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Conjugated linoleic acid in buffalo (*Bubalus bubalis*) milk from Argentina

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Conjugated linoleic acid (CLA), a naturally occurring anticarcinogen found in dairy products, has received increasing attention in recent years. Twelve lactating Murrah river buffaloes (*Bubalus bubalis*) were used to study profile long-chain fatty acids in milk fat. Buffaloes were fed with natural pasture *ad libitum*. Milk samples were collected in the morning. Animals were separated by stage lactation (3–4 months). The composition of milk samples and fatty acids profile was analyzed. Milk was refrigerated, homogenized and pasteurized for evaluating any possible effect of treatment on cis9, trans11 octadecadienoic acid (CLA) level. Results show that 59% of long chain fatty acids are saturated. C16:0 was the most abundant fatty acid in buffalo milk and C18:1 the major unsaturated fatty acid. CLA level in buffalo milk was 4.83 mg/g of fatty acid methyl ester (FAME). There was a positive correlation between CLA and trans-11 C18:1 vaccenic acid. Any treatment of buffalo raw milk modified significantly CLA content or other fatty acids. Milk fat content of trans11 C18:1 and CLA were closely related (r=0.90). A high quality of buffalo milk was obtained after a diet based only on cultured of pasture carried out in Northwest Argentine.

Konjugierte Linolsäure in argentinischer Büffelmilch (*Bubalus bubalis*)

Konjugierte Linolsäure (CLA), ein natürlich vorkommendes Antikarzinogen in Milchprodukten, findet in den letzten Jahren zunehmende Aufmerksamkeit. 12 laktierende Murrah-Flussbüffel (*Bubalus bubalis*) wurden zur Untersuchung des Profils der langkettigen Fettsäuren in Milchfett eingesetzt. Die Büffel erhielten natürliches Weidefutter *ab libitum*. Milchproben wurden morgens genommen. Die Tiere wurden nach Laktationsstadien (3-4 Monate) getrennt. Die Zusammensetzung der Milchproben und das Fettsäurenprofil wurden analysiert. Die Milch wurde gekühlt, homogenisiert und pasteurisiert, um jede mögliche Auswirkung der Behandlung auf die cis9, trans11 Octadecadiensäure (CLA)-Level festzustellen. Die Ergebnisse zeigen, dass 59% der langkettigen Fettsäuren gesättigt waren. C16:0 war die häufigste Fettsäure in Büffelmilch, C18:1 die wesentliche ungesättigte Fettsäure. Der CLA-Level in Büffelmilch betrug 4,83 mg/g Fettsäure-Methylester (FAME). Es bestand eine positive Korrelation zwischen CLA und trans-11 C18:1 Vaccensäure. Jegliche Behandlung der Büffel-Rohmilch veränderte signifikant den CLA-Gehalt oder den anderer Fettsäuren. Die Milchfettgehalte von trans11 C18:1 und CLA hatten einen engen Bezug (r=0,90). Eine hohe Büffelmilchqualität wurde bei einer Fütterung ausschließlich auf Kulturweideland in Nordwest-Argentinien erzielt.

38 Buffalo milk (conjugated linoleic acid) **38 Büffelmilch** (konjugierte Linolsäure)

1. Introduction

Conjugated linoleic acid (CLA) is a term used for a mixture of positional and geometric isomers of linoleic acid in which double bonds are conjugated. CLA is formed like biohydrogenation intermediate produced in rumen during hydrogenation of polyunsaturated 18-atom fatty acids. CLA has been reported to prevent carcinogenesis (1, 2), atherosclerosis (3) and modulate immune activity (4). There are many CLA isomers, being cis9, trans11 and t10, cis12 the most biologically effective. The first microorganism discovered which produced CLA was anaerobic ruminal bacterium, *Butyrivibrio fibrisolvens* (5). However, major source is endogenous synthesis of CLA in body tissues. The specific enzyme catalyzing endogenous synthesis is Δ^9-desaturase being trans11 C18:1 principal substrate (6). Meat and milk of ruminant animals are the richest sources of CLA, which contain cis9, trans11 isomer as major CLA. The effect of CLA is observed to minimal dosis (6). CLA milk content is diet-dependent pasture producing higher CLA levels than silage or grain (7). Dairy products at high levels of conjugated linoleic acid are promising as functional foods due to numerous health benefits. There are no published data on fatty acids profile in Murrah buffalo milk, especially on the CLA level. All data existing are of Italian river buffalo (8, 9), the principal Mozzarella cheese producer. In Argentine, where buffalo was introduced recently, there is not reported CLA milk content. In Northwest Argentina (Tucumán) natural pasture is very important as cattle-food. The aim of this study was to determine Murrah buffalo milk composition and fatty acids profile, specially CLA concentration, in pastured-feeding animals.

2. Materials and methods

2.1 Reagents and standards

Fatty acids methyl esters (FAME) from C14:0 to 18:3 and anhydrous sodium sulfate were obtained from Sigma Chemical Co. (St. Louis, MO) (All 99% pure). c9, t11 octadecadienoic acid was used as CLA standard. All solvents used were HPLC grade (E. Merck AG, Darmstadt, Germany).

2.2 Milk Samples and analyses

Twelve lactating Murrah buffaloes were separated according to lactation period (3–4 months). All animal used in this study were multiparous. Milk samples were collected at morning (7 a.m.), during autumn season. Milk composition (fat, protein, lactose, total solids and not fat solids) was determined by automated infrared analysis using Milkoscan apparatus (Foss Electric, Denmark).

2.3 Lipid extraction

Lipids milk was extracted using chloroform/methanol 2:1 (v/v) according FOLCH procedure (10), and derivatized to methyl ester (FAME) according to CHIN *et al.* (11). Pentadecanoic acid was used as internal standard. Fatty acid methyl esters were analyzed by GC. GC analysis was carried out by using an Agilent Technologies Model 6890N gas chromatograph equipped with a flame ionization detector and a model 7683 automatic injector with a 10 µl syringe.

One µl of fatty acid preparation was injected to a HP-5 capillary column (30 m x 0.32 mm i.d. x 0.25 µm of thickness). GC conditions were injector temperature 250 °C; initial oven temperature 50 °C was increased to 150 °C at 20 °C/min and held for 50 min, then increased to 225 °C at 10 °C/min and held for 20 min; and detector temperature, 250 °C. Carrier gas nitrogen was used. Fatty acids were identified by comparison of the retention times with the methylated standards.

2.4 Treatment of milk

Aliquot of milk samples were refrigerated (4 °C) during 3 h after collecting samples. Other aliquot was homogenate and other was pasteurized to low temperature (62.3 °C for 30 min). Lipids in each treatment were extracted according above method, and compared with control. Duplicated samples were processed for each animal and treatment.

2.5 Statistical analysis

All data obtained were statistically analyzed by ANOVA test. Significant level was $p<0.05$. The results are expressed as means ± standard deviation.

3. Results and discussion

Data on milk composition are shown in Table 1. Fat content of buffalo milk was 8.73% and protein was 4.35%. These values were little higher than SPANGHERO and SUSMEL reported (12) and little lower than those found by POLIDORI (9). Total solids (TS) and not fat solids (NFS) were elevated, too.

Results indicated that 59% of long-chain fatty acids in buffalo milk are saturated: the predominant fatty acid was palmitic (C16) followed by stearic (C18). (Table 2). Between unsaturated (41%) the oleic is higher than others fatty acid. The elevated content in polyunsaturated fatty acid is remarkable, linoleic acid being the most abundant (16.46 mg/g of FAME), which due to pasture has high C18:2 content.

Milk CLA content was 4.83 mg/g of FAME. There is not large variation in CLA level in milk fat from individual buffalo. The range of variation obtained was 3.2 to 6.5 mg/g of FAME. Many factors can influence CLA level and by manipulating animal diet it is possible increase CLA in milk (13, 14, 15). Seasonal factors (16) and lactation number (17) have influence, too. The mean concentration of vaccenic acid (VA) in the milk fat was 39.51 mg/g of FAME and ranged for individual samples from 24.3 to 56.7 mg/g of FAME.

There was a positive correlation between CLA and VA content in milk fat ($r=0.90$) (Fig. 1). A strong positive relation between CLA and VA was observed previously in cows milk fat (18) which suggest that the first two steps in the biohydrogenation of linoleic to stearic acid were not rate limitating. Relationship between VA and CLA is possible due to relation between precursor-product for endogenous synthesis of CLA. Endogenous synthesis occurred by the following reaction:

$$\text{Trans11 C18:1 (VA)} \xrightarrow{\rho^9\text{-desaturase}} \text{cis 9, trans 11 C18:2 (CLA)}$$

This metabolic pathway and relation between CLA and VA was studied by PALMQUIST (19) in cows milk.

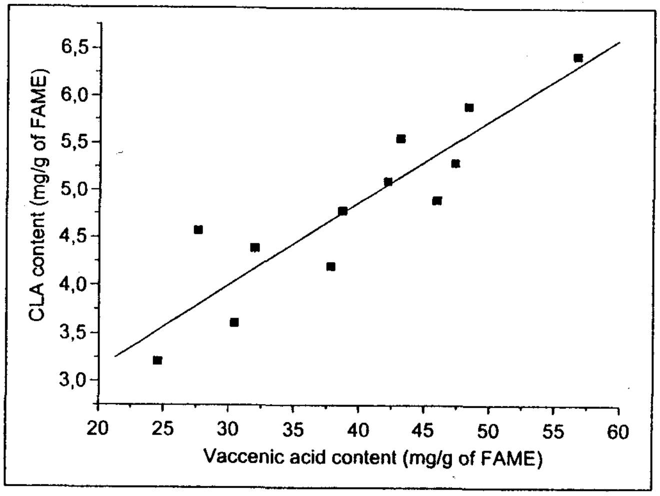

Fig. 1: Relationship between CLA and VA concentration in the milk fat of dairy Murrah buffalo fed with pasture. CLA = 1.38 + 0.087 (VA), r = 0.90.

Table 1: Milk composition of Murrah buffalo in North-west Argentine

Buffalo milk composition	Value[1]
Proteins (%)	4.35±0.22
Lipids (%)	8.73±0.71
Carbohidrate (%)	4.75±0.61
TS (%)[2]	19.04±1.12
NFS (%)[3]	9.68±1.09
pH	6.61±0.10

[1]Means of duplicate sample analysis ± standard deviation. [2]TS: Total Solids. [3]NFS: Not Fat Solids

Table 2: Profile of long chain fatty aids of Murrah Buffalo's milk

Fatty acid	Concentration[2]	SD[1]
C14:0	68.01	10.99
C14:1	5.04	1.30
C15:0	5.83	1.50
C16:0	319.41	44.08
C16:1	10.24	6.67
C17:0	4.39	2.08
C18:0	111.49	24.80
C18:1 trans11	39.51	9.53
C18:1 cis9	271.56	27.85
C18:2	16.46	3.41
C18:3	5.04	1.30
CLA[3]	4.83	0.92

[1]SD: standard deviation. [2]Means of duplicate sample analysis (mg/g of FAME). [3]All conjugated linoleic acid isomer

Treatment effects on milk fatty acid composition are shown in Table 3. Any treatment caused significantly variation on CLA level ($p>0.05$) or another fatty acids. Fatty acid profile was similar in all cases and relationship between CLA and VA is similar too (data not shown). These results are very important because of it can be possible processing raw milk without interfering fatty acid profile of milk.

Table 3: CLA and VA Content in different treatment of raw milk

FAME[1]	Control	Refrigeration	Homogenization	Pasteurization
CLA	4.83±0.92	4.51±1.31	4.62±1.22	4.63±1.09
VA	39.51±9.53	38.65±8.71	36.41±10.23	36.13±9.37
P		>0.05	>0.05	>0.05

[1]Means of fatty acid methyl ester (mg/g of FAME)

High fat and elevated CLA contents and increased percentage of yield in buffalo milk are important characteristics to make dairy products which can be used as functional foods. Our study shows that buffalo can be a good alternative for northwest Argentine where pasture is very important as cattle-food. So that, regional elaboration of dairy products could be possible. There is another method to increase CLA level moreover modifying animal diet. Many bacteria used in dairy industry are shown to have isomering effect on linoleic acid (20, 21). It might be possible to use bacteria as additive in food that cause a good effect on human health (22) and elaborate a probiotic food. The addition of probiotic cultures in yogurt or cheese production can be a viable alternative to modify somewhere fatty acid profile and to increase CLA absorption by human.

Many studies were carried out with Mediterranean buffalo milk in Italy, but very little is reported concerning Murrah buffalo milk. We found higher levels of C18:0 and C18:1 than POLIDORI (9) who reported 95.1 mg/g of FAME for C18:0 and 251.6 for C18:1 and we have found 111.5 and 271.5 mg/g of FAME, respectively (Table 2). Values of C16:0 and C18:2 were similar in both studies.

There are no published data about CLA level in buffalo milk. CLA content in our study was very close to that of WHITE *et al.* (7) on Jersey cows fed pasture, who reported values of 5.9±0.5 mg/g of FAME.

Most studies about CLA values were done in cow's milk, but not in other ruminant species. This study reported CLA concentration in buffalo milk fed only natural pasture in a small farm of Northwest of Argentine. In our country most buffaloes are Murrah; for this reason it is important to study their milk composition.

The nutritional value of buffalo milk as an important source of energy and high quality protein has been probed in our study. Buffalo milk has higher fat than other ruminant species, making it an exceptional product for dairy industry. The results of the present study showed that it is possible to obtain a good buffalo milk quality in Northwest Argentine feeding animals only with natural pasture. As no significant treatment effect is observed, milk can be processed to dairy products without affecting CLA content.

The screening of lactic acid bacteria able to isomer linoleic acid to CLA in food elaborate from buffalo milk is in progress.

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