http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 15 (14): 690-693, 2012
ISSN 1028-8880 / DOI: 10.3923/pjbs.2012.690.693

Enrichment of Milk with Conjugated Linoleic Acid by Supplementing Diets with Fish and Sunflower Oil

S.A.H. Abo El-Nor and Mostafa S.A. Khattab
Department of Dairy Science, National Research Centre, Dokki, Cairo, Egypt

Abstract: There is an increase interesting in enrichment of milk with Conjugated Linoleic Acid (CLA) due to its anti-oxidative and anti-carcinogenic properties. The objective of this study was to investigate the effect of supplementing diets fed to lactating goats with sunflower, fish oil and its blend. Eight lactating Nubian goats were fed a base diet (T1), diet supplemented with 2% sunflower oil (on dry matter (DM) basis) (T2), diet supplemented with 2% fish oil (T3) and diet supplemented with 2% sunflower and fish oil (T4) for 84 day. Milk composition milk fat, protein (%) decreased in T2, T3 and T4 compared with control (T1) while there was no significant differences between treatments in milk lactose content. CLA content in milk fat was higher in response to fish oil or sunflower and fish oil blend compared with control (T1). The results indicated that supplementing diets fed to lactating goats with sunflower, fish oil increased CLA contents in the milk 2-4 times than control.

Key words: Conjugated linoleic acid, milk, goat, fish oil, sunflower oil

INTRODUCTION

Over the last years, Conjugated Linoleic Acids (CLA) have been intensively studied for their activity and its benefits. As a reason of the beneficial effects, the most two CLA isomers, 9, 11-cis, trans and 10, 12 -trans, cis CLA, have been highly studied for its biological effects and activity. Conjugated Linoleic Acid (CLA) is a mixture of seventeen positional and geometric isomers of linoleic acid with conjugated double bonds located at positions 11, 13-, 10,12-, 9,11-, 8,10- or 7,9- on the carbon chain (Mir *et al*., 1999). *Butyrivibrio fibrisolvens* bacteria are the producer of the CLA isomers in the rumen (Mir *et al*., 2000) the CLA isomers could be intermediary compound in the successive bio-hydrogenation of linoleic acid to stearic acid. The cis, trans/trans, cis-8,10-, - 9,11-, -10, 12- and -11, 13- isomers accounts for the major ones, however, the most abundant CLA isomer is cis-9, trans-11-octadecadienoic acid.

CLA appears to have anti-oxidative and anti-carcinogenic properties (Mir *et al*., 1999, 2000). In addition, CLA has been shown to stimulate immune response and protect against arteriosclerosis (Cook *et al*., 1993; Lee *et al*., 1994). Many studies have been carried out to investigate the impact of addition of different sources of oils such as fish oil, sunflower oil, olive oil and soybean oil in lactating animals' diets in a try to increase Conjugated Linoleic Acid (CLA) concentration in milk.

The current study aimed to compare two sources of oil (fish, sunflower oil) on the productive performance of lactating Nubian goat and its effect on the CLA concentration in the milk.

MATERIALS AND METHODS

The present study was performed at the Agricultural Experimental Station and Dairy Science Department, National Research Centre, Dokki, Giza, Egypt.

Experimental animals: Eight lactating Nubian goats aged 3 years were used in the present study. Live body weight ranged between 35 and 40 kg. Animals were assigned randomly into four groups (two animals each group) using 4×4 Latin square design. The experimental period was extended for 84 days and consisted of four periods (21 days each).

Experimental rations: The intended ratio of concentrate to roughage was 62:38 on Dry Matter (DM) basis. The goats were individually fed according to (NRC, 1985). All supplements were first calculated on DM basis then mixed with the concentrate feed mixture (Table 1). Concentrates were offered twice daily during milking times at 7:00 a.m. and 7:00 p.m. Egyptian clover was offered at 11:00 am and overnight. Fresh water was available to the animals all time.

Corresponding Author: Mostafa Sayed Khattab, Department of Dairy Science, National Research Centre, Dokki, Cairo, Egypt

The experimental diets were as follow:

- Control diet was 62% concentrate feed mixture, 38% Egyptian clover on DM basis [T_1]
- Control diet+2% sunflower oil [T_2]
- Control diet+0.5% fish oil [T_3]
- Control diet+1.5% sunflower oil+0.5% fish oil [T_4]

Milk sampling: Animals were milked handily twice daily at 7:00 a.m. and 7:00 p.m. Milk yield was recorded, samples of milk were collected from each animal at morning and evening during the last three days of each experimental period. Composite milk samples (relative to the quantity of milk produced) were taken from the two milking to determine the components of milk.

Chemical analysis

Feed and feces samples analysis: The dry matter contents of feed and faeces were determined by oven-drying for 4 h at 105°C according to AOAC (1984) method No. 930.15. Ash analysis was conducted at 550°C for 4 h based on the AOAC (1984 method No. 942.05. Nitrogen was measured using a mixed catalyst Kjeldahl method (AOAC, 1984); method No. 988.05. The crude protein content was calculated by multiplying nitrogen by 6.25. Ether extract was determined by the Soxhlet method with petroleum ether as a solvent following AOAC (1984) method No. 963.15. The Total Mixed Ration (TMR) samples were also analyzed for Acid Detergent Fiber (ADF) (method 973.18c; AOAC (1984) and Natural Detergent Fiber (NDF) (Van Soest *et al*., 1991) using α-amylase (A3306; Sigma Chemical Co., St. Louis, MO) and sodium sulfite corrected for ash concentration adapted for an Ankom 200 fiber analyzer (Ankom Technology, Fairport, NY).

Milk samples analysis: Milk samples were analyzed for Total Solids (TS), Solids Not Fat (SNF), Total Protein (TP), fat and lactose using infrared spectroscopy (Bentley 150, Infrared Milk Analyzer, Bentley Instruments, USA). Milk Fatty Acids Methyl Esters (FAME) were prepared by base-catalyzed methanolysis of the glycerides (KOH in methanol) according to International Standard (ISO-IDF, 2002). FAME were separated using a Cp-Sil 88 fused-silica capillary column (100×0.25 mm i.d.×0.2 μm film thickness, Chrompack, Middelburg, Netherlands) on a Perkin-Elmer chromatograph (model 8420, Beaconsfield) equipped with a flame ionization detector. The column was held at 100°C for 1 min after injection, temperature-programmed at 7°C min^{-1} to 170°C, held there for 55 min, then temperature-programmed at 10°C min^{-1} to 230°C and held there for 33 min. Helium was the carrier gas with a column inlet pressure set at 30 psig and a split ratio of 1:20. The injection volume was 0.2 μL. Total run time was of 105 min.

Table 1: Chemical composition of concentrate experimental ration

Items	Percent
Ingredient	
Egyptian clover	38
Corn, ground	30
Wheat bran	12
Bean, cracked	18
Glycerol-mineral/vitamin	2
Chemical composition	
CP	15.93
ADF	21.79
NDF	29.39
Hemicellulose	7.60
Ether extract	6.12
Ash	6.03
NE_L (kcal kg^{-1})	1.73

Statistical analysis: Data were statistically analyzed using the GLM procedures of SAS (2004) according to procedures outlined by Snedecor and Cochran (1982) significant level was 0.05.

RESULTS AND DISCUSSION

Milk yield and composition: The effects of supplementation of ration with oils on milk yield and milk composition are shown in Table 2. The differences between T2, T3, T4 in either milk yield or 4% fat corrected milk were not significant; while were significantly differ with control (T1); the highest value of daily milk yield (1386 g day^{-1}) was obtained in goats fed on control diet while the lowest value was recorded for T3 (1113 g day^{-1}). Milk fat percentage was decreased for T2, T3 and T4 compared to T1; differences between groups were significant ($p<0.05$). The values of milk fat % were 4.8, 4.5, 4.2 and 3.6 for T1, T2 and T4, respectively.

The results of the present study are in agreement with those reported by Dhiman *et al*. (1995), Donovan *et al*. (2000) and Abu-Ghazaleh *et al*. (2003). The addition of polyunsaturated oils in free form tends to depress milk fat percentage (Selner and Schultz, 1980).

Table 2: Effect of experimental ration on milk yield and milk composition

	Treatments			
Items	T1	T2	T3	T4
Milk yield (g/head/day)	1386.00^a	1177.00^b	1113.00^b	1188.00^b
FCM (g/head/day)	1552.3	1265.30^b	1164.40^b	1116.70^b
Milk composition (%)				
Fat	4.80^a	4.50^b	4.20^b	3.60^c
Protein	4.00^a	2.70^b	3.00^b	2.80^b
Lactose	4.50	4.35	4.64	5.21
TS	14.0^a	11.55^b	11.84^b	11.61^b
SNF	9.80	7.05	7.64	8.01
Ash	0.70	0.60	0.48	0.48

Different superscripts in the same row mean significant difference at $p \leq 0.05$ level

Table 3: Effect of dietary supplementation with sunflower oil, fish oil and their blend on the conjugated linoleic acid (CLA) content in milk

	T1	T2	T3	T4
C 14	16.00^{a}	14.20^{b}	13.90^{b}	13.80^{b}
C 15	0.76^{b}	0.59^{b}	1.03^{a}	0.60^{b}
C 16	4.53^{a}	4.39^{b}	4.37^{b}	4.17^{c}
C 17	1.37	1.39	1.10	1.27
C 18	24.80^{b}	26.00^{a}	25.20^{ab}	25.50^{ab}
C 18:1 n-9	45.90^{a}	39.79^{b}	29.90^{c}	30.93^{c}
C 18:2 cis	5.20^{c}	11.60^{b}	16.20^{ab}	20.40^{a}
C 18:2 trans	0.49^{b}	0.76^{b}	6.70^{a}	0.58^{b}
C 18:3	0.13	0.36	0.02	0.53
C 18:2 n-6	0.82^{c}	0.92^{c}	1.58^{b}	2.22^{a}
CLA	5.69^{c}	12.36^{b}	22.90^{a}	20.98^{a}
ω-3	0.13	0.36	0.02	0.53
ω-6	2.71	2.07	8.92	3.92
ω-9	45.90	39.16	29.90	30.93
MCF (C14-C16)	21.29^{a}	19.18^{b}	19.30^{b}	18.57^{b}
LCF (>C16:0)	78.71	80.82	80.70	81.43

MCF: Medium chain fatty acids, LCF: Long chain fatty acids, Different superscripts in the same row mean significant difference at $p \leq 0.05$ level

These results may be due to the effect of supplementing rations with oils which may reduce the digestibility coefficients of nutrients and the increase of intakes passage from the rumen.

From the current results it could be noted that total solids, fat, protein contents were decreased in groups that fed diets supplemented with oil (T2, T3 and T4) compared with control diet (T1).

Milk fatty acids and CLA contents: Effect of dietary supplementation with sunflower oil, fish oil and their blend on the Conjugated Linoleic Acid (CLA) content in milk are shown in Table 3. The contents of myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) acids were significantly ($p<0.05$) decreased by supplementation with sunflower oil, fish oil and their blend. While, $C_{15:0}$ were significantly higher in T3 compared with other treatments. Milk from goats fed the T2, T3 and T4 diets had lower ($p<0.05$) concentration of medium-chain fatty acids (C14:0-C16:0) and a greater ($p<0.05$) concentration of long-chain fatty acids compared with the T1 diet (Table 3). Feeding polyunsaturated fat is typically associated with a decrease in the de novo synthesis of short and medium-chain fatty acids (Casper *et al*., 1988; Abu-Ghazaleh *et al*., 2002), with the greatest decrease when a high linoleic acid source was fed (Casper *et al*., 1988; Kelly *et al*., 1998; Abu-Ghazaleh *et al*., 2003). The decrease in medium-chain fatty acids may represent an improvement in the profile of milk fat fatty acids as these fatty acids have been reported to constitute the hypercholesterolemic portion of milk fat (Ney, 1991). Stearic acid ($C_{18:0}$) was higher when supplemented with sunflower oil, difference between experimental groups are significant at $p \leq 0.05$. Oleic acid ($C_{18:1}$) was significantly ($p \leq 0.05$) decreased and was lower for T2, T3 and T4 diets. Goats fed diet supplemented with oil (T2, T3 and T4) produced milk fat CLA 2-4 times higher than that in control; differences among groups are significant at $p \leq 0.05$. The two source of oils used in the study were chosen due to their richness with polyunsaturated fatty acids specially in Linoleic acid $C_{18:2}$ (sunflower oil) and Oleic acid $C_{18:1}$ (fish oil), (Griinari *et al*., 2000; Piperova *et al*., 2002).

The linoleic acid $C_{18:2}$ content in fish oil was 589.9 mg g^{-1} of fat which is lower than that in sunflower oil (751.9 mg g^{-1}). But CLA content in milk fat was higher in response to fish oil or sunflower and fish oil blend supplement compared with control (T1) (Table 3). this result may be due to an interaction between the high content of oleic acid $C_{18:1}$ in fish oil and richness of sunflower oil with linoleic acid. Milk fat contains vary in levels of CLA in milk among herds (Kelly and Bauman, 1996). The substantial variation in content of CLA in milk fat between herds suggests that diet has a major influence. Previous study has suggested that the bio-hydrogenation sequence of linoleic acid can lead to an increase in CLA levels in milk fat (McGuire *et al*., 1996). The results of the present study indicate that other fatty acids might contribute to CLA production.

CONCLUSION

Results of the current study showed that feeding lactating goats sunflower oil, fish oil or blend of them decreased milk production and increased the content of milk cis-9, trans-11 CLA. The high increase in milk cis-9, trans-11 CLA occurred within fish oil and blend of fish oil plus sunflower oil supplements to the diet.

REFERENCES

AOAC, 1984. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington DC., USA.

Abu-Ghazaleh, A.A., D.J. Schingoethe, A.R. Hippen and L.A. Whitlock, 2002. Feeding fish meal and extruded soybeans enhances the Conjugated Linoleic Acid (CLA) content of milk. J. Dairy Sci., 85: 624-631.

Abu-Ghazaleh, A.A., D.J. Schingoethe, A.R. Hippen and K.F. Kalscheur, 2003. Milk conjugated linoleic acid response to fish oil supplementation of diets differing in fatty acid profiles. J. Dairy Sci., 86: 944-953.

Casper, D.P., D.J. Schingoethe, R.P. Middaugh and R.J. Baer, 1988. Lactational responses of dairy cows to diets containing regular and high oleic acid sunflower seeds. J. Dairy Sci., 71: 1267-1274.

Cook, M.E., C.C. Miller, Y. Park and M. Pariza, 1993. Immune modulation by altered nutrient metabolism: Nutritional control of immune-induced growth depression. Poult. Sci., 72: 1301-1305.

Dhiman, T.R., K.V. Zanten and L.D. Satter, 1995. Effect of dietary fat source on fatty acid composition of cow's milk. J. Sci. Food Agric., 69: 101-107.

Donovan, D.C., D.J. Schingoethe, R.J. Baer, J. Ryali, A.R. Hippen and S.T. Franklin, 2000. Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. J. Dairy Sci., 83: 2620-2628.

Griinari, J.M., B.A. Corl, S.H. Lacy, P.Y. Chouinard, K.V.V. Nurmela and D.E. Bauman, 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ9-desaturase. J. Nutr., 130: 2285-2291.

ISO-IDF 2002. Milk Fat: Preparation of Fatty Acid Methyl Esters. ISO, Bruxelles, Belgium, Pages: 6.

Kelly, M.L. and D.E. Bauman, 1996. Conjugated linoleic acid: A potent anticarcinogen found in milk fat. Proceedings of the Cornell Nutrition Conference, (CNC'96), Cornell University, Ithaca, New York, USA., pp: 124-133.

Kelly, M.L., J.R. Berry, D.A. Dwyer, J.M. Griinari, P.Y. Chouinard, M.E. Van Amburgh and D.E. Bauman, 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr., 128: 881-885.

Lee, K.N., D. Kritchevsky and M.W. Pariza, 1994. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis, 108: 19-25.

McGuire, M.A., M.K. McGuire, M.A. Guy, W.K. Sanchez and T.D. Shultz *et al*., 1996. Short-term effect of dietary lipid concentration on content of conjugated linoleic acid (CLA) in milk from dairy cattle. J. Anim. Sci., 74: 266-266.

Mir, Z., L.A. Goonewardene, E. Okine, S. Jaegar and H.D. Scheer, 1999. Effect of feeding canola oil on constituents, conjugated linoleic acid (CLA) and long chain fatty acids in goats milk. Small Ruminant Res., 33: 137-143.

Mir, Z., L.J. Paterson and P.S. Mir, 2000. Fatty acid composition and conjugated linoleic acid content of intramuscular fat in crossbred cattle with and without Wagyu genetics fed a barley-based diet. Can. J. Sci., 80: 195-197.

NRC, 1985. Nutrient Requirements of Sheep. 6th Edn., National Academy Press, Washington, D.C., USA.

Ney, D.M., 1991. Potential for enhancing the nutritional properties of milk fat. J. Dairy Sci., 74: 4002-4012.

Piperova, L.S., J. Sampugna, B.B. Teter, K. Kalscheur and M.P. Yurawecz *et al*., 2002. Duodenal and milk trans octadecenoic acid and Conjugated Linoleic Acid (CLA) isomers indicate postabsorptive synthesis is the predominant source of cis9-containing CLA in lactating dairy cows. J. Nutr., 132: 1235-1241.

SAS, 2004. Statistical Analysis Systems. Version 9.2, SAS Institute, Cary, NC.

Selner, D.R. and L.H. Schultz, 1980. Effects of feeding oleic acid or hydrogenated vegetable oils to lactating cows. J. Dairy Sci., 63: 1235-1241.

Snedecor, G.W. and W.G. Cochran, 1982. Statistical Methods. 7th Edn., Iowa State University Press, Ames, Iowa, USA.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.