EFFECT OF FEEDING DIFFERENT OIL SOURCES ON CONJUGATED LINOLEIC ACID CONTENT IN EGYPTIAN BUFFALO MILK.

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SUMMARY

onjugated linoleic acid (CLA), a naturally occurring anticarcinogen found in dairy products, is an intermediary product of ruminal biohydrogenation of polyunsaturated fatty acids. The aim of the present study was to determine the effect of different dietary oils, which vary in fatty acid composition, on CLA concentrations in milk of lactating buffaloes. Eight Egyptian lactating buffaloes were assigned randomly into four groups (two animals / each group) using 4x4 Latin square design. Each experimental period lasted for 3 weeks. In each period, buffaloes of each group were fed the same basal diet and received one of the following treatments; $[T_1]$ control (without oil), $[T_2]$ control diet + 2% sunflower oil (SFO), $[T_3]$ control diet + 2% olive oil (OLO), $[T_4]$ control diet + 1% SFO + 1% OLO. All oils supplements were calculated on DM basis then mixed with the concentrate feed mixture. The differences between treatments in milk yield, 4% fat corrected milk and milk composition were not significant; although the highest value of daily milk yield (10.29 kg/day) was detected in buffaloes fed on diet supplemented with olive oil. Buffaloes fed diet supplemented with oils mix produced milk fat cis-9, trans-11 CLA four times higher than control. Supplementation of plant oils tended to improve milk production of lactating buffaloes, and the CLA concentration in milk fat was significantly increased. Clearly CLA contents in milk fat can be enhanced by the addition of polyunsaturated fatty acids to the diet. The results of the present study indicate that other fatty acids might contribute to CLA production.

Keywords: conjugated linoleic acid; milk; buffalo; plant oil

INTRODUCTION

Conjugated linoleic acid (CLA) is a term used to describe a mixture of positional and geometric isomers of linoleic acid C_{18:2} (Aydin, 2005; Stanton et al., 1997), that contain conjugated unsaturated double bonds separated by single carbon-carbon bond instead of a methylene group (Chin et al., 1992; Dhiman et al., 1999 a.b; Parodi., 1999; Dhiman et al. 2000; Donovan et al., 2000; Griinari et al., 2000; Abu-Ghazaleh et al., 2002 and Parodi., 2003).

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CLA is a group of unsaturated fatty acid isomers that occur naturally in foods derived from ruminants, and is found in its highest concentration in bovine milk (Chin et al., 1992; Dhiman et al., 1999a; Aydin et al., 2005).

The CLA is formed as an intermediate during the biohydrogenation of linoleic acid ($C_{18:2}$) to stearic acid ($C_{18:0}$) in the rumen by *Butyrivibrio fibrisolvens* (Kepler *et al.*, 1966) and other rumen bacteria (Kritchevsky, 2000) or from the endogenous conversion of trans-11 $C_{18:1}$ (Transvaccenic acid, TVA), another intermediate of rumen biohydrogenation of linoleic acid or linolenic acid ($C_{18:3}$) by the Δ -9 desaturase enzyme in the mammary gland (Corl *et al.*, 2001; Griinari and Bauman, 1999).

An increasing interest on CLA is attributed to its potential health benefits such as anticarcinogenic, antidiabetic and antiadipogenic effects (Banni et al., 2003; Belury, 2003; Kritchevsky, 2003; Pariza, 1999). Its role on vitamin A metabolism (Carta et al., 2002), bone modeling (Watkins et al., 2003) and immune response (Cook et al., 2003) has also been reported.

Of the two physiologically important isomers, cis-9, trans-11 CLA is the most prevalent one comprising 80 to 90% of total CLA in food products from ruminants, whereas trans-10, cis-12 CLA is present in small amounts at 3-5% of total CLA (Parodi, 2003). Dietary supplementation of plant oils high in linoleic acid gave the greatest response, and there is a clear dose-dependent increase in milk fat content of CLA (Kelly et al., 1998).

The present study was conducted to evaluate the effect of dietary supplementation with sunflower oil, olive oil and their blend on the conjugated linoleic acid (CLA) content as a functional constituent in Egyptian buffalo milk.

MATERIALS AND METHODS

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

Experimental animals:

Eight lactating Egyptian buffaloes aged 5 - 6 years (at the third and fourth lactation) were used in the present study. Live body weight ranged between 473 and 586 kg. Animals were assigned randomly into four groups (two animals / each group) using 4x4 Latin square design. The experimental period was extended for 84 days and consisted of four periods (21 days each). Milk yield was recorded daily.

Experimental rations:

The intended ratio of concentrate to roughage was 60:40 on dry matter (DM) basis. The buffaloes were individually fed according to (Ghoneim, 1967). All supplements were first calculated on DM basis then mixed with the concentrate feed mixture. Concentrates were offered twice daily during milking times at 6:00 am and 6:00 pm, Berseem clover was

offered at 9:00 am, while rice straws was offered overnight. Fresh water was available to the animals all time.

The experimental diets were as follows:

- 1- Control diet was 60% concentrate feed mixture (CFM) without any supplements, 30% Berseem clover and 10% rice straw on DM basis [T_i].
- 2- Control + 2% sunflower oil [T₂]
- 3- Control + 2% olive oil $[T_3]$
- 4- Control + 1% sunflower oil + 1% olive oil [T₄].

Chemical compositions of concentrate feed mixture (CFM), Berseem clover (B) and rice straw (RS) are shown in Table (1).

Table (1): Chemical composition of concentrate feed mixture (CFM), Berseem clover (B) and rice straw (RS), (% on dry matter basis).

74	Diet ingredients			
Item	CFM	В	RS	
Dry matter	91.32	13.28	93.12	
Organic matter	87.58	86.72	84.16	
Nitrogen free extract	54.68	45.85	44.05	
Crude protein	15.20	17.82	3.68	
Ether extract	4.52	2.67	2.21	
Crude fiber	13.18	20.38	34.22	
Ash	12.42	13.28	15.84	

Plant Oils:

The plant oils used in this study are Sunflower oil (SFO) and olive oil (OLO) and were purchased from the Egyptian market. Fatty acids composition of sunflower oil (SFO) and olive oil (OLO) are shown in Table (2).

Milk Sampling:

Animals were hand-milked twice daily at 6:00 a.m. and 6:00 p.m. Milk yield was recorded daily, 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.

Table (2): Fatty acids composition of used plant oils

Fatty Acids	SFO	OLO
Tally Acids —	mg / g	; oil
C16:0	52	84
C16:1	1	8.2
C18:0	32	29
C18:1	148	696.2
C18:2	751.9	162
C18:3	5	8.3
C20:0	2.3	4,5
C20:1	1.6	4.1
C22:0	6.2	2.3
C22:1	-	1.4

Chemical Analysis:

The chemical composition of different feedstuffs samples were analyzed according to the A.O.A.C (2000) while nitrogen free extract (NFE) content was calculated by difference. Milk total solids (TS) content was determined by the Majonnier method according to Laboratory Manual (1949), fat percentage was determined by Gerber method for milk according to British Standard Institution (1951), fat corrected milk (4%) was calculated according to Gaines (1928) equation, Lactose content was determined calorimetrically according to Barnett and Abd El-Tawab (1957). Total protein content was determined by the semi-micro kjeldahl distillation methods according to Ling (1963). Solids not fat (SNF) content was calculated by the difference between total solids and fat content.

Fatty acids profile and Conjugated linoleic acid isomers analysis:

- 1. Milk fat separation: Milk fat was separated for fatty acids analysis according to Luna et al. (2005).
- 2. Fatty acids methylation: Milk Fatty acids were methylated according to Park et al. (2002).
- 3. Chromatographic procedures: Methylated samples were analyzed for Fatty acids and CLA using a Gas Chromatograph (Hewlett Packard GC system 6890; Wilmington, DE) equipped with a flame ionization detector and a CP-7489 fused silica capillary column (100m×0.25mm i.d. with 0.2-µm film thickness; Varian, Walnut Creek, CA). The Gas chromatograph oven parameters, gas variables have been described by Moore et al., (2005). Peaks were identified by comparing the retention times with those of the corresponding standards (Supelco, Bellefonte, PA; Matreya, Inc., State College, PA; Fluka, Purum, PA).

Statistical analysis:

The data were analyzed using general linear method of statistical analysis system (SAS, 2001), Duncan multiple range test (Duncan, 1955) was carried out for separation among means. Data of milk yield, milk composition and milk CLA concentrations were analyzed according to repeated measurements where the model was:

$$Y_{iik} = \mu + T_i + A_K (T_i) + P_i + (TxP)_{ii} + E_{iik}$$

Where:

Y = is the effect of the observation, $\mu = is$ the overall mean, T = is the effect of the treatment. A (T) = the animal within treatment, P = the effect of the period.

TxP =the interaction between treatment and period, E =the experimental error.

RESULTS AND DISCUSSION

Milk yield and composition:

The effects of supplementation of ration with plant oils on milk yield and milk composition are shown in table (3). The differences between treatments in either milk yield or 4% fat corrected milk were not significant; although the highest value of daily milk yield (10.29 kg/day) was obtained in buffaloes fed on diet supplemented with olive oil. Milk fat percentage was decreased for all treatments compared to control group; differences between groups were not significant. The lowest value of % milk fat obtained for treatment 3 (OLO); whereas, milk fat yield showed no significant differences between treatments.

Table (3): Effect of dietary supplementation with sunflower oil, olive oil and their blend on milk yield and milk composition.

	Treatments				
Item	T1 (Control)	T2	T3	T 4	± SE
Milk yield (Kg/head/day)	9.84	10.10	10.29	10.24	0.290
4% FCM (Kg/head/day) Milk Composition	13.42	13.58	13.48	13.63	0.416
Fat %	6.44	6.26	6.04	6.21	0.071
Fat yield (g/head/day)	633.24	632.77	621.36	636.35	0.020
Protein %	4.15	4.15	4.05	4.15	0.030
Protein yield (g/head/day)	410.77	418.70	417.45	423.38	0.014
Lactose%	4.79	4.91	5.06	5.04	0.064
TS%	16.51	16.23	16.14	16.49	0.118
SNF%	10.08	9.97	10.11	10.28	0.071
Ash%	1.02	1.03	1.00	1.10	0.021

Data in the same row was not significantly different.

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

Milk fatty acids and CLA contents:

Effect of dietary supplementation with sunflower oil, olive oil and their blend on the conjugated linoleic acid (CLA) content in milk are shown in table (4). The contents of myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) acids were significantly ($p \le 0.05$) decreased by supplementation with SFO, OLO and their blend. Stearic acid ($C_{18:0}$) was higher when supplemented with olive oil, difference between experimental groups are significant at $p \le 0.05$. Oleic acid ($C_{18:1}$) was significantly ($p \le 0.05$) increased and was relatively higher for diet supplemented with oils mix. Buffaloes fed diet supplemented with oils mix produced milk fat cis-9, trans-11 CLA four times higher than control.

Table (4): Effect of dietary supplementation with sunflower oil, olive oil and their blend on the conjugated linoleic acid (CLA) content in milk.

Different superscripts in the same row means significantly different at P\(\sigma 0.05\) level

Fatty acid	T1 (Control)	T2	Т3	T4	±SE	
	mg/g Fat					
C14:0	164.2ª	139.6°	142.3 ^b	138.2 ^d	3.99	
C16:0	453.6ª	437.6°	439.8 ^b	417.2 ^d	4.91	
C18:0	248.8 ^d	252.5°	260.4ª	255.3 ^b	1.60	
C18:1	98.2 ^d	111.5 ^b	108.3°	121.9ª	3.20	
C18:2	21.5 ^d	34.2 ^b	28.9°	38.8ª	2.43	
C18:3	8.4 ^b	8.3°	8.5ª	8.1 ^d	0.056	
cis-9, trans-11 CLA	5.2 ^d	16.2 ^b	11.6°	20.4ª	2.13	
trans-10, cis-12 CLA	0.1 ^b	0.1 ^b	0.2ª	0.1 ^b	0.016	

Milk fat containing cis-9, trans-11 CLA produced from all plant oil diets was 2-4 times higher than that in control; differences among groups are significant at $p \le 0.05$. Dietary supplementation with plant oils high in linoleic acid gave the greatest response, and there was a clear dose-dependent increase in milk fat content of CLA (Kelly et al., 1998). The two plant oils used in the study were chosen due to their richness with polyunsaturated fatty acids specially in Linoleic acid $C_{18:2}$ in SFO and in monounsaturated fatty acid like Oleic acid $C_{18:1}$ in OLO, Griinari et al. (2000) and Piperova et al. (2002).

The linoleic acid C_{18:2} content in OLO was 162 mg/g of fat which is lower than that in SFO (Table 2), but the *cis-9*, *trans-11* CLA content in milk fat was slightly higher in response to SFO and OLO blend (Table 4), this result may be due to an interaction between the high content of oleic acid C_{18:1} in OLO and richness of SFO with linoleic acid.

Milk fat contains between 3 and 6 mg of CLA/g of fat, but the levels of CLA in milk can vary widely 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.

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Previous work has suggested that the biohydrogenation 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

The results of the present study indicate that conjugated linoleic acid content in milk of lactating buffaloes can be increased via dietary manipulation. The greatest CLA content was observed when the mix of sunflower oil and olive oil was added to the diet. CLA concentration averaged four times higher in milk fat of T4 than that observed in control. Under the Egyptian situation, buffalo is the first milking animal and its milk is preferred due to its high fat content, which may contain the highest CLA yield compared to other species. The addition of plant oils rich in unsaturated fatty acid, particularly linoleic acid markedly enhanced CLA content in Egyptian buffalo milk fat.

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تأثير التغنية على مصادر غنية بحمض اللينوليك على محتوى حمض اللينوليك المرتبط في لبن الجاموس المصرى.

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حمض اللينوليك المرتبط (CLA) هو مركب ذو فعل مضاد للسرطان موجود بصورة طبيعية في منتجات الالبان المختلفة، وهو عبارة عن مركب وسطى ينتج من الهدرجة الحيوية للأحماض الدهنية عديدة عدم التشبع داخل كرش الحيوان. الهدف من هذا البحث هو تحديد مدى تأثير. اضافة زيوت مختلفة في تركيبها من الأحماض الدهنية الى الأعلاف الحيوانية وذلك على محتوى حمض اللينوليك المرتبط CLA في البان الجاموس الحلاب.

ثمانية من الجاموس الحلاب تم توزيعهم عشوانياً على اربع مجموعات (حيوانين لكل مجموعة) وذلك باستخدام تصميم المربع اللاتيني 4x4حيث كل مرحلة تجريبية امتنت اثلاثة اسابيع. في كل مرحلة يحصل الجاموس على احد هذه المعاملات; معاملة 1) عليقة الكونترول بدون اضافة اى زيوت، معاملة 2) عليقة الكونترول + 2% زيت عبلا الشمس، معاملة 3) عليقة الكونترول + 1% زيت عباد الشمس بالشمس، معاملة 3) عليقة الكونترول + 1% زيت عباد الشمس بالشمس المادة الجافة المأكولة ثم تخلط جيدا بمخلوط الإعلاف المركزة.

الفروق بين المعاملات في كل من انتاج اللبن، اللبن المعدل لنسبة دهن 4% و نسبة الدهن كانت غير معنوية، وأعلى قيمة لإنتاج اللبن وجدت في الجاموس الذي تغذى على العليقة الكونترول مضافاً اليها زيت الزيتون. الجاموس في المعاملة 4 نتج عنه اربع أمثال كمية مشابه حمض اللينوليك المرتبط cis-9, trans-11 وذلك بالمقارنة بالكونترول.

إضافة زيوت نباتية الى علائق الجاموس الحلاب تميل الى تحصين انتاج اللبن بالإضافة الى زيادة معنوية فى إنتاج حمض اللينوليك المرتبط وكان من الواضح ان محتوى الألبان من حمض اللينوليك المرتبط يمكن زيادته عن طريق إضافة مصادر غنية بأحماض عديدة عدم التشبع الى العلائق, ونتائج هذا الدراسة تشير الى ان أحماض دهنية اخرى قد تدخل فى عملية إنتاج حمض اللينوليك المرتبط.