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Buffalo's Milk Composition and Its Fat Properties as Affected by Feeding Diet Supplemented with Flaxseed or Fibrolytic Enzymes in Early Lactation

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ABSTRACT The effects of feeding lactating dairy buffaloes with crushed flaxseed or fibrolytic enzymes supplemented diets on milk production, composition and milk fat properties was studied. Thirty five lactating dairy buffaloes were randomly divided into 5 groups and fed on: control diet without supplementation, diets supplemented with low (LCF) or high (HCF) level of crushed flaxseed and diets supplemented with low (LFE) or high (HFE) fibrolytic enzymes activity. The results revealed that crushed flaxseed supplementation positively affected the milk yield and composition. Milk yield was higher in LCF and HCF diets than in control buffaloes and LCF was more effective than HCF diets. Fibrolytic enzymes supplementation had no effect on milk yield. On the other hand, feeding lactating dairy buffaloes with LCF or LFE-supplemented diets caused an increase in iodine value, long chain fatty acids (Fas), mono-unsaturated fatty acids (MUSFAs), poly- unsaturated fatty acids (PUSFAs), unsaturated fatty acids/saturated fatty acids (USFAs/SFAs) ratio accompanied with a decrease in Plonisk value, acid value, short chain FAs and medium chain FAs compared with HCF, HFE supplemented and control diets. Conjagated lenoliec acid (CLA) content was increased in milk fat from LCF and decreased in milk fat from LFE compared to milk fat from control buffaloes. In addition, milk fat from lactating buffaloes fed different rations supplemented with crushed flaxseed or fibrolytic enzymes characterized with the same oxidative stability or more stable compared with those from control buffaloes.

INTRODUCTION

Polyunsaturated fatty acids (PUFA) such as the n-3 fatty acids (FA) are not synthesized by humans and consumers are increasingly aware of the potential health benefits of these FAs. As a result, there is growing interest to manipulate dairy animal diets to increase concentrations of PUFA in milk fat. Milk fat contains low concentrations of n-3 FA and high levels of SFA, particularly $C_{16:0}$, which has hypercholesterolemia properties (Kennelly 1996). Increasing the level of n-3 FA and other PUFA and reducing the proportion of $C_{16:0}$ can therefore be considered an attractive way to modify milk composition which would increase human consumption of milk and dairy products.

Oilseeds are rich in PUFA which can be fed to dairy animals to modify the milk FA profile and produce more nutritionally beneficial milk for human consumption (Kennelly 1996). 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 (Petit 2003). Feeding flaxseed to dairy cows decreases the concentrations of short- and medium-chain FA and increases the long-chain FAs (Mustafa et al. 2003; Petit 2003), MUSFA, PUSFA and conjugated linoleic acid (CLA) in milk fat (Glasser et al. 2008; Caroprese et al. 2010).

Exogenous enzymes have been investigated primarily as enhancers of ruminal fiber digestion (Beauchemin et al. 2003). Proposed modes of action include solublization of dietary fiber before entering the rumen, provision of readily fermentable substrate for ruminal microorganisms and/or enhancement of microbial enzymatic activities in the rumen resulting more soluble carbohydrates (Gado et al. 2009). Direct-fed enzymes can also enhance microbial colonization of feed by increasing numbers of ruminal fibrolytic microbes (Nsereko et al. 2000) to increase rate of degradation of fiber in the rumen (Giraldo et al. 2008), rumen microbial protein synthesis (Yang et al. 1999) and for stomach digestibility (Nsereko et al. 2002). Therefore, the aim of this study was to evaluate the effects of feeding lactating dairy buffaloes with diets supplemented with crushed flaxseed

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as fatty acids source or fibrolytic enzymes as degradable carbohydrate producer on milk production and composition, and milk fat properties.

MATERIAL AND METHODS

Materials

Flaxseed was obtained from El-Harraz Market, Cairo, Egypt. The flaxseeds dried at 55 ^{O}C for 48 hr, then ground to pass a 1-mm screen with a mill. The chemical composition and fatty acids content of ground flaxseed are shown in Table 1. High fibrolytic enzymes (HFE) product (patent, 22155, Molecular Biology Lab., Ain Shams University, Cairo, Egypt) and low fibrolytic enzymes (LFE, under test) were made from natural sources of anaerobic ruminal bacteria according to (Gado 1997) as shown in Table 2.

Table 1: Chemical composition and fatty acids profile of ground flaxseed

Items	*Flaxseed*
Composition (%)	
Moisture content	4.68
Ash	4.10
Total protein	23.76
Total carbohydrates	18.84
Crude fiber	10.09
Total fat	43.21
Fatty Acids (%)	
C16:0 (Palmtic acid)	6.738
C16:1n-7 (Palmitoleic acid)	0.100
C18:0 (Stearic acid)	4.616
C18:1n-9 (Oleic acid)	21.819
C18:2 n-6 (Linoleic acid)	15.608
C18:3 n-3 (α-Linoleic acid)	50.509
Saturated fatty acids (SFA)	11.354
Unsaturated fatty acids (USFA)	88.036
Mono-unsaturated fatty acids (MUFA)	21.919
Poly-unsaturated fatty acids (PUFA)	66.117
Total n-6	15.608
Total n-3	50.509
SFA / PUFA	0.129

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 2010 to April 2011. Thirty- five lactating Egyptian buffaloes averaging 561±15 kg of body weight (blocked for similar expected calving dates) were randomly divided into 5 groups, housed in tie stalls and fed individually. Buffaloes within groups were assigned randomly to one of five total mixed rations (TMR) composed of 9.89% moisture, 10.34% ash, 16.2% total protein, 46.15% total carbohydrate, 23.59% crude fiber and 3.72% total fat (Table 1) with no additives (control), treated animals were fed on control diet supplemented with low (LCF, 221 g/animal/day) or high (HCF, 442 g/animal/day) crushed flaxseed, and control diet supplemented with low (LFE, 40 g/animal/day) or high (HFE, 40 g/animal/day) fibrolytic enzymes. All diets were designed to have similar concentrations of crude protein and net energy and formulated to meet the animal's requirements (NRC 2001). Diets were fed twice daily at 08:00 and 16:00 hr. Animals were milked twice daily at 06:00 and 16:00 hr. and milk production was recorded at every milking.

Table 2: Fibrolytic enzymes (U/g) composition

Enzymes	*LFE*	*HFE*
Cellulase	0.892	7.05
Xylanase	0.058	2.32
α-amylase	3.39	61.5
Proteases	1.56	29.2

LFE, low fibrolytic enzymes; HFE, high fibrolytic enzymes

METHODS

Samples Collection

Milk samples were obtained once every month from each buffalo for two consecutive milking and pooled within treatment to obtain one composite milk sample per treatment for determination of milk composition and milk fat properties. Milk samples were stored at 4 ± 2^{O}C until analysis.

Milk Fat Separation

The fat of pooled milk samples were separated to skim milk and cream using laboratory separator. Milk fat was prepared from cream by the method of Amer et al. (1985) and frozen at -20^{O}C until the determination of milk fat constant, oxidative stability and milk FAs profile.

Chemical Analysis

Milk total solids, fat, total nitrogen and ash content were determined according to AOAC

(2007, methods 990.20, 2000.18, 991.20 and 945.46 respectively). Protein content was obtained by multiplying percentage of total nitrogen by 6.38. pH values were measured using a digital pH meter (HANNA, instrument, Italy).

Milk Fat Properties

Iodine value of milk fat was determined as the weight of iodine absorbed by 100 parts by weight of the sample according to Egan et al. (1981). Soluble volatile fatty acid (Reichert value) and insoluble volatile fatty acid (Polenske value) were determined according to the method described by international dairy federation (IDF 1966). Acid value of milk fat was determined according to AOAC (2007, method 969.17).

Fatty Acids Profile

Fatty acids of extracted butter oil were methylated according to method of AOAC (2007). Fatty acids were identified by comparison of gas chromatography peaks with peaks of known standards according to Petit and Côrtes (2010).

Oxidative Stability of Milk Fat

Extracted milk fat of each group was stored in 250 ml conical flask in an electrical oven at 80^{O}C. Oxidative stability was followed by determining the peroxide value (PV) and ρ-ansidine value (ρ-PV) at regular intervals (daily for 8 days) as described by Egan et al. (1981).

Statistical Analysis

All results were analyzed using the MIXED procedure of SAS (2004). Data of milk production, milk composition and milk fatty acids profile were analyzed as a complete random design where treatment was the main source of variation. Data were expressed as mean values when there was no interaction between month and treatment (that is, $P>0.10$). When a significant F-test was detected (that is, $P<0.05$), treatment means were separated using Duncan's multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

Milk Yield and Composition

As shown in Table 3, crushed flaxseed supplementation positively affected the milk yield and composition. Milk yield was significantly ($P<0.05$) higher in LCF and HCF than in control buffaloes and higher in LCF than in HCF ($P<0.05$). In particular, milk yield of LCF and HCF were 18.8 and 9.87% higher than milk yield of control buffaloes respectively. However, milk production is not always positively respond to supplemental fat (Drackley et al. 2003; Rego et al. 2005; Martin et al. 2008; Côrtes et al. 2010) because milk yield in previous studies could depend on several factors, including the source of fat and the level of supplementation (Caroprese et al. 2010). Milk yields of total solids, fat, protein, lactose, ash and solids not fat were significantly higher in LCF and HCF than in control. Caroprese et al. (2010) suggested that the increase in milk protein yield could be the result of a decrease in the ruminal protein degradability of the whole flaxseed supplementation. The administration of whole flaxseed could have increased the flow of nitrogen to the duodenum because of its greater bypass protein content. As a consequence, the amino acids availability for protein synthesis in the mammary gland increased. No significant differences were found in yield of milk components between LCF and HCF even if yield of milk components was numerically higher in LCF than in HCF. On the other hand, milk fat percentage of LCF and HCF were higher than milk fat percentage of control buffaloes. A similar observation was made by Caroprese et al. (2010) for cow fed a diet supplemented with whole flaxseed. However, insignificant reduction in total solids and protein percentage was found in LCF followed by HCF then control buffaloes.

Fibrolytic enzymes supplementation (LFE and HFE) had no significant effect on the milk yield compared with control diet. On contrast, Yang et al. (2000) and Gado et al. (2009) reported a 7-15% higher in milk production of dairy cows fed fibrolytic enzymes and the authors explained the improvement as being due to increased nutrient digestibility and microbial protein synthesis. Also, no significant differences was found in milk yields of total solids, fat, protein, lactose, ash and solids not fat among treatments. Milk fat percentage was higher in LFE than in control buffaloes ($P<0.05$) and the differences in total solids, protein, lactose, ash and solids not fat percentages were not significant. Conversely, a reduction in total solids, fat, protein percentage was found in HFE compared

Table 3: Milk yield and composition of lactating buffaloes fed different rations supplemented with crushed flaxseed or fibrolytic enzymes

Items	*Treatments*					
	Control	*LCF*	*HCF*	*LFE*	*HFE*	*SEM*
Yield (g/day)						
Milk	7500^{c}	8910^{a}	8240^{b}	7400^{c}	7780^{bc}	11.90
Total solids	1309^{b}	1485^{a}	1422^{a}	1314^{b}	1247^{b}	0.266
Fat	512.5^{b}	627.3^{a}	613.9^{a}	556.3^{b}	515.8^{b}	0.167
Protein	326.0^{b}	366.2^{a}	346.1^{a}	318.9^{b}	309.3^{b}	0.125
Lactose	370.5^{b}	419.6^{a}	393.9^{b}	372.6^{b}	367.0^{b}	0.166
SNF	797.1^{b}	858.0^{a}	808.3^{a}	758.5^{b}	731.1^{b}	0.854
Ash	60.9^{b}	72.3^{a}	68.3^{a}	60.8^{b}	60.4^{b}	0.098
Milk Composition (%)						
Total solids	17.45	16.67	17.26	17.75	16.03	0.156
Fat	6.83^{b}	7.04^{a}	7.45^{a}	7.51^{a}	6.63^{b}	0.033
Protein	4.34	4.11	4.20	4.31	3.98	0.045
Lactose	4.93	4.71	4.78	5.03	4.72	0.044
SNF	10.63	9.63	9.81	10.25	9.40	0.098
Ash	0.81	0.81	0.83	0.82	0.78	0.020
pH value	6.76	6.72	6.77	6.68	6.74	0.026

^{a,b} Means with different superscripts are significant (P<0.05) difference.
Control, buffaloes fed diets without supplementation; LCF, buffaloes fed 221 g crushed flaxseed/head/day; HCF, buffaloes fed 442 g crushed flaxseed/head/day; HFE, 0.058 U/g xylanase, 3.39 U/g α-Amylase, 0.892 U/g cellulose and 1.56 U/g protease; HFE, 2.32 U/g xylanase, 61.5 U/g α-Amylase, 7.05 U/g cellulose and 29.2 U/g protease.

with LFE and control buffaloes. pH value of milk had not significantly affected by the different diet supplementation. Our results are consistent with Dhiman et al. (2002) who reported that exogenous enzyme supplementation has had little effect on milk components when cows have been in positive energy balance. However, in another study, Reddish and Kung (2007) did not observe any effect on milk yield or milk composition for lactating cows fed a supplemental cellulase-xylanase enzyme mixture.

Milk Fat Properties

Milk fat properties of lactating buffaloes fed different rations supplemented with crushed flaxseed or fibrolytic enzymes are presented in Table 4. No significant differences were found for iodine, Reichert, Plonisk, peroxide and ρ-ansidine values among LCF, HCF and control buffaloes even if iodine and Reichert values were numerically higher and Plonisk value was lower in milk fat from LCF than in milk fat from control buffaloes. However, acid value of milk fat from LCF and HCF was lower than milk fat from control buffaloes (P<0.05). Feeding buffaloes on a high HFE increased the soluble volatile fatty acid (Reichert value) and decreased the peroxide value (P<0.05). While feeding buffaloes on a LFE elevated the iodine value and reduced insoluble volatile fatty acid (Polenske value) compared with control buffaloes (P>0.05). From these results we can concluded that milk fat properties are slightly affected by the type and level of the feeding diet supplementation.

Milk Fat Composition

As shown in Table 5, crushed flaxseed or fibrolytic enzymes supplemented diets signifi-

Table 4: Properties of milk fat from lactating buffaloes fed different rations supplemented with crushed flaxseed or fibrolytic enzymes

Milk fat properties	*Treatments*					
	Control	*LCF*	*HCF*	*LFE*	*HFE*	*±SEM*
Iodine value	34.15	36.46	34.19	36.06	34.84	0.355
Reichert value	28.32	30.24	29.64	28.20	30.36	0.441
Polenske value	2.20	1.80	2.00	1.70	1.90	0.054
Acid value	0.37^{a}	0.26^{b}	0.28^{b}	0.41^{a}	0.41^{a}	0.018
Peroxide value (mEq/kg)	0.53^{a}	0.57^{a}	0.56^{a}	0.52^{ab}	0.47^{b}	0.013
ρ-ansidine value	0.43^{ab}	0.48^{a}	0.44^{ab}	0.43^{ab}	0.39^{b}	0.01

^{a,b} Means with different superscripts are significant (P<0.05) difference.

Table 5: Fatty acids profile of milk fat from lactating buffaloes fed different rations supplemented with crushed flaxseed or fibrolytic enzymes

Fatty acids g/100g milk fat	*Treatments*					
	Control	*LCF*	*HCF*	*LFE*	*HFE*	*±SEM*
Short chain fatty acids (C_{4-8})	5.27[a]	4.25[b]	4.89[a]	4.43[b]	5.17[a]	0.356
Medium chain fatty acids (C_{10-14})	17.26[a]	14.87[b]	16.79[a]	16.28[a]	16.83[a]	0.452
Long chain fatty acids (C_{15-20})	77.47[b]	80.88[a]	78.32[ab]	79.29[ab]	78.00[b]	0.552
Saturated fatty acids	69.32[a]	63.60[c]	67.62[b]	66.56[bc]	67.2[b]	1.523
Unsaturated fatty acids	30.68[b]	36.40[a]	32.38[b]	33.43[ab]	32.80[b]	2.896
Mono unsaturated fatty acids	30.11[b]	33.42[a]	31.25[b]	29.82[b]	29.92[b]	0.855
Poly unsaturated fatty acids	0.56[c]	2.98[a]	1.13[b]	3.61[a]	2.88[ab]	0.566
USFA/SFA	0.442[c]	0.572[a]	0.485[b]	0.502[b]	0.488[b]	2.112
CLA	0.28[b]	0.52[a]	0.30[b]	0.13[c]	0.24[b]	0.035

[a,b,c] Means with different superscripts are significant (P<0.05) difference.
Control, buffaloes fed diets without supplementation; LCF, buffaloes fed 221 g crushed flaxseed/head/day; HCF, buffaloes fed 442 g crushed flaxseed/head/day; HFE, 0.058 U/g xylanase, 3.39 U/g α-Amylase, 0.892 U/g cellulose and 1.56 U/g protease; HFE, 2.32 U/g xylanase, 61.5 U/g α-Amylase, 7.05 U/g cellulose and 29.2 U/g protease.

cantly affected milk fat FAs profile. The content of short chain FAs in milk fat from LCF and LFE recorded the lowest values (P<0.05) than other treatments. No differences emerged among treatments for the content of medium chain FAs except LCF which had significantly lower value. Milk fat from LCF showed higher (P<0.05) long chain FAs compared to control, while other treatments were not significantly affected long chain FAs compared to control. SFAs were lower (P<0.05) and USFAs were higher (P<0.05) in milk fat of LCF compared with other treatments. PUSFAs was higher (P<0.05) in LCF and LFE milk fat followed by HCF and HFE then control. These results are in agreement with Mustafa et al. (2003) and Caroprese et al. (2010) who reported similar changes in milk fat when flaxseed supplements were used. The decrease in USFAs with HCF compare with LCF may be attributed to the negative effect of higher amount of oils on the rumen microflora (bacteria and protozoa) activity which inhibits the USFAs production. Latham et al. (1972) reported that less triglyceride was hydrolyzed as the roughage was reduced and the flaxseed content ration increased. The PUSFAs content and USFA/SFA ratio of milk fat from all treatments were significantly higher (P<0.05) than milk fat from control and the increase was more pronounced in LFE and LCF (P<0.05). Milk fat from LCF had a higher CLA content (P<0.05) than milk fat from control buffaloes; whereas, milk fat from LFE had a lower CLA content (P<0.05) than milk fat from control. In this concern, Petit and Côrtes (2010) found that flaxseed supplementation enhanced the linolenic acid proportion in milk fat compared to the control diet. Moreover, milk fat from HCF and HFE had no significant deference in CLA compared with control.

Oxidative Stability of Milk Fat

Oxidative stability of milk fat (peroxide value and ρ-ansidine value) from lactating buffaloes fed different rations supplemented with crushed flaxseed or fibrolytic enzymes is shown in Figures 1a,b. Peroxide value (PV) is the number of milligram-equivalents oxygen per kilogram milk fat, while ρ-ansidine value (ρ-AV) is level of aldehydes, principally 2-alkenals. No significant differences were found in PV and ρ-AV for milk fat among all treatments until 5th day at 80^{O}C, even if PV and ρ-AV were numerically higher in milk fat from control buffaloes than in of milk fat from other treatments. On day 6th, milk fat from HCF and HFE recorded the lowest increasing rate (p<0.05) compared with LCF, LFE and control buffaloes. Although, the increasing rate of PV and ρ-AV of milk fat from LCF and LFE, which had highest USFAs, were lower than milk fat from control buffaloes (P>0.05). These results may attributed to flaxseed contain some constituents provide protection against lipid peroxidation. Oomah (2003) reported that flaxseed is a leading source of the n-3 FA and α-linolenic acid and of phenplic compounds commonly known as lignans (> 500 μg/g).

CONCLUSION

Feeding lactating dairy buffaloes with crushed flaxseed supplemented diet increased

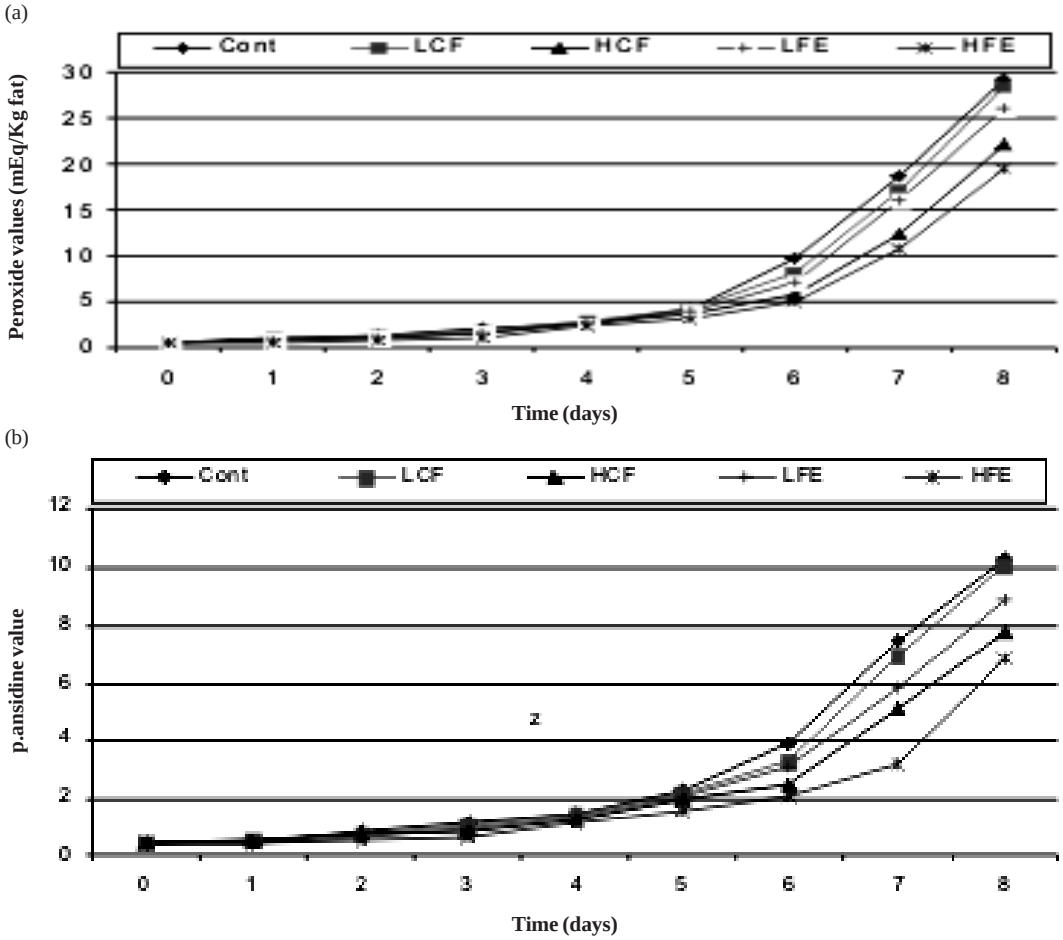

Fig. 1. Oxidative stability of milk fat (a, peroxide values and b, ρ-ansidine values) from lactating buffaloes fed different rations supplemented with crushed flaxseed or fibrolytic enzymes

milk yield, composition and milk fat percentage; while, fibrolytic enzymes supplementation had no effect on milk yield and composition. Both crushed flaxseed and fibrolytic enzymes supplementation (especially low level) improved milk fat FAs profile, PUSFAs, CLA and milk fat stability. Finally, crushed flaxseed and fibrolytic enzymes supplementation to lactating buffalos can contribute to improve the health properties of milk. The changes induce in FAs profile of milk fat can be affect the chemical and physical properties of some dairy products, which needs to more evaluation in future.

REFERENCES

Amer MA, Kupranyez DB, Baker BE 1985. Physical and chemical characteristics of butter fat fractions obtained by crystallization from molten fat. *J Am Oil Chem Soc*, 62: 1551-1557.

AOAC (Association of Official Analytical Chemists) 2007 . *Official Methods of Analysis*, 19th Edition. Washington, DC, USA.

Beauchemin KA, Yang WZ, Morgavi DP, Ghorbani, GR, Kautz W, Leedle JAZ 2003. Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. *J Anim Sci*, 81: 1628-1640.

Caroprese M, Marzano A, Marino R, Gliatta G, Muscio A, Sevi A 2010. Flaxseed supplementation improves fatty acid profile of cow milk. *J Dairy Sci*, 93: 2580-2588.

Côrtes C, Silva-Kazama DC, Kazama R, Gagnon N, Benchaar C, Santos GTD, Zeoula LM, Petit HV 2010. Milk composition, milk fatty acid profile, digestion, and ruminal fermentation in dairy cows fed whole flaxseed and calcium salts of flaxseed oil. *J Dairy Sci*, 93: 3146–3157.

Dhiman TR, Zaman MS, Gimenez RR, Walters JL, Treacher R 2002. Performance of dairy cows fed forage treated

with fibrolytic enzymes prior 518 to feeding. *Anim Feed Sci Technol,* 101: 115-125.

Drackley JK, Cicela TM, LaCount DW 2003. Responses of primiparous and multiparous Holstein cows to additional energy from fat or concentrate during summer. *J Dairy Sci*, 86: 1306-1314.

Duncan DB 1955. Multiple range and multiple F test. *Biometrics*, 11: 1-42.

Egan H, Kirk RS, Sawyer R 1981. *Pearson's Analysis of Foods*. 8th Edition. Edinburgh London Melbourne and New York: Churchill Livingstone.

Gado H 1997. Effect of enzymatic treatments for poor quality roughages on fiber digestibility and nitrogen metabolism in Baladi goats. *Egypt J Nutr Feeds*, 50-56.

Gado HM, Salem AZM, Robinson PH, Hassan M 2009. Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. *Anim Feed Sci Technol*, 154: 36-46.

Giraldo LA, Tejido ML, Ranilla MJ, Carro MD 2008. Effects of exogenous fibrolytic enzymes on in vitro ruminal fermentation of substrates with different forage: Concentrate ratios. *Anim Feed Sci Technol*, 141: 306-325.

Glasser F, Ferlay A, Chilliard Y 2008. Oilseed lipid supplements and fatty acid composition of cow milk: A meta-analysis. *J Dairy Sci*, 91: 4687-4703.

IDF 1966. *Determination of Soluble and Insoluble Volatile Fatty Acid Values of Milk Fat. FIL-IDF 37*. Brussels: International Dairy Federation.

Kennelly JJ 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. *Anim Feed Sci Technol*, 60: 137-152.

Latham MJ, Storry JE, Elisabeth Sharpe M 1972. Effect of low-roughage diets on the microflora and lipid metabolism in the rumen. *Applied Microbiol*, 24: 871-877.

Martin C, Rouel JP, Jouany JP, Doreau M, Chilliard Y 2008. Methane output and diet digestibility in response to feeding dairy cow's crude linseed, extruded linseed, or linseed oil. *J Anim Sci*, 86: 2642-2650.

Mustafa AF, Chouinard PY, Christensen DA 2003. Effects of feeding micronised flaxseed on yield and composition of milk from Holstein cows. *J Sci Food Agric*, 83: 920-926.

NRC (National Research Council) 2001. *Nutrient Requirements of Dairy Cattle*. 7th Revised Edition. Washington, DC, USA.: National Academy Press.

Nsereko VL, Beauchemin KA, Morgavi DP, Rode LM, Furtado AF, McAllister TA, Iwaasa, A.D, Yang WZ, Wang Y 2002. Effect of a fibrolytic enzyme preparation from Trichoderma longibrachiatum on the rumen microbial population of dairy cows. *Can J Microbiol*, 48: 14-20.

Nsereko VL, Morgavi DP, Rode LM, Beauchemin KA, McAllister TA 2000. Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of alfalfa hay fiber by mixed rumen microorganisms in vitro. *Anim Feed Sci Technol*, 88: 153-170.

Oomah BD 2003. Processing of flaxseed fiber, oil, protein, and lignan. In: LU Thompson, SC Cunnane (Eds.): *Flaxseed in Human Nutrition*. Washington DC: AOAC Press.

Petit HV 2003. Digestion, milk production, milk composition, and blood composition of dairy cows fed formaldehyde treated flaxseed or sunflower seed. *J Dairy Sci*, 86: 2637-2646.

Petit HV, Côrtes C 2010. Milk production and composition, milk fatty acid profile, and bloodcomposition of dairy cows fed whole or ground flaxseed in the first half of lactation. *Anim Feed Sci Technol*, 158: 36-43.

Reddish MA, Kung JL 2007. The effect of feeding a dry enzyme mixture with fibrolytic activity on the performance of lactating cows and digestibility of a diet 618 for sheep. *J Dairy Sci*, 90: 4724- 4729.

Rego OA, Rosa HJD, Portugal P, Cordeiro R, Borba AES, Vouzela CM, Bessa RJB 2005. Influence of dietary fish oil on conjugated linoleic acid, omega-3 and other fatty acids in milk fat from grazing dairy cows. *Livest Prod. Sci*, 95: 27-33.

SAS (Statistical Analysis System) 2004. *SAS User's Guide Statistical, Release 6.12 Education.* NC. USA: SAS Institute Inc. Cary.

Yang WZ, Beauchemin KA, Rode LM 1999. Effects of a 673 enzyme feed additive on extent of digestion and milk production 674 of lactating dairy cows. *J Dairy Sci*, 82: 391-403.

Yang WZ, Beauchemin KA, Rode LM 2000. A comparison of methods of adding fibrolytic enzymes to lactating cow diets. *J Dairy Sci*, 83: 2512-2520.