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Additional Information

1 **Short Communication: Goat colostrum quality. Litter size and lactation number**
2 **effects**

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ABSTRACT

27 The quality of colostrum of Murciano-Granadina goats was studied to establish the
28 transition period and the time when milk can be marketed. Forty-three dairy goats were
29 used, 19 primiparous (15 single births; 4 multiple births) and 24 multiparous (10 single
30 births; 14 multiple births). Samples were collected every 12 h during the first week
31 postpartum. Physico-chemical parameters and somatic cell count were determined.
32 Analysis of variance with repeated measures was used to study the effect of different
33 factors: postpartum time, litter size, lactation number, their interactions and production
34 level on colostrum.

35 Postpartum time had a significant effect on all parameters studied, which decreased
36 along the first week of lactation, while lactose, pH and conductivity increased. Based on
37 these results, colostrum secretion takes place until 36 hours postpartum (hpp). In
38 relation to other factors of variation studied, the lactation number influenced ($P < 0.05$)
39 most colostrum components, while the litter size only affected ($P < 0.05$) the pH value,
40 protein and lactose content. The production level influenced only the protein and dry
41 matter contents, with an inverse relationship. Milk produced during the period between
42 36 and 96 hpp is considered transition milk, which should not be commercialized. After
43 4 days postpartum (96 hpp) milk could be marketed, ensuring that composition does not
44 present a risk in the dairy industry.

45 **Keywords:** colostrum, physico-chemical parameters, Murciano-Granadina goats

46 Feeding goat kids colostrum is a very important livestock practice within artificial
47 rearing systems and it is used mainly in intensive farming. This system minimizes or
48 annuls the mother-kid link, which is established along the first hours postpartum (hpp)
49 (Ramírez et al., 1996), thus helping artificial nipples to be accepted. Besides, newborns
50 are hypogammaglobulinemic at birth (Rodríguez et al., 2009), colostrum intake during a

51 first 2 postpartum days reduces mortality, because it provides antibodies
52 (immunoglobulins) to avoid possible diseases and infections; their survival is related to
53 colostrum quality and the volume ingested (Argüello et al., 2004; Keskin et al., 2007).

54 Different studies have evaluated goat colostrum composition (Argüello et al., 2006;
55 Yang et al., 2009; Moreno-Indias et al., 2012). Colostrum is characterized by high fat as
56 well as protein and mineral contents when compared to milk. It also presents some
57 interesting components from a biological viewpoint, such as protective substances
58 (immunoglobulins, lactoferrin, lysozymes, etc.), growth factors (vitamins and amino
59 acids), among others. The physico-chemical characteristics and production period of
60 colostrum may vary according to different factors such as production, feeding, breed,
61 length of dry period, season of the year and animal health status (Csapó et al., 1998;
62 Caja et al., 2006).

63 From a practical point of view, suitably delimiting the colostrum production period is
64 interesting for farmers as they can offer good quality colostrum to feed goat kids. On
65 the other hand, it would also be useful for producers to know from what time milk can
66 be commercialized because, according to the legislation of many countries, milk
67 intended for human consumption may not contain colostrum (Commission Regulation,
68 (EC) 1662/2006). Also, for the dairy industry it is important that milk does not contain
69 colostrum, since the presence of a large amount of soluble proteins can negatively affect
70 the production and standardization of some milk products (Raynal-Ljutovac et al.,
71 2005).

72 Murciano-Granadina is the most common Spanish goat breed, being well adapted to the
73 Mediterranean livestock system. Some authors have studied different aspects of
74 Murciano-Granadina goat's milk production and composition (Díaz et al., 2012; León et

75 al., 2012). Nonetheless, information about Murciano-Granadina goat's colostrum
76 quality is rather limited (Quiles et al., 1991; Caja et al., 2006).

77 Therefore, this study aims to establish the physico-chemical characteristics of
78 Murciano-Granadina goat's colostrum by evaluating the influence of hours postpartum,
79 litter size, lactation number and the animal's production level; besides, the period of
80 transition from colostrum to milk is determined, establishing the time when goat's milk
81 can be marketed.

82 Experimental animal procedures were approved by the Ethical Committee of Universitat
83 Politècnica de València (UPV, Spain). Forty-three dairy goats of the Murciano-
84 Granadina breed belonging to the experimental flock of the Animal Science and
85 Technology Institute of UPV were used. Of these, 19 were primiparous (15 single births
86 and 4 multiple births); and 24 were multiparous (10 single births and 14 multiple
87 births), divided as follows: 3 of the second lactation, 10 of the third lactation and 11 of
88 the fourth lactation or more. The mean gestation length for the experimental goats was
89 151 ± 10 days, and the length of dry period was approximately 103 days. The animals
90 presented a suitable health status and were fed the same ration, basically alfalfa hay
91 (1,050 g), barley straw (330 g), orange pulp (1,615 g), barley (520 g), beet pulp (205
92 g), soya (275 g), treacle (40 g) and a vitamin-mineral corrector, using a mixer-feeder
93 wagon, which covers lactation requirements according to INRA (2007).

94 After birth, goat kids were separated from their mothers and underwent artificial
95 rearing. Machine milking (high line; CASSE 2x12x6; Alfa Laval[®], Lund, Sweden) was
96 performed every 12 h (8 am and 8 pm) during the first week of lactation. To obtain
97 colostrum and milk samples (300 mL), a complete milking was carried out, measuring
98 the total volume with milk-recording jars (3.5 L, Sneder[®], Industrias Berango S.L,

99 Vizcaya, Spain). The samples were stored at 4 °C until their subsequent analysis, and 3
100 aliquots of 1.5 mL were frozen (-40 °C) for IgG quantification.

101 The physico-chemical parameters were analyzed in triplicate on the same day of
102 sampling, and the pH value was determined using a portable pH meter (Crison, model
103 Basic 20, Crison Instrument, Barcelona, Spain). Colostrum density was determined
104 applying a densimeter (ranging between 1000 and 1100 kg/m³ (Proton 20 °C, GAB
105 Sistemática Analítica S.L, Barcelona, Spain). Electrical conductivity was measured
106 using a conductivity meter (Crison, model Basic 30, Crison Instruments, Barcelona,
107 Spain), and the results were expressed in millisiemens per centimetre at 25 °C (mS/cm).

108 To determine titratable acidity (AOAC, 2000), 1 mL phenolphthalein indicator (1 %)
109 was added to 10 mL of milk, and the mixture was titrated with Dornic NaOH, 0.111 M
110 (Suministros Químicos Arroyo, S.L, Santander, Spain) to a permanent light pink color.

111 Titratable acidity was expressed as ° Dornic using the relation: 1 °D = 0.1 mL Dornic
112 NaOH (1°D is equivalent to 0.01 % lactic acid concentration). The freezing point was
113 measured with a thermistor cryoscope (Cryostar 1, Funke- Dr.N. Gerber Labortechnik
114 GmbH, Berlin, Germany).

115 Gross composition analysis (fat, protein, lactose, dry matter) and somatic cell count
116 (SCC) were analyzed at LICOVAL (Interprofessional Milking Laboratory of the
117 Valencian Community, Spain). Composition was determined by infrared
118 spectrophotometry equipment (MilkoScan FT120, Foss, Hillerød, Denmark) (IDF,
119 2000), using a specific calibration for goat's milk; SCC was determined according to
120 the electronic fluoro-opto method (IDF, 2006) employing a Fossomatic 5000 (Foss,
121 Hilleørød, Denmark).

122 IgG analyses were done using the Calokit-Cabra[®] (Zeu-Inmunotec S.L, Zaragoza,
123 Spain), a direct ELISA sandwich-type immunoenzymatic assay, permitting IgG

124 quantification (mg/mL), with a range of 0.01-50 mg/mL. The kit is supplied with a
125 microtiter plate for 96 tests together with reagents. Colostrum and milk samples were
126 analyzed in duplicate following the manufacturer's instructions.

127 Statistical analyses were performed using SAS, version 9.2, 2001 (SAS Institute, Inc.,
128 Cary, NC). The SAS Proc Mixed procedure for repeated measurements was used to
129 determine the colostrum production period during the first lactation week. The
130 statistical model included the effect of hours postpartum:

$$131 \quad Y_{ijk} = \mu + HPP_i + G_j + \varepsilon_{ijk}$$

132 where: Y_{ijk} = dependent variable, μ =mean, HPP_i = hours postpartum, G_j = goat and ε_{ijk} =
133 residual error. SAS Proc Mixed was used to evaluate the effect of different variation
134 factors on colostrum quality characteristics:

$$135 \quad Y_{ijkl} = \mu + HPP_i + NL_j + LS_k + C_l(NL_j LS_k) + COV + \varepsilon_{ijkl}$$

136 where: Y_{ijkl} = dependent variable, μ = mean, HPP_i = hours postpartum, NL_j = lactation
137 number, LS_k = litter size, COV = covariate (mean production) and ε_{ijkl} = residual error.

138 Table 1 provides the physico-chemical characteristics of colostrum and milk of
139 Murciano-Granadina goats along the first 156 hpp. All parameters lowered along the
140 studied period ($P < 0.001$), except pH, electrical conductivity and lactose which
141 increased ($P < 0.001$). No significant differences were found with respect to the freezing
142 point ($P > 0.05$).

143 Changes in parameters studied (Table 1) were distinct for each of them. The pH value
144 rose considerably at 12 hpp, and continued its gradual increase until 156 hpp; however
145 density and dornic acidity dropped sharply at 12 hpp, and decreased until 156 hpp. The
146 results were similar to those reported by Argüello et al. (2006) in Majorera goats and
147 Vilar et al. (2008) in Saanen goats. After 60 hpp, the density value was similar within
148 the ordinary goat's milk range (1,030-1,034 g/L; Ludeña et al., 2006). Electrical

149 conductivity, increased from birth to 24 hpp, and remained constant ($P > 0.05$) until the
150 last day of the experiment. By contrast dry matter and fat content drastically lowered
151 from delivery to 24 hpp, and then dropped gradually until 156 hpp. Dry matter evolved
152 similarly to that reported by Keskin et al. (2007) in Damascus goats, although Yang et
153 al. (2009) found lower percentages in Saanen goats when compared to those presented
154 herein, as that breed presents a higher production capacity than Murciano-Granadina
155 (Haenlein, 2007). Fat results obtained differed from other authors; Keskin et al. (2007)
156 observed a significant decrease until day 5 postpartum; and, conversely, Argüello et al.
157 (2006) indicated that fat increased until 24 hpp, and thereafter decreased gradually.
158 Both protein content and IgG lowered markedly up to 36 hpp, which dropped as the
159 lactation period advanced. Protein evolution and values were similar to those provided
160 by Argüello et al. (2006) and Vilar et al. (2008). Other authors found higher percentages
161 of protein (Csapó et al., 1994; Hadjipanayiotou, 1995; Chen et al., 1998) for the first
162 secretions obtained after delivery. IgG content agreed with the data reported by other
163 authors (Argüello et al., 2006; Moreno-Indias et al., 2012). Chen et al. (1998) and Yang
164 et al. (2009) found higher values during the first 5 and 2 days postpartum, respectively,
165 which could be related to the methodology employed, as the electrophoresis method
166 tends to overestimate IgG values in comparison with the ELISA method (Rudovsky et
167 al., 2007).

168 Lactose increased up to 36 h, and then more gradually until 156 h. Several authors have
169 also described an increasing lactose evolution (Argüello et al., 2006; Yang et al., 2009;
170 Moreno-Indias et al., 2012) although, in some cases, percentages were lower than those
171 obtained in the present study. The lactose follows an evolution similar to milk
172 production, increases from partum to reach its maximum value and then decreases
173 (Mioč et al., 2008).

174 SCC decreased from delivery to 60 hpp, and no differences were found until lactation
175 day 7. Information on SCC in colostrum is very scarce. Recently, Moreno-Indias et al.
176 (2012) found a higher SCC in Majorera goat's colostrum than that presented herein. In
177 goat milk the SCC increases as lactation advances and there are many factors (day
178 lactation, parity, infection...) that contribute to high values of SCC (Zeng et al., 1997;
179 Dulin et al., 1983).

180 Based on the results obtained and depending on the parameter employed, it can be
181 established that the Murciano-Granadina goat colostrum production period takes place
182 during the first 36 hpp. This result is similar to that reported by Quiles et al. (1991) who
183 considered only the protein fraction, and established that transition should take place on
184 the 2nd day after delivery, in the same breed. Also, Yang et al. (2009) in Saanen goats,
185 found no significant differences between 24 hpp and 48 hpp for the composition
186 parameters studied. However, Vilar et al. (2008) considered colostrum to be exclusively
187 the secretion occurring between 0 h and 12 hpp.

188 Furthermore, it is possible to establish the time when milk can be marketed by the dairy
189 industry with no drawbacks given the presence of colostrum. In relation to the baseline
190 levels of IgG content in goat's milk, there are several studies (Levieux et al., 2002;
191 Raynal-Ljutovac et al., 2005) which indicate that this content is lower than 1 mg/mL
192 after the first week of lactation. In France, the quality payment system for goat's milk
193 (Pirisi et al., 2007) establishes a classification based on the IgG content and other
194 parameters; this system applies a bonus or penalty by fixing the milk price according to
195 each parameter. Milk category C presents the maximum penalty on IgG values above 1
196 mg/mL. The secretion of 36 hpp should not be commercialized as its IgG content is 2.16
197 mg/mL, however, after 96 hpp (4 days) milk can be marketed because its IgG content is
198 0.93 mg/mL, which would not present problems in milk processing. Thirty-six to 96

199 hpp may be considered transition milk, which could be used to feed goat kids, although
200 it does not have a good immunological quality.

201 Table 2 shows the effect of the lactation number (LN) on colostrum quality
202 characteristics (secretion corresponding to 0, 12, 24 and 36 hpp). LN influences ($P <$
203 0.05) the majority of components. Hence, primiparous goat's colostrum is characterized
204 by high dornic acidity levels and freezing point, as well as a higher content of dry
205 matter, protein and lactose than that of multiparous goats; conversely, it presents lower
206 SCC and electrical conductivity values.

207 The study conducted by Argüello et al. (2006) observed that LN had no effect on any of
208 the parameters studied. However Vilar et al. (2008) reported that only pH was affected
209 by LN and that those of a 3rd lactation gave higher values. In a study which evaluated
210 conductivity as a tool for mastitis detection in Murciano-Granadina's milk, Romero et
211 al. (2012) observed that both conductivity and SCC were lower in primiparous than in
212 multiparous goats (conductivity: 5.07 mS/cm vs. 5.45 mS/cm and SCC: 107×10
213 cel/mL^{-1} vs. $494 \times 10 \text{ cel/mL}^{-1}$, respectively); results agree with this work. Several
214 authors have indicated that SCC increases with increasing parity (Salama et al., 2003).
215 The results obtained may be due to the fact that milk production is lower in primiparous
216 than multiparous dairy goats, and can produce a component concentration effect. Indeed
217 the production level increases with the LN (Zeng and Escobar, 1995).

218 The influence of litter size (LS) was significant ($P < 0.05$), but only in relation to pH,
219 protein and lactose contents (Table 3). Thus, the colostrum of goats of single births
220 contained a higher value of pH and percentage of protein and lactose than colostrum of
221 goats of multiple births. There are only a limited number of studies concerning the
222 effect of LS on colostrum characteristics, with different and sometimes contradictory
223 results (Zygoiannis, 1994; Csapó et al., 1994; Argüello et al., 2006). From these

224 results, it can be deduced that increased prolificity may be accompanied by increased
225 milk production, and consequently a dilution of the components.

226 The animal's production level significantly influenced ($P < 0.05$) dry matter and protein
227 percentages, and an inverse relation with the volume produced was found; when milk
228 production is greater occurs dilution effect, therefore the percentage of these parameters
229 is lower (dry matter: $\beta_{x_e} = -0.0032$ and protein: $\beta_{x_e} = -0.6018$). No statistical interactions
230 were detected between time and LS or LN effects, or between LS and LN.

231 In conclusion, the colostrum secretion period in Murciano-Granadina goats is
232 established between 0 and 36 hpp. Milk produced between 36 and 96 hpp is considered
233 transition milk, which should not be commercialized as it may cause technological
234 problems in milk processing, but which may be used in artificial rearing of goat kids.
235 After 4 days postpartum, milk could be marketed, thus ensuring that composition does
236 not present a risk in the dairy industry.

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Table 1. Evolution of the physico-chemical characteristics of goat's colostrum and milk during the first 156 hours postpartum (n=602)

Parameter	Hours Postpartum (hhp)														SEM
	0	12	24	36	48	60	72	84	96	108	120	132	144	156	
Production (mL)	1,786.5 ^a	822.2 ^a	897.9 ^{bc}	810.9 ^d	892.2 ^c	907.5 ^{bc}	913.0 ^{bc}	954.4 ^{bc}	914.4 ^{bc}	958.4 ^{bc}	993.9 ^{bc}	1,031.2 ^{bc}	955.9 ^{bc}	1,028.9 ^{bc}	±50.773
Acidity (°Dornic)	36.29 ^a	25.10 ^b	21.99 ^c	19.27 ^{def}	19.33 ^{def}	18.04 ^{def}	18.58 ^{def}	17.69 ^{ef}	17.43 ^{ef}	17.99 ^{def}	16.30 ^{fg}	15.27 ^g	14.94 ^g	15.34 ^g	±0.773
pH	6.38 ^a	6.54 ^{bc}	6.58 ^{bcde}	6.58 ^{bcde}	6.56 ^{bcd}	6.58 ^{bcde}	6.59 ^{bcde}	6.6 ^{cdef}	6.62 ^{defg}	6.63 ^{efg}	6.66 ^{fgh}	6.68 ^{gh}	6.7 ^h	6.7 ^h	±0.0227
Conductivity	4.45 ^a	4.92 ^b	4.99 ^c	5.07 ^c	4.96 ^c	5.03 ^c	4.97 ^c	5.25 ^c	5.00 ^c	5.14 ^c	5.0 ^c	5.17 ^c	5.07 ^c	5.06 ^c	±0.0944
Density (g/L)	1,052.8 ^a	1,039.6 ^b	1,037.7 ^{bc}	1,035.3 ^d	1,034.7 ^{de}	1,033.1 ^e	1,031.8 ^{ef}	1,033.7 ^e	1,031.8 ^{ef}	1,031.5 ^{ef}	1,031.3 ^f	1,030.7 ^f	1,030.1 ^f	1,030.3 ^f	±2.7456
Freezing Point (-°C)	0.484	0.539	0.545	0.553	0.551	0.548	0.553	0.561	0.560	0.548	0.549	0.547	0.549	0.554	±40.34
Dry Matter (%)	29.04 ^a	23.39 ^b	19.26 ^c	18.47 ^{cd}	18.24 ^{cde}	18.16 ^{de}	17.48 ^{defg}	17.19 ^{efg}	17.68 ^{def}	17.28 ^{efg}	17.6 ^{defg}	16.85 ^{fg}	17.26 ^{efg}	16.5 ^g	±0.4137
Fat (%)	9.53 ^a	9.05 ^a	7.42 ^b	7.26 ^{bc}	7.25 ^{bc}	7.05 ^{bc}	6.98 ^{bc}	6.87 ^{bc}	7.26 ^{bc}	6.81 ^{bc}	7.43 ^b	7.31 ^b	6.87 ^{bc}	6.59 ^c	±0.272
Protein (%)	13.64 ^a	8.04 ^b	6.24 ^c	5.58 ^{de}	5.42 ^{de}	5.36 ^{de}	4.99 ^{ef}	4.75 ^{fgh}	4.76 ^{fg}	4.74 ^{fgh}	4.48 ^{gh}	4.31 ^{gh}	4.25 ^h	4.33 ^{gh}	±0.208
IgG¹ (mg/mL)	28.23 ^a	11.10 ^b	5.03 ^c	2.16 ^d	2.01 ^{de}	1.86 ^{de}	1.31 ^{de}	1.03 ^{de}	0.93 ^{de}	0.75 ^e	0.77 ^e	0.69 ^e	0.68 ^e	0.73 ^e	±1.308
Lactose (%)	2.9 ^a	3.6 ^b	4.14 ^c	4.37 ^d	4.45 ^{de}	4.67 ^{efgh}	4.55 ^{ef}	4.59 ^{ef}	4.68 ^{fgh}	4.74 ^{gh}	4.76 ^h	4.79 ^h	4.80 ^h	4.82 ^h	±0.716
LSCC²	6.01 ^a	6.09 ^a	5.99 ^a	5.93 ^{ab}	5.85 ^b	5.72 ^c	5.65 ^{cd}	5.57 ^d	5.63 ^{cd}	5.57 ^d	5.64 ^{cd}	5.53 ^d	5.58 ^d	5.54 ^d	±0.0801

^{a-h}The means in the same row with different letters differ significantly ($P < 0.05$); ¹IgG: Immunoglobulin G; ²LSCC: Log₁₀ Somatic Cell Count.

Table 2. Effect of lactation number on Murciano-Granadina goat colostrum obtained during the first 36 hours postpartum

Parameter	Lactation Number		L.S ¹
	1 st Lactation ± SEM	≥ 2 nd Lactation ± SEM	
Acidity (°Dornic)	28.88 ± 1.28	23.11 ± 1.10	**
pH	6.47 ± 0.03	6.53 ± 0.03	ns
Conductivity (mS/cm)	4.53 ± 0.13	5.41 ± 0.11	***
Density (g/L)	1,039.40 ± 3.35	1,040.58 ± 0.03	ns
Freezing Point (-°C)	562.66 ± 7.83	537.87 ± 6.74	***
Dry Matter (%)	23.82 ± 0.46	21.37 ± 0.55	**
Fat (%)	8.33 ± 0.39	8.19 ± 0.33	ns
Protein (%)	9.17 ± 0.46	7.79 ± 0.39	*
IgG ² (mg/mL)	15.20 ± 3.04	12.85 ± 2.37	ns
Lactose (%)	3.95 ± 0.12	3.52 ± 0.10	*
LSCC ³	5.79 ± 0.12	6.18 ± 0.12	ns

¹LS: Level of significance *** P < 0.001; ** P < 0.01; * P < 0.05 ns: P > 0.05; ²IgG: Immunoglobulin G; ³LSCC: Log₁₀ Somatic Cell Count.

Table 3. Effect of litter size on Murciano-Granadina goat colostrum obtained during the first 36 hours postpartum

Parameter	Litter Size		
	Single \pm SEM	Multiple \pm SEM	L.S ¹
Acidity ($^{\circ}$ Dornic)	24.64 \pm 0.97	27.31 \pm 1.35	ns
pH	6.53 \pm 0.02	6.48 \pm 0.03	*
Conductivity (mS/cm)	5.05 \pm 0.10	4.89 \pm 0.13	ns
Density (g/L)	1,039.65 \pm 2.55	1,040.33 \pm 3.05	ns
Freezing Point ($^{\circ}$ C)	531.29 \pm 5.90	535.24 \pm 7.28	ns
Dry Matter (%)	23.03 \pm 0.48	22.16 \pm 0.61	ns
Fat (%)	8.36 \pm 0.37	8.19 \pm 0.39	ns
Protein (%)	9.10 \pm 0.43	7.86 \pm 0.35	*
IgG ² (mg/mL)	11.07 \pm 2.37	16.98 \pm 3.25	ns
Lactose (%)	3.92 \pm 0.09	3.57 \pm 0.12	*
LSCC ³	5.97 \pm 0.08	5.92 \pm 0.09	ns

¹LS: Level of significance *** P < 0.001; ** P < 0.01; * P < 0.05 ns: P > 0.05; ²IgG: Immunoglobulin G; ³LSCC: Log₁₀ Somatic Cell Count.