Chemical and microbiological characteristics of Llama's (*Lama glama*) milk from Argentina

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The objective of this study was to evaluate the chemical characteristics, fatty acid composition and microbiological quality of llama's (*Lama glama*) milk from Tucumán, Argentina. Good nutritional properties were determined showing a value of 4.33 % for protein, 6.34 % for lactose and 4.55 % for fat. Saturated, monounsaturated and polyunsaturated fatty acids (FA) showed a value of 64.8, 31.1 and 4.0 g/100g of fat, respectively. Among polyunsaturated FA, conjugated linoleic acid (CLA) showed an average of 0.7 g/100 g of fat. Cholesterol content in llama milk was 84.8 µg/ml resulting lower compared with ruminants. For mesophillic microorganism microbiological counts revealed a value of 4.34×10^3 CFU/ml. A dominance of cocci (2.18×10^3 CFU/ml) on Lactobacilli (3.0×10^1 CFU/ml) was observed. Low coliform and *Enterobacteriaceae* counts were determined (4.4. and 1.6×10^1 CFU/ml, respectively). Somatic cell count was low compared to ruminant milk. High nutritional and microbiological quality was determined for llama milk, from animals fed on natural pasture during summer season.

Chemische und mikrobiologische Eigenschaften von Lama-(*Lama glama*) Milch aus Argentinien

In der Studie sollten die chemischen Eigenschaften, die Fettsäurenzusammensetzung und die mikrobiologische Qualität von Lama-Milch aus Tucuman in Argentinien, analysiert werden. Gute ernährungsphysiologische Eigenschaften wurden ermittelt mit Werten von 4,33% für Eiweiß, 6,34% für Laktose und 4,55% für Fett. Die gesättigten, einfach ungesättigten und vielfach ungesättigte Fettsäuren ergaben Werte von 64,8, 31,1 bzw. 4,0 g/100 g Fett. Unter den vielfach ungesättigten Fettsäuren hatte die konjugierte Linolsäure (CLA) einen Durchschnittswert von 0,7 g/100 g Fett. Der Cholesterolgehalt von Lama-Milch betrug 84,8 µg/ml und lag damit niedriger im Vergleich zu Wiederkäuermilch. Bei den mesophilen Mikroorganismen lagen die Gesamtkeimzahlen bei $4{,}34 \times 10^3$ KbE/ml. Dabei dominierten Kokken ($2{,}18 \times 10^3$ KbE/ml) und Laktobazillen ($3{,}0 \times 10^1$ KbE/ml. Die Zahlen an coliformen Keimen und Enterobacteriaceen waren mit 4,4 bzw. $1{,}6 \times 10^1$ KbE/ml niedrig. Auch der somatische Zellgehalt lag im Vergleich zu Wiederkäuermilch niedrig. Somit ergaben sich gute Werte für die ernährungsphysiologische und mikrobiologische Qualität der Lama-Milch bei Haltung der Tiere auf natürlichen Weiden während der Sommersaison.

38 Llama milk (chemical and microbiological characteristics)

38 Lama-Milch (chemische und mikrobiologische Eigenschaften)

1. Introduction

Llama (*Lama glama*) is a domestic South American camelid belonging to Artiodactyla Order (Tylopoda). There are among 3.78 millions of llamas in South America, distributed among Columbia, Bolivia, Perú and Argentina (8). The multiple purpose of llama includes meat and fiber production, being the main specie of the South American camelid by their abundance. This animal has an important role in the economy and culture of many South American countries.

Llama's milk characteristics have not been thoroughly investigated in Argentina, especially in Northwest (Tucumán) where many people of the mountain zone keep llama for meat and fiber production. Authors only reported the protein, fat and carbohydrates content (6). Moreover, data on milk composition from other countries are limited (23, 38) and no data are available for fatty acid composition of new world camelids.

Knowledge about milk composition facilitates the nourishment of nursing llamas (crias) when a supplemental feeding is required and it helps for a better understanding of the nutrient requirement of the dam too.

Fatty acid composition of milk fat has much interest by its implication on human health. Unsaturated fatty acids are healthier than saturated fatty acids. Among polyunsaturated the conjugated linoleic acid (CLA) content is of interest. This compound is found in ruminant milk and is formed during ruminal biohydrogenation or by a desaturase activity in the mammary gland. CLA represents a mixture of positional and geometrical isomers of linoleic aid with conjugated double bonds. The main isomer present in milk fat is *cis*9, *trans*11 and it has important physiological functions such as anticarcinogenic acitivity (12; 19), atherosclerosis inhibition (18), immuno-modulation (16) and hypocholesterolemic action (25). Animals grazing on natural pasture have more CLA content than others (20; 21).

Camelids are not true ruminants, but they have a stomach with three compartments (C1-C3) with similar functional properties of ruminant's stomach.

South America is the main recognized agricultural entity for domestic camelids (llama and alpaca). In Northwest Argentina, in the mountain region, many farms are dedicated to camelid herding and people of the zone often consume llama milk as an alternative food.

In the absence of reliable data on llama milk composition, the aim of this work was to determine the chemical and microbiological characteristics and fatty acid composition of llama, obtained after a feeding based on natural pasture.

2. Materials and methods

2.1 Milk samples

Ten llamas (n=10) were used in the present study, belonging to a regional farm of the mountain zone at 2300 m ("La Carolina"). Animals were among 2 to 4 months of lactation period. Milk samples were collected in the morning by hand milking. Teats were water washed and dried with paper towels. Mammary glands were visually examined and palpated for lesions or abnormal consistency. The first milliliters were discarded and the rest was collected in sterile flasks. Samples delivered by refrigerated transport (4°C) to our laboratory were immediately processed or maintained at -20 °C until analysis.

2.2 Chemical analysis

Milk samples were analyzed for proteins, no fat solids (NFS), fat and density by ultrasound method (EKO-MILK analyzer, Milkana Kam 98-2A). Lactose was determined by the UV lactose/galactose method (Boehringer Mannheim, Germany). Total solids (TS) were analyzed according to IDF-FIL (10). Somatic cells were counted according to the method recommended by IDF-FIL (11) using milk immediately after its arrival to our laboratory; pH was measured by Metrohm peachimeter (model 692, Herisau, Switzerland) and acidity by titulation of fresh milk with NaOH (2) expressed as Dornic grade (°D). Water was determined by difference (100 – TS), so as ash (NFS - [protein+ lactose]).

Cholesterol content was measured by enzymatic kit (Colestat, Wiener Lab., Rosario, Argentina). Gross energy (GE) was determined using the following equation according to Perrin (27):

$$GE\ (MJ/100g) = 39.8\ (fat\%) + 23.9\ (protein\%) + 16.7\ (lactose\%)$$

2.3 Microbiological analysis

To analyze the microbiological quality of fresh milk, aliquots of 1 ml were taken and decimally diluted in sterile 0.1 % (w/v) peptone water (Sigma) and plated in duplicate on selective media.(i) Mesophillic microorganisms were determined on Plate Count Agar (PCA, Sigma) incubated aerobically at 30°C for 48 h (ii) *Lactobacilli* on MRS agar (5) were acidified with acetic acid down to a pH of 5.5 and were anaerobically incubated at 37°C for 48 h (iii) Total Cocci on LAPTg agar, anerobically incubated at 37°C for 48 h (iv) M17 agar was used for Lactococci enumeration after anaerobically incubation at 37°C for 48 h. (v) Determination of total coliform microorganisms was carried out on Mac Conkey agar incubated aerobically at 30°C for 48 h and *Enterobacteriaceae* (vi) on Violet Red Bile agar at 37°C for 24 h. (vii) The counting of yeasts was determined on potato dextrose agar incubated at 30°C for 72-96 h. Results were expressed as CFU/ml.

2.4 Lipid analysis

Lipids were extracted using chloroform/ methanol solution (2:1, v/v) according to FOLCH *et al.* (7). Fatty acid methyl esters (FAME) analyses were performed according to CHIN *et al.* (4), using HCl methanolic solution.

One microlitre of fatty acid methyl esters, dissolved in hexane, was injected to an Agilent Technologies gas chromatograph (Model 6890N, USA) equipped with a flame ionization detector and automatic injector (Model 7683, USA) into a HP-88 capillary column (100 m x 0.25 mm x 0.20 µm, Agilent Technologies, USA). GC conditions were: injector temperature, 255 °C; the initial oven temperature of 75°C was increased to 165°C at 8°C/min and held there for 35 min, increased to 210°C at 5.5°C/min and held for 2 min and then increased to 240°C at 15°C/min and held for 3 min. Detector temperature was 280°C. Nitrogen was used as carrier gas at flow rate of 18 ml/min, at 38 psi. Fatty acids were identified by comparison of retention times with the methylated standards (Sigma, St. Louis, MO, USA). CLA and fatty acids methyl esters were identified and quantified by comparison with the retention times and peak areas of Sigma standards. Results were expressed as g/100 g of fatty acids

2.5 Somatic cells count

Somatic cell count was performed in a Zeiss microscopeusing the technique described by the International Dairy Federation (11). Samples were heated in a water bath to 30-40°C. Then, 0.01 ml of milk sample was placed on a clean slide in a 1 cm^2 area. It was dried and dipped in dye solution for 30 min, dried again, dipped in tap water until all surplus dye was washed away and dried again.

2.6 Statistical analysis

All samples were analyzed at least in duplicate. All data were statistically evaluated by variance analysis (ANOVA) test (Minitab® Release 14 Statistical Software, 2003 Minitab Inc.). Differences were considered significant at $p < 0.05$. Samples were separated by two lactation periods (28): 4 from 50-60d and 6 from ≥61 d. There were no significant differences between both lactation groups and data were analyzed as pool. Results were expressed as mean ± standard deviation (SD).

3. Results

3.1 Chemical composition of Llama milk

The chemical properties of llama's milk are shown in Table 1. Fat content was 4.55 ± 0.66 %, similar to data reported by other authors (6; 28). Other authors reported values of 2.7 % (23) and 5.6 % (13).

Table 1: Chemical characteristics of milks

	Value	Minimum	Maximum
Protein	4.33±0.17	4.18	4.67
Fat	4.55±0.66	3.82	5.60
Lactose	6.34±0.34	5.90	6.71
TS	16.32±0.76	15.2	17.3
NFS	11.7±0.47	11.2	12.3
Ash	0.88±0.15	0.70	1.10
pH	6.80±0.07	6.74	6.92
Acidy (°D)	15.4±0.89	13.5	16.3
Water	83.7±0.7	82.7	84.8
SCC (cells/ml x1000)*	130.9±47.8	41.6	194.1
Density	1.038±0.00	1.036	1.042
Gross energy (MJ/100g)	395.6±32.2	355.9	432.8
Cholesterol (µg/ml)	84.8±27.1	48	124

Values are expressed as mean ±SD of the duplicate analysis (n=20). : Samples were processed five (n=50).

Protein content was 4.33 ± 0.17 %, similar to results by FERNÁNDEZ and OLIVER (6). Lower protein level was

reported for llama's milk, varying from 3.4 to 3.9 % (23, 28).

Lactose content amounted to 6.34 ± 0.34 %. According to previous data Lactose varied from 5.7 to 6.5 % (6; 23; 28), and our results are in between.

Total solids present an average of 16.32 ± 0.76 %, similar to previous studies (28).

Llama's milk has a higher pH value than ruminants (30) and in our study reached 6.80 ± 0.07. Dornic acidity was 15.4 ± 0.89 and density 1.039 ± 0.002.

According to literature, llama's milk yield is around 2.5 Kg/d (29). In the present study milk yield was not determined, but data reported by other authors allow to compare llama milk production with that of sheep.

Gross energy (GE) showed a value of 395.6 ± 32.2 MJ/ 100g and this result is in agreement with the value determined by RIEK and GERKEN (28).

Cholesterol content was 84.8 ± 27.1 µg/ml. This result is lower than that of cow and sheep milks where it varied from 102 to 196 µg/ml (9; 32).

3.2 Fatty acid analysis

Fatty acid (FA) composition is shown in Table 2. Saturated FA content was 64.8 g/100 g FA, being palmitic (C16.0), miristic (C14:0) and stearic (C18:0) the most abundant. Among monounsaturated (31.1 g/100 g FA), oleic acid (C18:1 *cis*9) was predominant. Polyunsaturated FA showed a value of 4.0 g/100 g FA. Conjugated linoleic acid (CLA) was determined among PUFAs showing a value of 0.7 g/100 g FA. *Cis*9, *trans*11 was the only isomer present. To our knowledge no data exist about FA composition or CLA presence in llama's milk. However, CLA content in ruminant milk varied from 0.3 to 0.9 g/100 g FA (4; 26; 34).

Among *trans* FA, vaccenic (C18:1 *trans* 11) showed a level of 3.3 %, and it could serve as substrate for CLA production in the mammary gland. The CLA:VA ratio determined in this study is shown in Fig. 1. Correlation coefficient was r=0.69, lower than given for cow and buffalo milk (15, 17, 34).

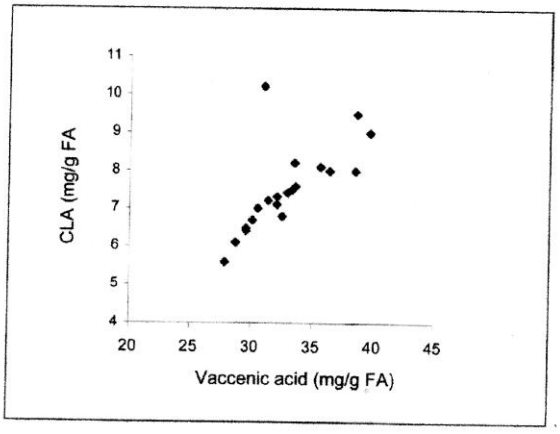

Fig. 1: CLA:VA ratio in llama milk

The literature indicates that digestive efficiency of camelids increases at higher altitudes (22, 31). Moreover, camelids are able to produce rumia process and have higher ability to degrade forage with low nutritional compound than ruminants. In our study a high short chain FA concentration was found, being similar to ruminant species.

3.3 Δ^9-Desaturase activity in mammary gland and atherogenicity index

The mammary gland of ruminants has substantial Δ^9-desaturase activity, enzyme which can be measured indirectly by comparing the product:substrate ratio of certain FA. Therefore, C14:1/ C14:1+C14:0 ratio is the best indicator because all C14:0 in milk fat comes from de novo synthesis in the mammary gland (21).

In the present study the C14:1/ C14:1+C14:0 ratio was determined as desaturase index (Table 2). Δ^9-desaturase index was 0.27. Lower values were determined by LOCK and GARNSWORTHY (20) in cow's milk, reporting an average value of 0.072.

Table 2: Fatty acid composition of llama Milk

Fatty acid (g/100 g FA)	Mean	SD[b]
C4:0	1.9	0.3
C6:0	2.4	0.5
C8:0	2.1	0.6
C10:0	2.5	0.3
C 12:0	1.5	0.2
C12:1	0.9	0.2
C13:0	0.7	0.1
C14:0	10.5	2.4
C14:1	3.9	0.6
C15:0	1.5	0.3
C16:0	28.6	2.0
C16:1	6.5	1.0
C17:0	2.4	0.2
C18:0	9.6	1.3
C18:1 *trans*-11	3.3	0.5
C18:1 *cis-9*	16.1	1.6
C18:2	1.3	0.3
C18:3	1.0	0.2
CLA *cis*-9,*trans*-11	0.7	0.2
C20:0	1.1	0.3
C20:1	0.3	0.1
C22:4	1.0	0.2
SCFA	6.4	
MCFA	21.5	
LCFA	71.9	
Saturated	64.8	
Monounsaturated	31.1	
Polyunsaturated	4.0	
Δ^9-Desaturase activity	0.27	
Atherogenicity index	2.06	

[a]Standard deviation. Samples were done in duplicate (n=20). SCFA: short chain fatty acids (C4-C8); MCFA: medium chain fatty acids (C10-C15); LCFA: long chain fatty acids (≥C16).

The atherogenicity index was calculated by using the following formula:

C12:0+4xC14:0+C16:0/MUFA+PUFA (3)

and characterizes the atherogenicity of dietary fat. Foods with high atherogenicity index are considered more detrimental for human health. In llama, the atherogenicity index was 2.07. CHILLIARD *et al.* (3) informed different values for goat's milk according to feeding and our results are in the mediuml reported.

3.4 Microbiological composition

Microbiological composition of milks is shown in Table 3. Mesophillic microorganisms were dominant in llama milk (4.34 x 10^3 CFU/ml). This result is in accordance with other ruminant milk of the region (14).

Coliform microorganisms showed a low count with a

value of 4.04 x 10^1 CFU/ ml for total coliform count and 1.6 x 10^1 CFU/ ml for the *Enterobacteriaceae* group.

Among lactic acid bacteria, a dominance of Cocci on Lactobacilli was determined (2.18 x 10^3 and 3.0 x 10^1 CFU/ml, respectively), Lactococci count being very low (0.94 x 10^1 CFU/ml). Yeast showed a value of 1.96 x 10^2 CFU/ ml.

Table 3: Microbiological counts of llama milk

Microorganism (CFU/ml)	Mean	SD
Aerobic mesophillic	4.34 x 10^3	1.77
Total coliform	4.04 x 10^1	1.19
Enterobacteriaceae	1.6 x 10^1	0.87
Total cocci	2.18 x 10^3	0.96
Lactococci	0.94 x 10^1	0.22
Lactobacilli	3.0 x 10^1	0.41
Yeast	1.96 x 10^2	0.43

3.5 *Somatic cells count*

Our results showed a SCC of 130.9 ± 47.8 x 1000 cell/ml. Other authors determined lower values (37.0 x 1000 cell/ml) in llama's milk (28). SCC varies in milk according to many factors, such as animal health, milking process and geographic site. Moreover, SCC varies among different ruminant species. In healthy cow's milk SCC are from 170.0 to 220.0 x 1000 cell/ml (1). For goat this value increases reaching values of 1.3 x 10^6 cell/ml in healthy animals (24).

In spite of the higher value of SCC reported by other authors, no mastitis was determined by teat palpated in the present work, and was confirmed by microbiological analysis of milk (coliform enumeration).

4. Conclusion

In the present study the chemical characteristics, FA composition and microbiological quality of llama's milk were determined. Samples were obtained from farms from northwestern Argentina where llama is raised for both meat and fiber. Llama milk showed good nutritional and microbiological quality. Among fatty acids, CLA was determined as *cis*9-*trans*11 isomer. The atherogenicity index of llama milk was lower than that of other ruminants so as cholesterol content. Our results fall within the range of the observed gross composition of llama milk during different lactation periods (28). All characteristics analyzed allow understanding the nourishment requirements of the cria (teke) and the dam. The quality of milk, used for human consumption in regions with high malnourishment index, was determined in the present study.

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