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Additional Information

1 **Effect of midline or low-line milking systems on lipolysis and milk composition in**
2 **dairy goats**

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25 **Abstract**

26 Two experiments were carried out to find out how milking in mid-line (ML) affects the
27 lipolysis level and milk composition in goat livestock, in comparison to low-line (LL)
28 milking. The first experiment took place, in triplicate, on an experimental farm. For
29 each replicate, a crossover design (62 goats, 2 treatments, ML and LL, in 2 periods each
30 lasting 4 days) was used. Milk samples were taken daily at 0 and 24 h after milking. In
31 the first experimental replicate, some enzymatic coagulation cheeses were made, which
32 were assessed by a panel of tasters at 50 and 100 days of maturation. In the second
33 experiment, the lipolysis level and composition of tank milk from 55 commercial dairy
34 goat farms (25 ML and 30 LL) were analysed, in milk samples taken in three different
35 weeks. The results of the first experiment showed that ML milking significantly
36 increased the free fatty acid (FFA) concentration in raw goat's milk (0.71 vs 0.40
37 mmol/l, respectively). However, in the milk samples taken from commercial farms the
38 FFA concentration remained unaffected by the milking pipeline height (0.59 vs 0.58
39 mmol/l for ML and LL, respectively). No significant differences were found in the milk
40 composition, nor in the sensory characteristics in the cured cheeses, which suggests that
41 factors other than the milkline height are able to influence the level of lipolysis under
42 commercial conditions. Therefore, ML milking should not be discouraged, provided
43 that the correct functioning and management of the milking operation and milk storage
44 on the farm is guaranteed.

45

46 **Keywords:** goat milk, lipolysis, milking system, mid-line milking, low-line milking.

47

48 **1. Introduction**

49 Milk fat lipolysis consists of enzymatic hydrolysis of the triglycerides of fat globules,
50 which leads to an increase in the concentration of free fatty acids (FFA) and,
51 consequently, of the acidity of the fat. In raw milk, lipolysis is mainly caused by the
52 action of lipoprotein lipase (LPL), a natural milk enzyme synthesized in secretory
53 epithelial cells (Chen *et al.*, 2003), although it may be partly haematic in origin
54 (Chilliard *et al.*, 2003). Other enzymes with significant lipolytic activity are lipases of
55 microbial origin, mainly from psychotropic germs (Ouattara *et al.*, 2004), and somatic
56 cell lipases (Gargouri *et al.*, 2008).

57 Different physiological, genetic and nutritional factors have been identified that may
58 influence the LPL activity in raw milk (Deeth, 2006; Chilliard *et al.*, 2014). The good
59 correlation between LPL activity and lipolysis could be due to a higher degree of
60 association of LPL to the fat phase in goat's milk (Chilliard *et al.*, 1984), thus increasing
61 the enzyme-substrate interactions, contrary to what occurs in cow's milk (Chilliard *et*
62 *al.*, 2003). On the other hand, it has also been shown that the factors that impair fat
63 globule membrane integrity, such as excessive shaking or abrupt changes in storage
64 temperature, can increase the lipolysis level in milk by exposing the triglycerides to
65 lipase action (Meffe, 1994; Chen *et al.*, 2003).

66 It should be noted that the hydrolytic release of FFA from triglycerides can have
67 negative consequences for the dairy industry. First, it may affect the technological
68 properties of milk, causing fat loss and delays in the growth of starter cultures used in
69 the production of fermented products such as cheese or yoghurt (IDF, 1991; Collomb
70 and Spahni, 1995). It may also give rise to the appearance of off-flavours, described as
71 rancid, butyric, astringent or even bitter, in milk and its by-products (Le Mens *et al.*,
72 1997; Deeth *et al.*, 2006). For this reason, FFA concentration is often used as an

73 indicator of the organoleptic quality of milk, which is occasionally included in
74 interprofessional regulations for payment by quality (Pirisi *et al.*, 2007; Skeie *et al.*,
75 2014). Additionally, another possible negative effect of lipolysis is that it could affect
76 the analytical results of milk composition obtained with infrared equipment (IDF,
77 2000), as the release of FFA from triglycerides due to lipase action changes the readings
78 (absorbances) from the equipment in certain wavelengths that affect the determination
79 of fat (fat wavelength A, 5.7 μm) and protein (protein wavelength: 6.5 μm). Similarly,
80 Robertson *et al.* (1981) in cow's milk stated that an increase in FFA of 1 meq/l resulted
81 in analyses with infrared equipment showing a decrease in fat (-0.033 %) and an
82 increase in crude protein content (+0.019 %). These analytical changes are also
83 reflected in the IDF standard for cow's milk analysis with mid-infrared based
84 equipment (IDF, 2000).

85 Although lipolysis progresses during milk storage, most of it occurs in the first 24 hours
86 of refrigeration (Wiking *et al.*, 2003; Ouattara *et al.*, 2004), before it reaches the dairy
87 industry. Therefore, the mechanical stress that affects milk in the milking systems
88 becomes a crucial aspect to preserve milk supply and quality.

89 Several works in cattle have demonstrated the relationship between certain milking
90 conditions and the increase in lipolysis in milk (Pillay *et al.*, 1980; Escobar and Bradley,
91 1990; Abeni *et al.*, 2005). One of these factors is milking with a milk line located above
92 the animals standing level, i.e. in mid-line (ML) or high-line (HL), with elevations
93 lower or higher than 1.25 m, respectively (ISO, 2007a). In this type of setup, the milk is
94 mixed with air so that it rises through the long milk tube, forming bubbles and,
95 therefore, being submitted to more turbulent agitation. In cattle, it has been shown that
96 HL milking, in comparison to low-line milking (LL), increases milk lipolysis (Gudding
97 and Lorentzen, 1982; Mikulová, 2011); the higher the milkline height or the air intake

98 caused by the milking cluster, the greater the increase (Judge *et al.*, 1977; Meffe, 1994;
99 Rasmussen *et al.*, 2006). However, in small ruminants information about this is scarce,
100 even though the use of ML milking has become increasingly popular in recent years.
101 This is because, with an equal number of milking stalls, the installation of an ML
102 usually cuts initial investment by around 25-35 % (Díaz *et al.*, 2004) compared to LL.
103 To the best of our knowledge, there are only two studies: one comparing ML vs LL in
104 sheep (Díaz *et al.*, 2004) and another comparing HL vs LL in goats (Morand-Fehr *et al.*,
105 1983), neither of which found statistically significant differences in FFA concentrations
106 in milk. Further studies along these lines would be warranted to determine whether the
107 use of ML or HL milking can negatively affect the quality of goat's milk; a crucial
108 aspect when considering that goat's milk is mainly used to manufacture cheese, whose
109 sensory characteristics might be affected by an increase in the FFA concentration as a
110 result of excessive lipolysis in the milk. In this sense, Morgan *et al.* (2001) noted a high
111 risk of obtaining lactic coagulation cheeses with unacceptable sensory characteristics
112 when the FFA concentration is equal to or greater than 1g oleic acid/100g milkfat (3.5
113 meq/100g milkfat) in goat's milk. However, there is no information on the effect of
114 lipolysis on the sensory quality of cheeses made by enzymatic coagulation, a processing
115 technique widely used in traditional goat cheese-making of Mediterranean countries.
116 There are no studies evaluating the effect of lipolysis in goat's milk on the results of
117 analyses performed with infrared equipment by milk quality laboratories. To this end,
118 the aim of our study was to assess the influence of ML milking system on lipolysis and
119 components of goat's milk that are routinely determined with infrared equipment, taking
120 milk from LL milking as reference. The effect of ML milking on the sensory features of
121 enzymatic coagulation goat cheeses was also evaluated.

122

123 **2. Material and Methods**

124 2.1. Experimental procedure

125 To meet the aforementioned objectives, two experiments were carried out: the first at
126 the dairy goat experimental farm of the Universitat Politècnica de València (UPV,
127 Valencia, Spain) and the second on commercial dairy goat farms, whose bulk milk was
128 routinely analysed at the Interprofessional Dairy Laboratory of the Valencian
129 Community Region (LILCOVAL, Valencia, Spain).

130 2.1.1. First experiment

131 This experiment was carried out in triplicate on the Universitat Politècnica de València
132 (UPV) experimental farm. Each replicate experiment was designed as follows: 62
133 Murciano-Granadina breed goats, halfway through the lactation period (4 ± 1 month of
134 lactation), were used. The animals were divided into two groups of 28 goats each,
135 according to production level and lactation number, with each group randomly assigned
136 to ML or LL milking for an initial 4-day period. Then, the treatments (ML and LL)
137 were switched between the two groups for a second experimental 4-day period.

138 The goats were milked once on a daily basis (8:30 a.m.) following a routine which
139 included machine stripping, manual teatcup removal and iodine post-dipping solution.

140 The milking parlour (2x12) had, two milking pipelines installed with 6 clusters in ML
141 and 12 clusters in LL. The ML milkline, dead in type, was 52 mm in diameter and 520
142 cm in length and was located at 112 cm above goat standing level. The LL milkline,
143 looped type, was 52 mm in diameter, 1,500 cm in length and located at 40 cm below
144 the standing level.

145 The AlmaticTM cluster G50 from DeLaval (Tumba, Sweden) was used in this study.
146 However, in the case of ML milking, a claw from DeLaval cluster SG-TF80 (claw
147 volume 100 ml), was incorporated.

148 A different nominal vacuum was set (40 kPa in ML and 37 kPa in LL) so that the
149 average teat-end vacuum, in the absence of milk flow during milking, was similar in
150 both types of milking systems. The effective reserve (ML: 750 litres/min; LL: 950
151 litres/min) complied with international recommendations (higher than 512 and 804
152 litres/min in ML and LL, respectively; extra air for automatic teatcup valves: 32
153 litres/min; ISO, 2007b). The pulsation rate (90 cycles/min) and ratio (60 %) were the
154 same in ML and LL. The air intakes in the milking cluster (7.5 litres/min) were
155 produced in the inlets at the base of the teatcup liners.

156 The milk from the two groups of animals was stored separately in tanks that were
157 empty at the start of each experimental period and which accumulated the milk
158 obtained during each 4-day set. Bulk milk samples (250 ml) were taken on a daily basis
159 from each batch of animals immediately after milking (0 hours) and 24 hours later, just
160 before starting the next milking, to determine the following variables: FFA content,
161 main milk components (fat, protein, lactose and dry matter), pH, freezing point,
162 somatic cell count (SCC) and total bacterial count (TBC). For the FFA analysis, a 30
163 ml aliquot was separated after sampling, adding hydrogen peroxide (0.02 %) following
164 IDF recommendations (1991). In addition, the bulk milk accumulated during each 4-
165 day period was used to prepare enzymatic coagulation cheeses, whose sensory
166 characteristics were assessed by a testing panel after 50, respectively, 100 days'
167 maturation. The cheesemaking could only be performed in the first experimental
168 replicate.

169 2.1.2. Second experiment

170 Of the 200 commercial dairy goat farms whose milk is usually analyzed by the
171 Interprofessional Dairy Laboratory of the Valencian Community Region (LILCOVAL,
172 Valencia, Spain), 55 were chosen at random, 25 with ML milking and 30 with LL. All

173 these commercial exploitations produce milk from Murciano-Granadina goats and carry
174 out a daily milking routine including machine stripping, manual teatcup removal and
175 some of them use an iodine post-dipping solution.

176 Milking parlours had one or two milking platforms (28 % and 62 %, respectively), most
177 of them having between 12 and 24 stalls per platform. In ML, there was usually a
178 milking cluster for every 2-4 stalls, whereas the most frequent setup in LL was one
179 milking cluster for every 1-2 stalls.

180 Bulk milk samples from commercial farms were taken in 50 ml flasks containing azidiol
181 as preservative (133 μ l/40 ml milk), as stipulated by the Spanish legislation (Real
182 Decreto 752/2011). Milk sampling was performed weekly during three consecutive
183 weeks, between April and May (one sample/week and farm) to determine the same
184 variables as in the first experiment.

185 2.2. Cheese procedure

186 Four batches of cheese were prepared in a commercial artisan cheese factory, with milk
187 collected by LL and ML milking from each of the two periods considered in the first
188 replicate of the experiment.

189 Pasteurised goat's milk (74 °C, 15 s) inoculated with starter cultures and spiked with
190 calcium chloride was coagulated by rennet at 32 \pm 1 °C. After coagulation (40 min), the
191 curd was cut and gently shaken for 20-30 min while the temperature was steadily
192 increased until reaching a maximum of 38 °C. After moulding, the cheeses (900-1000 g)
193 were pressed for two hours under increasing pressure until a pH value of 5.3-5.4 was
194 reached. Next, cheeses were salted immersed in brine (22 Bè) for 4 hours and then
195 placed in an airing chamber (4 °C, 75 % RH) for 48 hours. Finally, the cheeses were
196 kept in a ripening chamber (10-12 °C, 80-85 % RH) for a 100-day period.

197

198 2.3. Analytical procedure

199 FFA quantification was performed, in duplicate, at the Interprofessional Dairy
200 Laboratory of Cantabria Region (LILC, Santander, Spain) using the copper soap method
201 (IDF, 1991). The somatic cell count (SCC) was analyzed with Fossomatic 5000
202 equipment (Foss, Hillerød, Denmark) and the chemical composition of the milk (fat,
203 protein, lactose and dry matter) was analyzed using Milko Scan FT 6000 infrared
204 equipment (Foss). The freezing point and the pH of goat's milk were determined by
205 reference methods using a thermistor cryoscope (Cryostar, Fungger-Gerber, Germany)
206 and a conventional pH meter (Crison Instruments, Barcelona, Spain), respectively. The
207 total bacterial count (TBC) of the milk samples was determined from the standard plate
208 count at 30 °C (ISO, 2013).

209 Sensory analysis of cheeses made with milk from ML and LL milking was performed
210 by a panel of 62 tasters (balanced 50 % by gender and aged from 20 to 55 years)
211 through a triangular test (ISO, 2004). At each tasting session, the judges analyzed two
212 successive triads with cheeses from both experimental periods. These tests were
213 performed repeatedly at 50 and 100 days of maturation, obtaining a total of 248
214 evaluations.

215 2.4. Statistical analysis

216 In the first experiment, the milk quality variables (FFA content, main milk components
217 (fat, protein, lactose and dry matter), pH, freezing point, SCC and TBC) were analyzed
218 by PROC GLM procedures in SAS 9.2, with a model that included the following fixed
219 effects: Milkline (ML and LL), Post-milking time (0 h and 24 h), Replication of the trial
220 (1 to 3), Day of the period (1 to 4), their respective interactions, and the effect of the
221 group of animals within each replicate.

222 The variables of the second experiment were analyzed by PROC MIXED procedures in
223 SAS 9.2, as per Littell *et al.* (1998), using a model that considered the fixed effects of
224 the Milkline (ML and LL), the week of sampling (1 to 3) and their interaction, and the
225 random effect of the farm (1 to 55). In both analyses, when an interaction was non-
226 significant ($P>0.05$), the corresponding interaction term was pooled with the error.

227 The data obtained from the sensorial analysis of the cheeses (frequency of hits in the
228 triangular test) were analysed statistically based on the binomial distribution of the
229 parameter $p= 1/3$ with n responses (ISO, 2004).

230 **3. Results**

231 3.1. Goat's milk quality parameters

232 In the first experiment it was observed that ML milking caused a significant increase
233 ($P<0.001$) in the FFA concentration in goat's milk compared to LL milking (Table 1).
234 This was the case in milk samples taken immediately after milking (ML: 0.64 ± 0.020
235 mmol/l; LL: 0.35 ± 0.020 mmol/l) as well as in those taken after 24 hours of refrigerated
236 storage (ML: 0.77 ± 0.020 mmol/l; LL: 0.45 ± 0.020 mmol/l). A higher level of lipolysis
237 in ML than in LL was also found in each of the three experimental replicates, although
238 in the last replicate a smaller difference was observed (Milkline x Replicate interaction
239 significant, $P<0.05$; Figure 1).

240 The time elapsed since milking also significantly affected the FFA concentration in
241 goat's milk, in such a way that the milk samples taken after 24 h in refrigerated tank
242 storage presented higher values ($P<0.001$) than those taken immediately after milking
243 (Table 1). Concerning the evolution of lipolysis with storage days, Figure 2 shows how
244 the release of FFA in bulk milk tended to increase during the 4-day study period,
245 although the differences were only significant ($P<0.05$) when the values of the first day

246 were compared with those of the following days. All the interactions considered in the
247 model (except the Day x Milkline interaction, described above) were non-significant.

248 The rest of the milk variables analyzed (gross composition, pH-value, freezing point,
249 SCC, and TBC) were unaffected ($P>0.05$) by the milking pipeline height (Table 1). The
250 time elapsed since milking only significantly affected ($P<0.05$) the pH-value which was
251 higher (0.03) in milk samples taken immediately after milking than in those taken at 24
252 h post-milking. The storage Day factor and all the interactions included in the statistical
253 model did not significantly affect the aforementioned variables.

254 On the other hand, in the second experiment, carried out under commercial conditions,
255 the level of lipolysis in the milk did not differ significantly between ML and LL milking
256 (Table 2). Moreover, as shown in Figure 3, goat's milk from most commercial farms
257 presented an FFA concentration between 0.2 and 0.8 mmol/l, regardless of the type of
258 milking installation used. Nor did the two groups of farms differ significantly ($P>0.05$)
259 in milk gross composition (fat, protein, lactose, total solids), pH-value, freezing point,
260 SCC, and TBC (Table 2). The Milkline x Week interaction was also non-significant
261 ($P>0.05$) in all cases.

262 3.2. Sensory analysis of goat's milk cheeses

263 The characteristics of the goat's milk used in each of the cured cheese manufactures are
264 presented in Table 3 and the results of sensory analysis of the cheese samples in Table
265 4.

266 The outcomes show that the judges were not able to perceive significant differences
267 between the two types of cheeses ($P>0.05$) for either of the two maturing times
268 considered (50 and 100 days). On the other hand, when the tasters were successful in
269 differentiating the two types of samples, 63 % of the judgements considered that the

270 cheeses from ML milking presented more intense flavour features (stronger, more acid
271 or spicier) than the cheeses from LL milking, with the latter generally being the main
272 reason for their choice.

273 **4. Discussion**

274 The average FFA concentration found in this work for LL and ML milking in the first
275 experiment (0.40 and 0.71 mmol/l, respectively; 0.88 and 1.5 meq/100g milkfat), and in
276 the second (0.58 and 0.59 mmol/l; 0.96 meq/100g milkfat), falls within the range
277 reported by other authors (Žan *et al.*, 2006; Strzalkowska *et al.*, 2010; Chilliard *et al.*,
278 2014) for goat's milk.

279 The three replicates performed under experimental conditions coincide in demonstrating
280 that ML milking significantly increases the level of lipolysis in goat's raw milk,
281 compared to LL milking, presenting an increase in the FFA concentration of between 62
282 and 92 %. According to Meffe (1994), the height of the milkline above the animals
283 standing level can accentuate deterioration of the membrane of the fat globules through
284 two mechanisms. The first is that the rise of the milk through the long milk tube, mixed
285 with air and in a totally turbulent regime, causes an increase in the air-milk interface
286 (the more the higher the air/milk ratio), giving rise to a greater deformation and risk of
287 rupture of the membrane of the fat globules in said interface. The second mechanism,
288 less important than the previous one, derives from the friction of the milk against the
289 walls of the pipes, subjecting the fat globule to shear forces that can break its
290 membrane. Therefore, as in ML milking installations the length of the long milk tube is
291 usually almost double that in LL (in our case, 215 cm and 100 cm, respectively), it can
292 be assumed that the cited risk will increase. Moreover, it must be noted that in the three
293 assays performed, an increase in the FFA concentration in the milk after 24 hours in the
294 refrigerated storage tank (between 10 and 42 % depending on the type of line and assay)

295 was observed, which agrees with the findings of other authors in cow milk (Wiking *et*
296 *al.*, 2002; Ouattara *et al.*, 2004).

297 However, the fact that commercial farms did not show significant differences in the
298 FFA concentration in goat's milk depending on the type of milking installation (ML *vs.*
299 LL) suggests that the other factors able to influence lipolysis (physiological, genetic and
300 other features of the milking machine, among others; Deeth, 2006; Chilliard *et al.*,
301 2014) are more important overall than the effect of the milk line height alone. In fact,
302 some ML farms repeatedly had low FFA values (0.2-0.4 mmol/l), whereas other farms
303 using LL always presented FFA concentrations higher than 0.8 mmol/l. Thus, it does
304 not seem that the increase in lipolysis that can occur exclusively due to the effect of ML
305 milking is a decisive argument to discourage this type of milking installation.

306 In any case, the FFA concentration in milk from most commercial farms considered in
307 this study was lower than the threshold values applied in quality payment systems for
308 goat's milk used in some regions of France (1.77 meq/100 g milkfat; Pirisi *et al.*, 2007),
309 and Norway (1.33 meq/l; Skeie *et al.*, 2014), regardless of the type of milking system
310 employed. Thus, 97.2 % of goat's milk samples from farms using ML milking
311 presented a FFA concentration lower than the French threshold, more restrictive,
312 whereas for farms with LL milking, this percentage was of 93.9 %.

313 On the other hand, ML milking had no relevant effect on the analytical results of the
314 different milk components analysed by infrared equipment, nor in the experiment one
315 nor in the experiment two, as the differences found were quantitatively of low
316 importance (≤ 0.02 %) and did not reach significance in any case. Similarly, Kaylegian
317 *et al.* (2007) in cow milk, also found no changes in fat and protein values higher than
318 0.01 % when increasing FFA up to 0.2 meq/kg milk.

319 Regarding cheeses, no information is currently available on the maximum thresholds of
320 lipolysis (FFA) in the goat's milk of our indigenous breeds to avoid the deterioration of
321 the sensory characteristics of the flavour of the cheeses, in particular those made by
322 enzymatic coagulation. The organoleptic analysis results suggest that FFA values in
323 milk of 0.79 mmol/l compared to 0.45 mmol/l (1.73 meq/100g milkfat vs. 0.97
324 meq/100g milkfat) do not significantly alter the sensory characteristics of the enzymatic
325 coagulation cured cheeses ($P>0.05$). Nevertheless, these FFA levels are sufficient for
326 some consumers to be able to detect some more intense flavour features (i.e. stronger,
327 more acid or spicier) in the cheeses made with milk from ML milking. In any case,
328 these values are so far from those reported by other authors as causing off-flavours in
329 goat's cheeses (3.5 meq/100g milkfat, Morgan *et al.*, 2001). However, it seems prudent
330 not to directly extrapolate these results to our environment, given the differences in milk
331 composition and the cheese manufacturing process. This topic, therefore, remains open
332 for future studies.

333 **5. Conclusion**

334 In experimental farm conditions, it was found that ML milking significantly increased
335 the FFA concentration in raw goat's milk compared to LL milking system. However,
336 the results obtained on commercial farms failed to confirm these differences, which
337 points to the existence of other factors (related to the animals, feeding or other
338 conditions of the machine and/or milking routine used) that may have a greater
339 influence on the level of lipolysis of the milk than the mere fact of milking in ML or
340 LL. No differences were found in other milk quality parameters, nor were sensory
341 defects in the enzymatic coagulation cheeses perceptible by consumers. There is,
342 therefore, no reason to discourage farmers from this type of milking setup, provided that

343 the correct functioning and management of the milking operation and milk storage on
344 the farm is guaranteed.

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Table 1. Average values of parameters in the bulk milk samples of Murciano-Granadina breed goats according to the type of milking used (ML: mid-line; LL: low-line) and refrigerated storage time (0 and 24 h post-milking) obtained under experimental farm conditions. Statistical non-significance (N.S.= $P>0.05$) or significance ($*P<0.05$ and $***P<0.001$) of milking type and time effects are indicated as superscripts of their respective standard errors (SEM).

Parameter	Milking type			Refrigerated storage time (hours)		
	ML	LL	SEM	0	24	SEM
FFA (mmol/l)	0.71	0.40	0.015 ^{***}	0.50	0.61	0.015 ^{***}
Fat (% w/w)	4.54	4.55	0.018 ^{N.S.}	4.56	4.53	0.018 ^{N.S.}
Protein (% w/w)	3.33	3.33	0.008 ^{N.S.}	3.33	3.33	0.008 ^{N.S.}
Lactose (% w/w)	4.50	4.49	0.006 ^{N.S.}	4.49	4.49	0.006 ^{N.S.}
Dry mater (% w/w)	13.26	13.27	0.027 ^{N.S.}	13.28	13.25	0.027 ^{N.S.}
pH	6.74	6.74	0.008 ^{N.S.}	6.76	6.73	0.008 [*]
Freezing point (°C)	-0.554	-0.554	0.0016 ^{N.S.}	-0.553	-0.555	0.0016 ^{N.S.}
SCC log	6.11	6.11	0.009 ^{N.S.}	6.11	6.11	0.009 ^{N.S.}
TBC log	5.31	5.47	0.086 ^{N.S.}	5.29	5.49	0.090 ^{N.S.}

FFA: Free fatty acids (mmol/l); SCC log: Somatic cell count (cell/ml) logarithm; TBC log: Total bacterial count (cfu/ml) logarithm. Degrees of freedom for milking type and refrigerated storage time are respectively, 1 and 1.

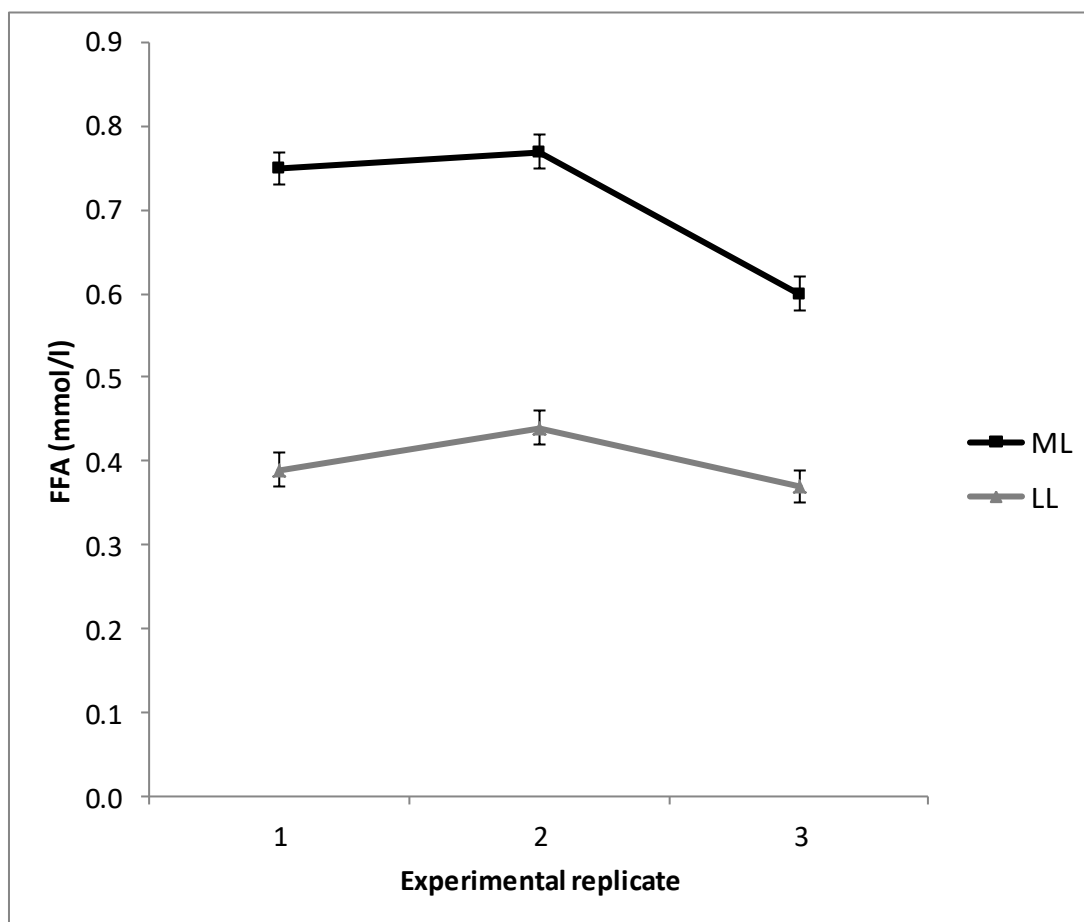


Figure 1. Free fatty acid (FFA) concentration (average value \pm SEM, expressed as mmol/l) in the bulk milk of Murciano-Granadina breed goats according to the type of milking (ML: mid-line; LL: low-line) in each replicate of the study conducted under experimental conditions.

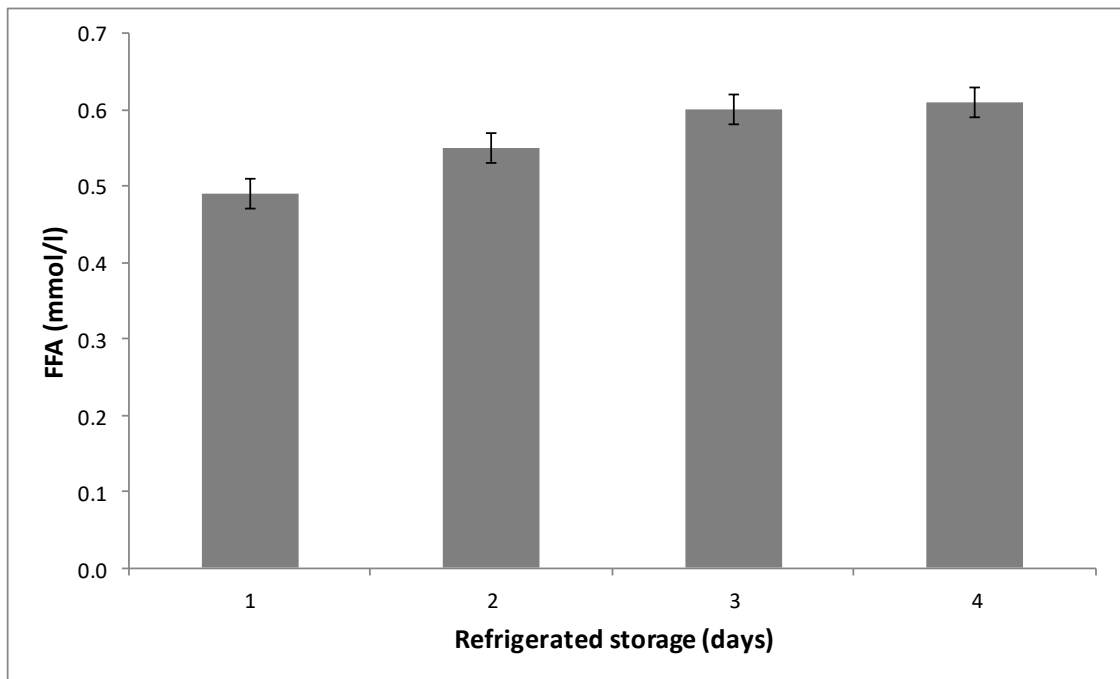


Figure 2. Evolution of free fatty acid (FFA) content (mmol/l) in bulk milk of Murciano-Granadina breed goats during a 4-day storage period (mean values of milk from mid-line and low-line milking).

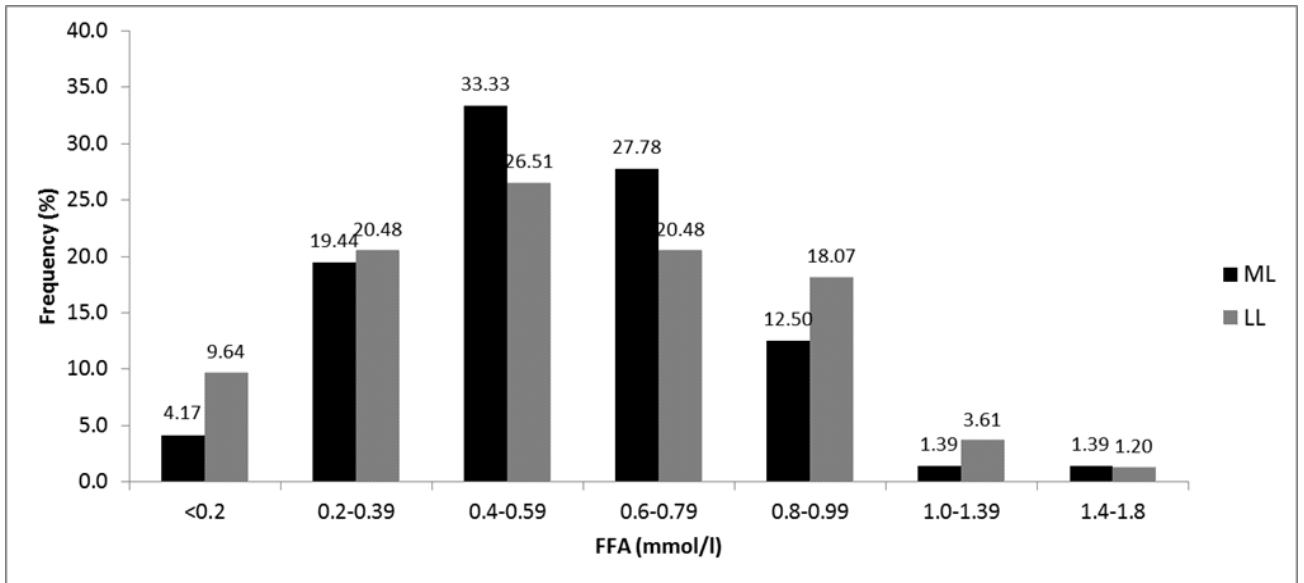


Figure 3. Frequency distribution (%) of free fatty acid (FFA) concentration (mmol/l) in goat milk samples from commercial farms according to the type of milking used (ML: mid-line; LL: low-line).

Table 2. Quality parameters (average value \pm SEM) for goat's milk in commercial farm bulk samples according to the type of milking used (ML: mid-line; LL: low-line)

Variable	Milking type		
	ML (n= 25)	LL (n= 30)	Sig.
FFA (mmol/l)	0.59 \pm 0.049	0.58 \pm 0.045	N.S.
Fat (% w/w)	6.15 \pm 0.137	6.02 \pm 0.126	N.S.
Protein (% w/w)	4.15 \pm 0.096	4.10 \pm 0.088	N.S.
Lactose (% w/w)	4.63 \pm 0.028	4.60 \pm 0.026	N.S.
Dry matter (% w/w)	15.78 \pm 0.222	15.59 \pm 0.203	N.S.
pH	6.74 \pm 0.013	6.73 \pm 0.012	N.S.
Freezing point ($^{\circ}$ C)	-0.556 \pm 0.0026	-0.559 \pm 0.0024	N.S.
SCC log	6.19 \pm 0.046	6.18 \pm 0.042	N.S.
TBC log	4.85 \pm 0.119	4.71 \pm 0.110	N.S.

FFA: Free fatty acid (mmol/l); SCC log: Somatic cell count (cell/ml) logarithm; TBC log: Total bacterial count (cfu/ml) logarithm; N.S.: Statistical non-significant ($P>0.05$).

Degrees of freedom for milking type are 53.

Table 3. Quality parameters of goat’s milk from mid-line (ML) and low-line (LL) milking used for the production of cured cheese in each of the two 4-day periods of the first experiment.

Variable	Period 1		Period 2	
	ML	LL	ML	LL
FFA (mmol/l)	0.76	0.43	0.83	0.47
Fat (% p/p)	4.54	4.53	4.56	4.70
Crude protein (% p/p)	3.46	3.57	3.45	3.49
Lactose (% p/p)	4.54	4.46	4.53	4.43
Dry matter (% p/p)	13.43	13.26	13.43	13.54
SCC (x1000 cells/ml)	1,242	1,167	1,320	1,450
TBC (ufc/ml)	175	160	153	169

FFA: Free fatty acids; SCC: somatic cell count; TBC: Total bacterial count.

Table 4. Results of the sensorial analysis triangular test on cheeses made with milk obtained in the first experimental replicate from mid-line (ML) or low-line (LL) milking, performed at 50 and 100 days of maturation.

Triangular test	Ripening time (days)	
	50	100
Judges	62	62
Triads *	124	124
Hits	46	48
Sig. Lev.	N.S.	N.S.

*: Two triads per judge (one in the first experimental period, and another in the second; N.S.:

Non-significant differences ($P>0.05$).