMI of cows in the mid (Secchiari *et al.*, 2003) and early stages of lactation (Petit, 2002). Feeding diets of 10% whole flaxseed in the DM had no effect on digestibility (Petit, 2002) although lower digestibility of dry matter, organic matter, natural detergent fiber, and acid detergent fiber has been reported for cows fed 12% (dry matter basis) whole flaxseed in the diet (Martin *et al.*, 2008). Recent results (Doreau *et al.*, 2009) reported similar ruminal digestion when dry cow's diet were supplemented with 7.5% rolled flaxseed, 7.5% extruded flaxseed, or 2.6% flaxseed oil. Discrepancies between experiments may result from differences in diet composition or in the number of animals used in each study.

Blood serum parameters:

Data in Table (3) showed that animals fed crushed flaxseed supplemented rations were not affect serum total protein, albumin and globulin concentrations. These results are parallel with values of crude protein and organic matter digestibilities of the experimental ration (Table, 2). Serum total lipid and cholesterol were decreased ($P<0.05$) with animals fed crushed flaxseed compared with that fed control diet. Blood serum glutamic-oxaloacetate-transaminase (GOT) and glutamic-pyruvate-transaminase (GPT) values were not affected by treatments. The concentrations of GOT and GPT were in the normal range for healthy animals. The results of blood serum parameters were indicated the healthy effect of flaxseed supplementation to buffalo's diets as a decrease of total lipids and cholesterol concentrations. Petit (2002) reported that plasma total cholesterol and HDL cholesterol concentrations were lower for cows fed flaxseed than control cows. Fat supplementation is known to increase blood cholesterol (Garcia-Bojalil *et* *al.*, 1998), although the types of fatty acids would seem to differ. Petit *et al.* (2002) observed that lactating cows fed flaxseed had a lower blood cholesterol concentration compared with control diet. While, Gonthier *et al.* (2005) reported that plasma cholesterol was greater ($P < 0.01$) for cows fed flaxseed than for those fed the control diet. These results indicated that tested additives to lactating buffalo's rations were not negatively affected liver activity or animal's health.

Table (2): Apparent nutrients digestibilities of buffaloes fed on rations supplemented with crushed flaxseed.

Item	Control	Low Flaxseed	High Flaxseed	±SE
No. of animals	3	3	3	
Live body weight	561.2	560.9	561.1	2.366
Dry matter intake (g/h/d)	15.98	15.99	15.63	1.356
Dry matter	69.0	69.89	68.95	1.157
Organic matter	69.0	70.75	70.04	1.331
Crude protein	69.6	70.70	70.79	1.372
Crude fiber	62.0	62.54	63.24	2.836
Ether extract	66.05	67.54	67.08	0.337
Nitrogen free extract	67.0	67.61	67.88	0.807

Each value represents an average of nine samples.

Table (3): Blood serum parameters of buffaloes fed on rations supplemented with crushed flaxseed.

Item	Control	Low Flaxseed	High Flaxseed	±SE
Total protein (g/dl)	6.60	6.91	6.58	0.164
Albumin (g/dl)	3.41	3.75	3.49	0.085
Globulin (g/dl)	3.19	3.16	3.09	0.138
A/G ratio	1.08	1.21	1.13	0.046
Total lipids (mg/dl)	297.8^{a}	283.1^{b}	292.1^{ab}	2.472
Cholesterol (mg/dl)	145.8^{a}	132.8^{b}	124.7^{b}	2.871
GPT (Units/ml)	15.42	15.64	15.72	0.184
GOT (Units/ml)	34.13	35.70	35.27	0.432

Each value represents an average of twenty eight samples.

^{a,b} *means at the same row with different superscript are significantly (P<0.05) different.*

Milk yield and composition:

Milk production and milk analysis data are shown in table (4). Milk production was higher ($P<0.05$) with buffaloes fed 17g/kg DM flaxseed compared with other groups. Also, 4% fat corrected milk yield was higher ($P<0.05$) buffaloes fed flaxseed compared with that fed control. An effect of period of lactation for milk yield and FCM were found ($P<0.05$). At wk 4 after the trial, buffaloes was recorded the highest milk and FCM yields which gradually decreased to the lowest values at the wk 16 of lactation (Table 5). Flaxseed supplementation positively affected the milk fat percentage ($P<0.05$). In particular, milk fat content of crushed flaxseed buffaloes were 7.15 and 13.39% higher than milk fat content of control buffaloes. No significant differences for milk lactose, total solids, solids not fat and ash contents were observed among treatments (Table 4). An effect of time of sampling for milk fat percentage was found ($P < 0.05$). At wk 12 of the trial, fat percentage was recorded the highest values where the lowest values was recorded at wk 4 of trial while, milk protein percentage was decreased ($P<0.05$) while milk urea-N was insignificantly decreased gradually by advanced the period of lactation (Table 5). Generally, feed efficiency calculated as milk yield/DMI and 4% FCM/DMI were significantly improved by crushed flaxseed supplementation compared with control (Table 4). Also, feed efficiency was decreased ($P<0.05$) gradually with advanced of lactation (Table 5). This study showed higher milk production and FCM with buffaloes fed flaxseed which are in the line of Moallen (2009) who reported that supplementation of extruded flaxseed to cows ration at a rate of 40 g/kg DM increased milk yield by 2.7%. These results conflicts with the findings of other studies, which found a reduction in milk production (Petit et al., 2005) or no response as a result of feeding whole flaxseed (Mustafa et al., 2003; Martin et al., 2008; Cortes et al., 2010), flaxseed oil (Caroprese *et al.*, 2010) or extruded flaxseed (Gonthier et al., 2005). However, in those studies the flaxseed was supplemented at more than 100 g/kg in the diet, on a DM basis, compared with 17 or 34 g/kg DM in the present study. The increase in milk fat with animals fed crushed flaxseed was expected, given that they received about 0.19 and 0.38% more dietary fat than control animals. Flaxseed supplementation improved energy utilization for milk synthesis compared with control animals (Caroprese *et al.*, 2010). Other milk components were not significantly affected by flaxseed supplementation. Similar results are obtained by Caroprese *et al.* (2010) and Moallem (2009) while Cortes *et al.* (2010) fond a higher milk protein with cows fed flaxseed oil.

Table (4): Average daily milk yield and composition buffaloes fed on rations supplemented with crushed flaxseed.

Item	Control	Low Flaxseed	High Flaxseed	±SE
No of animals	7	7	7	
Milk yield (g/d)	7.80^{b}	8.91^{a}	8.24^{b}	0.111
4% FCM (g/d)	10.81^{b}	12.97^{a}	12.50^{a}	0.121
Milk composition %				
Fat	6.57^{c}	7.04^{b}	7.45^{a}	0.067
Protein	4.18	4.11	4.20	0.024
Lactose	4.75	4.71	4.78	0.065
TS	16.78	16.67	17.26	0.061
SNF	10.22	9.63	9.81	0.061
Ash	0.782	0.811	0.829	0.009
pH	6.56	6.52	6.57	0.026
Urea N	21.84	24.64	26.08	0.026
Feed efficiency:				
Milk yield/DMI	0.601^{b}	0.686^{a}	0.652^{ab}	0.952
FCM yield/DMI	0.833^{b}	0.999^{a}	0.990^{a}	0.856

Each value represents an average of fifty six samples.
$^{a, b}$ *means with different superscripts are significant (P<0.05) difference.*

Table (5): Average daily milk yield and composition of buffaloes fed on rations supplemented with crushed flaxseed during lactation period.

Item	Lactation period (week)				±SE
	4	8	12	16	
Live body weight	562.1	561.3	560.1	559.25	2.354
Dry matter intake (kg/h/d):	12.68	12.59	12.78	12.32	0.654
Milk yield (g/d)	9.17^{a}	8.69^{ab}	8.10^{abc}	7.24b^{c}	0.267
4% FCM (g/d)	12.94^{a}	12.52^{a}	12.04^{a}	10.68^{b}	
Milk composition %					
Fat	6.74^{c}	6.94^{b}	7.24^{a}	7.17^{a}	0.069
Protein	4.26^{a}	4.21^{a}	4.14^{ab}	4.05^{b}	0.029
Lactose	4.89	4.82	4.92	5.02	0.071
TS	16.69	16.76	17.13	17.04	0.063
SNF	9.94	9.82	9.89	9.88	0.065
Ash	0.802	0.800	0.827	0.799	0.012
pH	6.67	6.67	6.67	6.60	0.123
Urea N	25.83	21.68	22.57	26.67	0.789
Feed efficiency:					
Milk yield/DMI	0.723^{a}	0.690^{ab}	0.634^{b}	0.587^{c}	1.263
FCM yield/DMI	1.020^{a}	0.994^{ab}	0.942^{b}	0.867^{c}	1.254

Each value represents an average of twenty eight samples.
$^{a, b, c}$ *means with different superscripts are significant (P<0.05) difference.*

Milk Fatty Acid Profile:

Milk fat composition is presented in Table 6; proportions of short-chain FA (6:0, 8:0 and 10:0) in milk fat were not affected by treatments with the exception of 4:0 was appeared when low flaxseed was added to the diet compared with other treatments (table 6). Medium-chain FA (12:0, 14:0 and 16:0) proportions in milk fat were decreased (P<0.05) and 15:0 and 15:1 were appeared when low flaxseed was added to the diet compared with other treatments. On the other ward, 16:1 proportion was increased with high flaxseed supplementation to the diet (Table 6).

Supplementation of crushed flaxseed changed the FA profile of the milk fat so that the proportions of USFAs were increased and SFAs were decreased. The C17:0 was appeared with low flaxseed while 18:0 and 20:0 was significantly decreased with low flaxseed followed by high flaxseed supplementation and then control. The C18:1n-9t and C18:1n-9c proportions were significantly increased with low flaxseed supplementation compared with other treatments. Further analysis of the milk fat indicated that the contents of total C18:1 *trans* isomers in FA were 12.8% higher ($P<0.05$) (30.49 and 27.02 g/100g, respectively).

Table (6): Milk fatty acids composition of lactating buffaloes fed crushed flaxseeds.

Fatty acids (g/100g FA)	Control	Low Flaxseed	High Flaxseed	SEM
C4	0^{b}	0.21^{a}	0^{b}	0.075
C6	1.1	1.0	0.63	0.030
C8	0.87	0.67	0.81	0.069
C10	1.71	1.28	1.68	0.093
C12	2.18^{a}	1.7^{b}	2.31^{a}	0.413
C14.0	11.27^{a}	8.98^{b}	11.59^{a}	0.009
C14.1	0.67	0.62	0.67	0.223
C15.0	0^{b}	1.34^{a}	0^{b}	0.095
C15.1	0^{b}	0.57^{a}	0^{b}	0.847
C16.0	33.67^{a}	28.87^{b}	34.2^{a}	0.306
C16.1	0.45^{b}	0.49^{b}	2.3^{a}	0.035
C17.0	0^{b}	0.21^{a}	0^{b}	0.349
C18.0	17.82^{a}	15.49^{b}	16.09^{b}	0.619
C18.1N9T	27.02^{b}	30.49^{a}	26.45^{b}	0.126
C18.1N9C	1.78^{b}	2.56^{a}	1.83^{b}	0.017
C18:2 *trans*-10, *cis*-12	0.10	0.13	0.13	0.041
C18:2 *cis*-9, *trans*-11	0.18^{b}	0.39^{a}	0.18^{b}	0.025
Total CLA	0.28^{b}	0.52^{a}	0.30^{b}	0.035
C18.3N3	0.01^{b}	0.15^{a}	0.15^{a}	0.227
C18.3N6	0.27^{c}	1.81^{a}	0.73^{b}	0.061
N-6/N-3	27.0^{a}	12.06^{b}	4.86^{c}	0.125
C20.0	0.71^{a}	0.40^{b}	0.31^{c}	0.032
C20.1	0.19^{a}	0.19^{a}	0.0^{b}	0.411
C20.4	0.0^{b}	2.45^{a}	0.0^{b}	0.512

Each value represents an average of four samples.
[a, b, c] means with different superscripts are significantly ($P<0.05$) different..

Feeding flaxseed increased ($P<0.05$) the total conjugated linolenic acid (CLA) (C18:2 *trans*-10, *cis*-12 and C18:2 *cis*-9, *trans*-11) content of milk with the highest increase (85.6%) achieved by feeding the low flaxseed concentrate (Table 6). The crushed flaxseed supplementation significantly increased the proportion of n-3 (C18:3 N-3) and n-6 FAs (C18:3 N-6) but significantly decreased the n-6: n-3 ratio in milk fat from 27.0 in control milk fat to 12.06 and 4.86 with low and high flaxseed supplementation, respectively. The n-3 FA content was 15 times as high in the low and high flaxseed groups as in the control group ($P<0.05$). Moreover, the n-6 FA content was 6.7 and 2.7 times as high ($P<0.05$) in the flaxseed groups, respectively as in the control group. The C20:1 was not affected with low flaxseed and disappeared with high flaxseed compared with control. While, C20:4 was appeared only with low flaxseed supplementation compared with other treatments.

The main aim of this work was to evaluate the effect of crushed flaxseed supplementation on milk FA profile from buffaloes. Several feeding strategies have been studied that aim to reduce the concentration of saturated FA and increase the concentration of PUFA in milk (Mustafa et al., 2003; Sarrazin *et al.*, 2004; Petit and Côrtes, 2010). Milk from buffaloes receiving crushed flaxseed supplementation displayed an improvement in the FA profile, with an increase in the PUFA and monounsaturated FA content and a decrease in saturated FA content. These results are in agreement with recent studies (Mustafa *et al.*, 2003; Gonthier et al., 2005; Petit and Côrtes, 2010), which reported similar changes in milk fat when flaxseed supplements were used. In particular, in the present study the decrease in C14:0 and C16:0 contents in milk from animals fed flaxseed may be a positive goal from a human health perspective because high proportions of C14:0 and C16:0 has been associated with human cardiovascular problems (Noakes *et al.*, 1996). The increased content of C18 FA in the milk fat of cows fed the flaxseed diet has been reported by several authors (Mustafa *et al.*, 2003; Loor and Herbein, 2003). The increase of C18:1 can be the result of partial bio hydrogenation of C18:2 and C18:3 FA and of the desaturation of C18:0 in the mammary gland (Kennelly, 1996). Mir *et al.* (1999) and Zhang *et al.* (2006) reported 210% and 73% increase in CLA of goat's milk and ewe's milk, as a result of supplementation with canola oil and flaxseed, respectively. Mir *et al.* (1999) suggest that substantial increase in CLA content in milk of small ruminants can be achieved by feeding oilseeds rich in PUFA. The increased dietary intake of C18:3 in flaxseed-supplemented cows resulted in increased levels of C18:1 *trans*-11 and increased CLA C18:2 *cis*-9, *trans*-11 by Δ9-desaturase activity. The endogenous synthesis of CLA C18:2 *cis*-9, *trans*-11 by Δ9-desaturase of the mammary gland is strictly connected to C18:1 *trans*-11 content of milk, and previous studies stated that milk with high CLA content also had high C18:1 *trans*-11 content (Griinari *et al.*, 2000). Milk from flaxseed cows with the high CLA content was characterized by low atherogenic and thrombogenic indices, suggesting that its utilization has less detrimental effects concerning the atherosclerosis and coronary thrombosis risk associated with the consumption of milk and dairy products, being potentially healthier for humans (Zhang et al., 2006).

CONCLUSION

Flaxseed supplementation to lactating buffaloes can be used as a nutritional strategy to reduce concentrations of short-chain and saturated fatty acids and increase long-chain and polyunsaturated fatty acids in milk. A significant increase in CLA and linolenic acid of milk can also be achieved by flaxseed supplementation to buffaloes. Feeding flaxseed 17g/kgDM had a positive impact on milk yield and milk fat percentage. Under the conditions of the present study the recommended flaxseed supplementation to achieve the highest concentration of CLA and linolenic acid is 221 g/animal/day (17g/kgDM). In addition, flaxseed supplementation to dairy buffaloes can contribute to improve the health properties of milk and suggesting that its consumption benefits human health.

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تأثير إضافة بذور الكتان المجروشة على إنتاج اللبن وتركيبة ومحتواه من الأحماض الدهنية ومعاملات الهضم و مكونات الدم.

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تم استخدام ٢١ جاموسة حلابة فى بداية موسم الحليب بمتوسط وزن ٥٦١ ±١٠ كجم قسمت إلى ثلاثة مجموعات فى كل منها سبع حيوانات تغذت على العلائق التالية:

١ العليقة المقارنة :عبارة عن مخلوط العلف المركز والخشن بدون إضافات.
٢ المعاملة الأولى: العليقة المقارنة مضاف إليها 17 جم/كجم مادة جافة.
٣ المعاملة الثانية: العليقة المقارنة مضاف إليها 34 جم/كجم مادة جافة.

وكانت أهم النتائج المتحصل عليها كما يلى:

١- لم يتأثر المأكول من المادة الجافة ولا أوزان الحيوانات بإضافة بذور الكتان.
٢- لم تتأثر معاملات الهضم ولا مكونات الدم بإضافة بذور الكتان ما عدا الدهون الكلية والكوليستيرول فى الدم الذين انخفض تركيزهما بإضافة بذور الكتان.
٣- ارتفع إنتاج اللبن مع المعاملة الأولى كما ارتفع اللبن المعدل نسبة الدهن بإضافة بذور الكتان. كما تحسنت نسبة الدهن بينما لم تتأثر باقى مكونات اللبن بإضافة بذور الكتان. كما تحسنت الكفاءة الغذائية بإضافة بذور الكتان مقارنة بالعليقة المقارنة.
٤- ارتفعت نسبة حمض اللينولينيك المرتبط (CLA) والأحماض الدهنية أوميجا ٣ والأحماض الدهنية غير المشبعة بينما انخفضت نسبة الأحماض الدهنية المشبعة خاصة C18:0 , C20:0 بإضافة بذور الكتان.

ويستخلص من هذة النتائج أنه يمكن استخدام إضافة بذور الكتان المجروشة بنسبة ٣٤ جم/كجم مادة جافة كسياسة غذائية لتحسين نسبة الأحماض الدهنية غير المشبعة خاصة CLA و أوميجا ٣ وخفض نسبة الأحماض الدهنية المشبعة فى دهن اللبن وكوليستيرول الدم لما له من فائدة صحية للمستهلك.