Gross, mineral and fatty acid composition of alpaca (*Vicugna pacos*) **milk at 30 and 60 days of lactation**

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ABSTRACT

The aim of this study was to investigate the composition of alpaca milk in order to improve knowledge of the nutritional needs of crias during their first months of life. Analyses of alpaca milk were performed in terms of chemical, mineral composition and fatty acid profile during the first two months of lactation. Percentages of fat, protein, casein and ash did not change in the first two months of lactation. Alpaca milk showed a similar protein content to sheep and camelid milk such as llama (*Lama glama*), lower casein content compared to ruminants, and similar fat percentages to cow and goat milk. The Ca and P content was similar to cow milk. Concerning the fatty acid profile, alpaca milk had higher conjugated linoleic acid (C18:2 cis 9, trans 11) and unsaturated fatty acid contents than ruminant milk, and a low percentage of fatty acids with chains <C14: 0. In fact, during the first months of life, alpaca offspring may not sufficiently exploit these fatty acids. Ruminant milk cannot be considered as an ideal surrogate for the nutritional needs of crias.

Keywords: Alpaca, crias, milk quality, milk feeding, fatty acids.

1. Introduction

Alpacas (*Vicugna pacos*) are camelids originating from the highlands of Peru, Chile and Bolivia, which are over 3800 meters above sea level (Parraguez et al., 2003).

South American camelids are classified in the order Artiodactyla, suborder Tylopoda, and family Camelidae, but are subdivided into Lamini and Camelini at the tribe level. Two New World genera, Lama and Vicugna, and one Old World genus, Camelus, are recognized. They are considered as 'pseudoruminants' since they have a stomach with three compartments rather than four, with similar functional properties to ruminant stomachs (Wheeler, 2012)

The alpaca is the smallest of the South American camelids: llama (Lama glama), guanaco (Lama guanicoe) and vicuña (Vicugna vicugna). While llamas are mainly used as pack animals in the areas of origin, and guanaco and vicuña live mainly in the wild (Pollard and Pollard, 2008), alpacas continuously attract business interest in farmers, including those far from their country of origin.

In fact, since the 1980s, alpacas have been exported from South America to other continents including Europe where they are reared primarily for their wool. These animals seem to adapt well to different environments (McGregor, 2002).

Research on alpaca has focused principally on the study of the wool and meat, and to date information on milk production and composition is scarce and partial. In fact, unlike other pseudoruminants such as camels, historically they have not been bred for dairy purposes (Medhammar et al., 2011).

Alpaca milk is used almost exclusively for feeding their offspring (crias). Crias double their weight in the first 60 days of life, when they are largely dependent on dam's milk to meet their nutritional needs (Chad et al., 2014). The milk is fundamental to satisfy the nutritional needs of crias during their first months of life, since feeding is one of the most important factors in production systems and is necessary for expressing the animal's production potential. Currently, there is a shortage of data on the amount of nutrients needed for optimal weight gain in alpacas. Although recommendations for energy requirements in llama have been published (National Research Council, 2007), they are limited to lactating llamas and are based on extrapolations from sheep and goat data.

In the event of the death of the dam with the subsequent low availability of milk and / or difficulty with sucking, it may be necessary to integrate the milk for crias. In these cases, cow or goat milk or formulas for lambs or zoo animals are generally used as replacers (Scroggins, 2012).

The aim of this preliminary investigation is to increase the knowledge of the composition of alpaca milk in order to better understand the nutritional needs of crias during their first months of life.

2. Materials and Methods

2.1 Animals and sampling

In October and November 2014, sixteen milk samples were collected from eight pluriparous alpaca from the Huacaya breed, which were homogeneous in terms of lactation phase. The alpacas were reared as fiber animals on the same farm in Tuscany and grazed on pastureland, following a semi-extensive breeding system. The animals were milked manually on the thirtieth and sixtieth day of lactation, at least four hours after separation from the suckling cria. Approximately 80 ml of milk were collected from both teats per individual. All the milk was evacuated. Milk letting agents were not used.

2.2 Chemical Analysis

The milk was transported to the laboratory in refrigerated tanks at -4°C. On each fresh milk sample, the following chemical analyses were carried out according to AOAC methods (2004): total fat, total protein, casein, ash, phosphorus by the colorimetric method, and Ca, Mg, K, Na, Zn by atomic absorption spectrophotometry.

2.3 Fatty acid profile

Milk fat extraction and analysis were performed following Rose-Gottlieb's method. Fatty acid methyl esters were prepared using methanolic sodium methoxide according to Christie (1982), and one µl of fatty acid methyl esters for each sample was injected with split injection mode into a Perkin Elmer Auto System (Norwalk, CT, USA). The instrument was equipped with an automatic injector, a flame ionization detector (FID) and a capillary column (Factor Four Varian, Middelburg, Netherlands; 30 m x 0.25 mm; film thickness 0.25 mm Middelburg, Netherlands). Helium was used as a carrier gas with a flow of 1 mL min^{-1}. The initial oven temperature was set at 50 ºC, after 5 min the temperature was increased at a rate of 3 ºC min^{-1} to 140 ºC and held for 2 min; then increased 1 ºC min^{-1} to 240 ºC and held for 20 min. Injector and detector temperatures were 270 ºC and 300 ºC, respectively. The peak areas of individual FAs were identified by comparison with fatty acid standard injection (Sigma Aldrich Chemical Co., St. Louis, MO, USA) and quantified as a percentage of the total fatty acids.

2.4 Statistical analysis

All the 16 milk samples (n=8x2) were analysed in duplicate and the data on quality characteristics, fatty acid and mineral composition were statistically analysed by a mathematical model for repeated measures, considering the day of lactation (30, 60 days) as a fixed effect, and the subject as a random effect. Statistical analysis was performed by JMP software (2002). The differences between the means were considered significant at $P < 0.05$.

3. Results and Discussion

3.1. Milk chemical composition

No significant changes were found in the percentages of fat, protein, casein and ash between 30 and 60 days of lactation (Table 1). However, a decreasing non-significant trend was observed in the percentage of milk macro constituents in the second month of lactation. Chad et al. (2014) also reported that the main components of milk did not change much during lactation. Milk protein was higher than in ruminant milk, such as cow and goat, and was more similar to sheep and camelid milk such as Llama (Park et al., 2007; Mayer et al., 2012; Medhammar et al., 2011).

The correct protein content in milk is specifically required to sustain the desired daily weight gain in animals reared for production, since proteins are fundamental for optimum growth and development. The right protein ingestion is fundamental especially during the first six weeks of the cria's life, when daily weight gain is highest (a desirable weight gain is from 110 to 230 gr/day during the first month) (Scroggins, 2012). A reduction in protein content in the diet of a rapidly growing cria could result in growth retardation (Scroggins, 2012). Therefore, goat or cow milk may not be suitable as an exclusive supplementation for crias.

Casein was on average 4.09% and 3.48% on the thirtieth and sixtieth days of lactation, respectively, (73% and 72% of the proteins) and lower than that reported for cows (Park et al., 2007). Casein content in milk is interesting due to its effects on the coagulation of milk in the stomach, and on digestion and absorption. In the literature no values have been reported concerning the amount of casein in alpaca milk.

As regards the nutritional requirements, milk fat content is important, since it ensures an adequate energy intake.

Fat percentages were similar to the average values of alpaca milk from California (Chad et al., 2014) and to cow and goat milk (Park et al., 2007; Martini et al., 2010), but lower than sheep and llama milk (Martini et al., 2008; Mayer et al., 2012; Riek and Gerken, 2006).

Ash was 0.66% and 0.67% on the thirtieth and sixtieth days of lactation, which was similar to llama (Schoos et al., 2008) but lower than alpaca milk from South America (1.4%-1.7 %) (Parraguez et al. 2003).

Here Table 1

3.2 Mineral composition

Minerals are critical nutrients. In addition to their structural function, they are also involved in the regulation of biochemical cellular equilibria. Both their excess and deficiency have detrimental effects on the health and production of livestock (Khan et al., 2012).

No significant changes were found in the mineral content during the period considered, except for an increase in zinc ($P<0.05$) (Table 2). To date, the data in the literature concerning the Zn content in alpaca milk show a lower average content than reported by Park et al. (2007) for ruminants.

The importance of zinc is linked to its role in several enzymatic activities. Its deficiency in farm animals could be also due to increased renal excretion during stress and disease states, and could cause skin lesions in some areas subject to mechanical injuries (breast and toenails).

In camelids idiopathic hyperkeratosis syndrome has been described, which is characterized by lesions on hairless areas of the body with a thickening of the skin and tightly adhering crusts (Van Saun, 2006).

The contents of calcium, magnesium and potassium were in agreement with Chad et al. (2014), whereas phosphorus showed a higher content, similar to cow (1190 mg/kg) and goat (1200mg/kg) (Park et al., 2007; Martini et al., 2010).

According to Van Saun (2008), phosphorus metabolism in ruminants and especially in camelids is unique because blood phosphorus is recycled to the rumen through saliva, providing the phosphorus needed for rumen microbes. Phosphorus is a critically limiting mineral for grazing animals in soils low in phosphorus. In general, a high molar calcium:phosphorus ratio in milk is recommended in order to maintain adequate calcium levels and prevent bone resorption. The metabolism of calcium and phosphorus is linked to vitamin D. In fact, the shortage of phosphorus in the diet of young animals leads to delayed development and rickets in crias (Van Saun et al., 1996).

Sodium was within the values reported for ruminants, but unlike the values described for alpacas by Chad et al. (2014); further research is needed to resolve these differences. In our study the sodium:potassium ratio was similar to sheep milk, in which potassium is about two or three times greater than sodium. Llama milk, on the other hand, contains four times more potassium than sodium (Medhammar et al., 2011). Sodium:potassium ratio in milk reflect the physiological status of the mammary gland and increase during the involution at the end of lactation in ruminant (Silanikove et al., 2013). In our study no changes in the sodium:potassium ratio between 30 and 60 days post lactation were found. Our results indicate that the metabolic adaptive response of the mammary gland to milk production in alpaca is similar to ruminants, in fact the tight junctions between the mammary epithelial cells are sealed at 30 and 60 days post lactation.

Here Table 2

3.3 Fatty acid profile

Table 3 reports the fatty acid profile of the milk alpaca at 30 and 60 days of lactation.

The fatty acid profile showed a few significant changes in the period of the study. The changes took place in C18:1 trans-11, C18:2 trans-9,12, C18:2 cis-9, cis-12 and C22:6 fatty acids, which increased ($P<0.05$) at sixty days of lactation. The balanced content of long chain unsaturated fatty acids in milk should be considered when choosing a milk replacer. However milk replacers often use vegetable oils as sources of fat and have a larger amount of C18:1 cis-9 and C18:2 cis-9, 12.

The proportions of saturated fatty acids (SFAs) were similar to other camelids, such as llama and dromedary, which have been reported at between 60% and 65% (Schoos et al., 2008; Medhammar et al., 2011), while the content of unsaturated fatty acids (UFAs) was higher than the average content of ruminant milk (Shingfield, 2005).

Similarly to Chad et al. (2014), we found lower short-chain fatty acids (with a carbon chain length shorter than 14) compared to ruminant and llama milk (Schoots et al., 2008; Medhammar et al., 2011). SCFA have also been reported at low or non-detectable concentrations in dromedary milk. It has been suggested that fatty acids with a carbon chain length of <C14 produced by cellulose fermentation in the rumen may be rapidly metabolized by tissue and are therefore excreted less in the milk (Medhammar et al., 2011). The low content of short fatty acids in alpaca milk could also be linked to differences in the expression of certain enzymes. In fact, it seems that camelids express the enzyme thioesterase II instead of the fatty acids synthetase (Grunnet and Knudsen, 1979). Currently, there are no reports in the literature on thioesterase II and FA synthetase for alpacas.

Since alpaca milk contains few short chains fatty acids the cria may have poor capacity to exploit them.

In addition, long chains constituted more than 40% of the total fatty acids. Concerning the individual fatty acids, to our knowledge there is only one study in the literature on Alpaca milk (Chad et al., 2014), which our results are in agreement with. However, there were some exceptions for some fatty acids (C18:1 trans-11, C18:2 cis-9, trans-11 and C18:2 cis 9, 12) registering higher values.

In agreement with the literature regarding llama and similarly to ruminant milk (Schoos et al., 2008; Park et al., 2007), the most representative fatty acid in alpaca milk was palmitic acid (C16:0).

In addition, the content of C17:0 was similar to cow milk (0.70%) (Vlaeminck et al., 2006), whereas C18:0 and C18:1 cis-9 were similar to llama and higher than goat, sheep and cow milk (Soyeurt et al., 2007; Schoos et al., 2008; Talpur, 2009).

CLA (conjugated linoleic acid) C18:2 cis-9, trans-11 was higher than cow milk and llama (0.4 g and 0.70 g 100 g^{-1} total FA, respectively) (Shingfield et al., 2006; Schoots et al., 2008; Medhammar et al., 2011).

CLA originates from the incomplete ruminal biohydrogenation of linoleic acid in feeding. CLA is absorbed from the small intestine, transported to the udder and included in the fat synthesis (Haug et al., 2007). In addition, most of the CLA cis-9, trans-11 in milk originates from vaccenic acid (C18:1 trans-11), which is an intermediate product from the biohydrogenation of unsaturated fatty acids in the rumen. After absorption and transportation by the blood to the udder, a portion of the vaccenic acid is desaturated by delta-9-desaturase to CLA (Kay et al., 2004). Since the alpaca is a pseudo-ruminant, similar biochemical reactions may also occur within the C1 stomach of this animal.

The n6/n3 ratio was more similar to the values reported by Jensen (1992) in cow milk (1.97) than goat and sheep milk (3.73 and 2.11 respectively) (Dønnem et al., 2011; Nudda and Pulina, 2014).

Here table 3

3. Conclusions

Alpaca milk showed a few similarities with the milk from commonly-reared species. The high percentage of protein suggests the need for a suitable protein level for the crias' growth. In addition, the low percentage of fatty acids <C14 would seem to indicate that the offspring of alpaca have a limited ability to use these fatty acids. In addition, a higher content of unsaturated fatty acids and CLA was found compared to the milk of ruminants. In conclusion, a good substitute for milk should take into account the nutritional needs of the crias, and ruminant milk cannot be considered as an ideal surrogate.

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Table 1. Gross composition of alpaca milk at 30 and 60 days of lactation (mean ±SE). Data on gross composition of cow, goat, sheep, llama and alpaca milk is added for comparison.

Table 2. Average mineral composition of alpaca milk at 30 and 60 days of lactation (mean±SE). Data on mineral composition of cow, goat, sheep, llama and alpaca milk is added for comparison.

Table 3. Average fatty acid composition of alpaca milk at 30 and 60 days of lactation. (mean±SE).

Table 1. Gross composition of alpaca milk at 30 and 60 days of lactation (mean±SE). Data on gross composition of cow, goat, sheep, llama and alpaca milk is added for comparison.

Parameter† (%)	Days of lactation				SEM
	30		60		
	Least square mean	SE	Least square mean	SE	
Fat	3.35	0.558	3.29	0.755	1.347
Protein	5.62	0.758	4.86	0.925	1.542
Casein	4.09	0.069	3.48	0.049	0.085
Ash	0.67	0.086	0.66	0.086	0.128

Parameter (%)	Milk				
	Cow[1]	Goat[2]	Sheep[3]	Llama[4]	Alpaca[5,6,7]
Protein	3.20	3.32	5.71	4.23	4.53-5.58
Fat	3.60	3.97	6.44	4.70	3.68
Ash	0.70	0.78	0.90	0.76	1.4 – 1.7
Casein	2.60	2.80	4.73	-	-

†Values are espressed as least square means of the duplicate analisys (n=8x2)

LMS: least square means; SE: standard error; SEM: standard error of the model

[1]Park et al., 2007; [2]Martini et al., 2010; [3]Martini et al., 2008; [4]Riek and Gerken, 2006; [5]Chad et al., 2014;[6]Medhammar et al., 2011; [7]Parraguez et al., 2003

Table 2. Average mineral composition of alpaca milk at 30 and 60 days of lactation (mean±SE). Data on mineral composition of cow, goat, sheep, llama and alpaca milk is added for comparison.

Parameter† (mg/kg)	Days of lactation 30 Least square mean	30 SE	Days of lactation 60 Least square mean	60 SE	SEM
Ca	1200	160.00	1100	160.00	0.057
P	1195	168.00	1229	182.00	0.046
Mg	200	18.70	100	20.30	0.005
K	1300	260.00	1560	270.00	0.07
Na	860	250.00	559	270.00	0.061
Zn	0.83[b]	0.076	1.08[a]	0.082	0.195
Ca:P ratio	1.10	0.106	0.91	0.115	0.270
Na:K ratio	0.32	0.068	0.26	0.068	1.910

Parameter (mg/kg)	Milk: Cow[1]	Goat[1, 2]	Sheep[1,4]	Llama[4]	Alpaca[5]
Ca	1220	1800	2000		1383
P	1190	1200	1500	1122	981
Mg	120	160	180	150	126
K	1520	1810	1360	1120	1302
Na	580	410	440	272	200
Zn	5.30	5.60	5.70		-
Ca:P ratio	1.02	1.58	1.33		1.41
Na:K ratio	0.38	0.23	0.32	0.24	0.15

†Values are espressed as least square means of the duplicate analisys (n=8x2)

a, b: Within a row, means without a common superscript differ at P<0.01

LMS: least square means; SE: standard error; SEM: standard error of the model

[1] Park et al., 2007; [2] Martini et al., 2010; [3] Martini et al., 2008; [4]Medhammar et al., 2011; [5] Chad et al., 2014

Table 3. Average fatty acid composition of alpaca milk at 30 and 60 days of lactation (mean±SE).

Fatty acid (g/100g of fatty acid methyl esters)	Days of lactation				SEM
	30		60		
	LMS	SE	LMS	SE	
C4:0	0.05	0.009	0.03	0.012	0.026
C6:0	0.23	0.027	0.20	0.037	0.078
C8:0	0.26	0.035	0.25	0.047	0.097
C10:0	0.79	0.106	0.61	0.143	0.327
C11:0	0.05	0.015	0.02	0.021	0.048
C12:0	0.55	0.049	0.53	0.067	0.131
C13:0	0.11	0.010	0.11	0.013	0.027
C14:0	8.00	0.377	7.72	0.510	1.048
C14:1	0.97	0.058	0.96	0.079	0.162
C15:0	1.64	0.112	1.3	0.151	0.318
C15:1	0.56	0.024	0.61	0.033	0.072
C16:0	33.92	0.657	33.00	0.889	1.936
C16:1	8.19	0.640	7.48	0.867	1.845
C17:0	0.69	0.059	0.69	0.080	0.164
C17:1	0.59	0.024	0.54	0.032	0.068
C18:0	13.80	0.706	15.00	0.956	2.172
C18:1 *trans-11*	3.95[b]	0.303	4.55[a]	0.411	0.926
C18:1 *cis-9*	16.84	0.447	16.25	0.605	1.275
C18:2 *trans-9,12*	0.35[b]	0.045	0.40[a]	0.060	0.089
C18:2 *cis-9,12*	2.24[b]	0.139	2.84[a]	0.188	0.392
C18:3n3	1.87	0.088	2.01	0.118	0.246
C20:0	0.19	0.021	0.20	0.029	0.060
C20:1	0.14	0.018	0.10	0.025	0.051
CLA *cis-9trans11*	1.83	0.119	1.67	0.161	0.359
C21:0	0.20	0.012	0.23	0.016	0.034
C20:2	0.02	0.003	0.02	0.004	0.007
C20:3n3	0.08	0.013	0.08	0.018	0.037
C20:3n6	0.07	0.007	0.07	0.009	0.019
C22:0	0.18	0.014	0.22	0.019	0.039
C22:1	0.09	0.009	0.10	0.013	0.027
C20:4n6	0.01	0.002	0.01	0.003	0.007
C23:0	0.13	0.014	0.12	0.019	0.039
C22:2	0.10	0.013	0.14	0.017	0.039
C20:5	0.03	0.013	0.04	0.017	0.035
C24:0	0.13	0.014	0.15	0.019	0.038
C24:1	0.04	0.012	0.06	0.016	0.034
C22:5	0.19	0.019	0.20	0.026	0.053
C22:6	0.03[b]	0.007	0.06[a]	0.009	0.020
SCFA (≤C10)	1.33	0.152	0.99	0.206	0.528
MCFA (≥C11≤C17)	55.70	0.985	55.76	1.333	3.832
LCFA (≥C18)	42.93	1.077	43.86	1.458	4.199
SFA	60.93	0.997	61.10	1.350	2.808
MUFA	32.09	0.921	31.11	1.247	2.577
PUFA	6.95	0.246	7.74	0.333	0.701
n6/n3 ratio	1.28	0.084	1.47	0.113	0.237

†Values are espressed as least square means of the duplicate analisys (n=8x2)

A,B: Within a row, means without a common superscript differ at P<0.05

a,b: Within a row, means without a common superscript differ at P<0.01

LMS: least square means; SE: standard error; SEM: standard error of the model; SCFA: Short Chain Fatty Acids; MCFA: Medium Chain Fatty Acids; LCFA: Long Chain Fatty Acids; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids