

1 **Gross, mineral and fatty acid composition of alpaca (*Vicugna pacos*) milk at 30 and**  
2 **60 days of lactation**

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15 **ABSTRACT**

16 The aim of this study was to investigate the composition of alpaca milk in order to  
17 improve knowledge of the nutritional needs of crias during their first months of life.  
18 Analyses of alpaca milk were performed in terms of chemical, mineral composition and  
19 fatty acid profile during the first two months of lactation. Percentages of fat, protein,  
20 casein and ash did not change in the first two months of lactation. Alpaca milk showed a  
21 similar protein content to sheep and camelid milk such as llama (*Lama glama*), lower  
22 casein content compared to ruminants, and similar fat percentages to cow and goat milk.  
23 The Ca and P content was similar to cow milk. Concerning the fatty acid profile, alpaca

24 milk had higher conjugated linoleic acid (C18:2 cis 9, trans 11) and unsaturated fatty acid  
25 contents than ruminant milk, and a low percentage of fatty acids with chains <C14: 0. In  
26 fact, during the first months of life, alpaca offspring may not sufficiently exploit these  
27 fatty acids. Ruminant milk cannot be considered as an ideal surrogate for the nutritional  
28 needs of crias.

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

30

### 31 **1. Introduction**

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

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

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

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

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

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

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

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

## 68 2. Materials and Methods

## 69 *2.1 Animals and sampling*

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

## 77 *2.2 Chemical Analysis*

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

## 82 *2.3 Fatty acid profile*

83 Milk fat extraction and analysis were performed following Rose-Gottlieb's method. Fatty  
84 acid methyl esters were prepared using methanolic sodium methoxide according to  
85 Christie (1982), and one µl of fatty acid methyl esters for each sample was injected with  
86 split injection mode into a Perkin Elmer Auto System (Norwalk, CT, USA). The  
87 instrument was equipped with an automatic injector, a flame ionization detector (FID)  
88 and a capillary column (Factor Four Varian, Middelburg, Netherlands; 30 m x 0.25 mm;  
89 film thickness 0.25 µm Middelburg, Netherlands). Helium was used as a carrier gas with  
90 a flow of 1 mL min<sup>-1</sup>. The initial oven temperature was set at 50 °C, after 5 min the  
91 temperature was increased at a rate of 3 °C min<sup>-1</sup> to 140 °C and held for 2 min; then  
92 increased 1 °C min<sup>-1</sup> to 240 °C and held for 20 min. Injector and detector temperatures

93 were 270 °C and 300 °C, respectively. The peak areas of individual FAs were identified  
94 by comparison with fatty acid standard injection (Sigma Aldrich Chemical Co., St. Louis,  
95 MO, USA) and quantified as a percentage of the total fatty acids.

#### 96 *2.4 Statistical analysis*

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

### 103 **3. Results and Discussion**

#### 104 *3.1. Milk chemical composition*

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

112 The correct protein content in milk is specifically required to sustain the desired daily  
113 weight gain in animals reared for production, since proteins are fundamental for optimum  
114 growth and development. The right protein ingestion is fundamental especially during the  
115 first six weeks of the cria's life, when daily weight gain is highest (a desirable weight  
116 gain is from 110 to 230 gr/day during the first month) (Scroggins, 2012). A reduction in

117 protein content in the diet of a rapidly growing cria could result in growth retardation  
118 (Scroggins, 2012). Therefore, goat or cow milk may not be suitable as an exclusive  
119 supplementation for crias.

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

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

127 Fat percentages were similar to the average values of alpaca milk from California (Chad  
128 et al., 2014) and to cow and goat milk (Park et al., 2007; Martini et al., 2010), but lower  
129 than sheep and llama milk (Martini et al., 2008; Mayer et al., 2012; Riek and Gerken,  
130 2006).

131 Ash was 0.66% and 0.67% on the thirtieth and sixtieth days of lactation, which was  
132 similar to llama (Schoos et al., 2008) but lower than alpaca milk from South America  
133 (1.4%-1.7 %) (Parraguez et al. 2003).

134

135 **Here Table 1**

136

137 *3.2 Mineral composition*

138

139 Minerals are critical nutrients. In addition to their structural function, they are also  
140 involved in the regulation of biochemical cellular equilibria. Both their excess and  
141 deficiency have detrimental effects on the health and production of livestock (Khan et al.,  
142 2012).

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

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

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

154 The contents of calcium, magnesium and potassium were in agreement with Chad et al.  
155 (2014), whereas phosphorus showed a higher content, similar to cow (1190 mg/kg) and  
156 goat (1200mg/kg) (Park et al., 2007; Martini et al., 2010).

157 According to Van Saun (2008), phosphorus metabolism in ruminants and especially in  
158 camelids is unique because blood phosphorus is recycled to the rumen through saliva,  
159 providing the phosphorus needed for rumen microbes. Phosphorus is a critically limiting  
160 mineral for grazing animals in soils low in phosphorus. In general, a high molar  
161 calcium:phosphorus ratio in milk is recommended in order to maintain adequate calcium  
162 levels and prevent bone resorption. The metabolism of calcium and phosphorus is linked

163 to vitamin D. In fact, the shortage of phosphorus in the diet of young animals leads to  
164 delayed development and rickets in crias (Van Saun et al., 1996).

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

176 **Here Table 2**

177

### 178 *3.3 Fatty acid profile*

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

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



186 The proportions of saturated fatty acids (SFAs) were similar to other camelids, such as  
187 llama and dromedary, which have been reported at between 60% and 65% (Schoos et al.,  
188 2008; Medhammar et al., 2011), while the content of unsaturated fatty acids (UFAs) was  
189 higher than the average content of ruminant milk (Shingfield, 2005).

190 Similarly to Chad et al. (2014), we found lower short-chain fatty acids (with a carbon  
191 chain length shorter than 14) compared to ruminant and llama milk (Schoots et al., 2008;  
192 Medhammar et al., 2011). SCFA have also been reported at low or non-detectable  
193 concentrations in dromedary milk. It has been suggested that fatty acids with a carbon  
194 chain length of <C14 produced by cellulose fermentation in the rumen may be rapidly  
195 metabolized by tissue and are therefore excreted less in the milk (Medhammar et al.,  
196 2011). The low content of short fatty acids in alpaca milk could also be linked to  
197 differences in the expression of certain enzymes. In fact, it seems that camelids express  
198 the enzyme thioesterase II instead of the fatty acids synthetase (Grunnet and Knudsen,  
199 1979). Currently, there are no reports in the literature on thioesterase II and FA synthetase  
200 for alpacas.

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

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

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

211 In addition, the content of C17:0 was similar to cow milk (0.70%) (Vlaeminck et al.,  
212 2006), whereas C18:0 and C18:1 cis-9 were similar to llama and higher than goat, sheep  
213 and cow milk (Soyeurt et al., 2007; Schoos et al., 2008; Talpur, 2009).

214 CLA (conjugated linoleic acid) C18:2 cis-9, trans-11 was higher than cow milk and llama  
215 (0.4 g and 0.70 g 100 g<sup>-1</sup> total FA, respectively) (Shingfield et al., 2006; Schoots et al.,  
216 2008; Medhammar et al., 2011).

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

225 The n6/n3 ratio was more similar to the values reported by Jensen (1992) in cow milk  
226 (1.97) than goat and sheep milk (3.73 and 2.11 respectively) (Dønnem et al., 2011; Nudda  
227 and Pulina, 2014).

228 **Here table 3**

229

230 **3. Conclusions**

231

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

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326

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

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

333 Table 3. Average fatty acid composition of alpaca milk at 30 and 60 days of  
334 lactation. (mean $\pm$ SE).

335



336 Table 1. Gross composition of alpaca milk at 30 and 60 days of lactation (mean±SE).  
 337 Data on gross composition of cow, goat, sheep, llama and alpaca milk is added for  
 338 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 <sup>1</sup>	Goat <sup>2</sup>	Sheep <sup>3</sup>	Llama <sup>4</sup>	Alpaca <sup>5,6,7</sup>
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	-	-

339 †Values are expressed as least square means of the duplicate analysis (n=8x2)

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

341 <sup>1</sup>Park et al., 2007; <sup>2</sup>Martini et al., 2010; <sup>3</sup>Martini et al., 2008; <sup>4</sup>Riek and Gerken, 2006; <sup>5</sup>Chad et  
 342 al., 2014; <sup>6</sup>Medhammar et al., 2011; <sup>7</sup>Parraguez et al., 2003

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344

345

346

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

Parameter† (mg/kg)	Days of lactation				SEM
	30		60		
	Least square mean	SE	Least square mean	SE	
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 <sup>b</sup>	0.076	1.08 <sup>a</sup>	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

350

Parameter (mg/kg)	Milk				
	Cow <sup>1</sup>	Goat <sup>1, 2</sup>	Sheep <sup>1,4</sup>	Llama <sup>4</sup>	Alpaca <sup>5</sup>
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

351 †Values are expressed as least square means of the duplicate analysis (n=8x2)

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

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

354 <sup>1</sup> Park et al., 2007; <sup>2</sup> Martini et al., 2010; <sup>3</sup> Martini et al., 2008; <sup>4</sup> Medhammar et al., 2011; <sup>5</sup> Chad et al., 2014

355

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

358

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 <i>trans-11</i>	3.95 <sup>b</sup>	0.303	4.55 <sup>a</sup>	0.411	0.926
C18:1 <i>cis-9</i>	16.84	0.447	16.25	0.605	1.275
C18:2 <i>trans-9,12</i>	0.35 <sup>b</sup>	0.045	0.40 <sup>a</sup>	0.060	0.089
C18:2 <i>cis-9,12</i>	2.24 <sup>b</sup>	0.139	2.84 <sup>a</sup>	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 <i>cis-9trans11</i>	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 <sup>b</sup>	0.007	0.06 <sup>a</sup>	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

359 † Values are expressed as least square means of the duplicate analysis (n=8x2)

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

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

362 LMS: least square means; SE: standard error; SEM: standard error of the model; SCFA: Short Chain Fatty Acids;  
363 MCFA: Medium Chain Fatty Acids; LCFA: Long Chain Fatty Acids; SFA: Saturated Fatty Acids; MUFA:  
364 Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids  
365