1   Original Research paper

2   **Effect of Stress on Somatic Cell Count and Milk Yield and Composition in Goats**

3   Amine Mehdid [a], Ana Martí-De Olives [b], Nemesio Fernández [a], Martín Rodríguez [a], Cristòfol Peris [a],*

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5   [a] Institut de Ciència i Tecnologia Animal, Universitat Politècnica de València, Camí de Vera s/n, 46022

6   València, Spain

7   [b] Departamento de Tecnología Agroalimentaria, Escuela Politécnica Superior de Orihuela, Universidad Miguel

8   Hernández, Orihuela, Spain

9   * Corresponding author at: Institut de Ciència i Tecnologia Animal, Universitat Politècnica de València, Camí de

10   Vera s/n, 46022 València, Spain; cperis@dca.upv.es

11   E-mail addresses: mma76@yahoo.fr (A. Mehdid), ana.marti@umh.es (A. Martí-De Olives),

12   nfernandez@dca.upv.es (N. Fernández), mrodriguez@dca.upv.es (M. Rodríguez), cperis@dca.upv.es (C. Peris)

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14   ABSTRACT

15   There is little information about the effect of the stress on Somatic Cell Count (SCC) and milk

16   yield and composition in goats. A total of 40 goats in their 4th month of lactation were

17   assigned to two groups: stress (STR) and untreated (CON). Goats of STR were exposed to

18   acute stress (visual and auditory stimulus from a barking dog for 20 minutes on day 0). After

19   the stress, average values of plasma cortisol were higher in STR than CON ($P < 0.001$);

20   likewise, in STR group cortisol was lower in parity 1+2 goats than parity $\geq$ 3 goats ($P < 0.05$).

21   Stress caused a considerable increase in SCC in parity $\geq$ 3 goats ($P < 0.05$), but not in parity

22   1+2 goats. On average, this increase of SCC was 6-fold compared to values prior to the stress,

23   and it was observed in both healthy and infected mammary glands. This increase was

24   transient, as SCC returned to normal values after 1 to 3 days. On day 1, stressed goats of

25   parity $\geq$ 3 produced 11% less milk compared with day 0 and, regarding milk composition,

26   only lactose showed a significant drop. Stressed parity 1+2 goats showed no changes in SCC

27   and milk yield and composition. We conclude that, in goats, stress is a non-infectious factor

28   that can interfere in the use of SCC as an indirect method of intramammary infection (IMI)

29   detection or, in bulk tank milk, as a commercial milk quality parameter.

30   ***Key words***: Somatic cell count; stress; cortisol; dairy goat; milk yield

31

1. Introduction

The somatic cells in milk are leukocytes (neutrophils, eosinophils, macrophages, lymphocytes) derived from blood circulation, as well as cellular debris and mammary epithelial cells, the former being the majority in ruminants (Boutinaud and Jammes, 2002). It is accepted that mammary inflammation, generally of infectious origin, is the main factor in increasing the somatic cell count (SCC)(Harmon, 1994; Raynal-Ljutovac et al., 2007), besides causing negative effects on milk production and quality (Le Maréchal et al., 2011; Raynal-Ljutovac et al., 2005, 2007; Silanikove et al., 2010;). For this reason, SCC is commonly used in cattle, sheep and goats as a sensitive marker of udder health condition and as a commercial milk quality parameter in bulk tank milk. However, to be able to interpret SCC properly, it is also necessary to take the influence of non-infection factors into account.

One particular feature in goats is that some non-infection factors, such as lactation stage and parity, have a greater influence on SCC than in sheep and cattle (Bergonier et al., 2003; Paape et al., 2007; Raynal-Ljutovac et al., 2007). Thus, in healthy udders the SCC shows a marked increase as the stage of lactation progresses and goats have more parities (De Crémoux et al., 1996; Dulin et al., 1983; Leitner et al., 2007; Luengo et al., 2004). Moreover, the SCC in goats has demonstrated high daily variability (Randy et al., 1988; Zeng et al., 1997), showing notable transient elevations of SCC lasting 1-3 days, in which the SCC increased 2-20 times within a day. Estrus has been shown to raise the SCC in goats (Christodoulopoulos et al., 2008; McDougall and Voermans, 2002; Moroni et al., 2007) and originate transient elevations of SCC which cannot be explained by variations in milk production (Mehdid et al., 2013). However, whether there are other non-infection factors that can also cause these SCC rises in goats remains unknown.

There are several factors on farms (management practices, food, type of housing, environmental conditions) that can end up causing different degrees of stress, affecting the animals' wellbeing. Stress triggers activation of the hypothalamus-pituitary-adrenal axis, an increase in glucocorticoid secretion and a rise in blood leucocytes, together with a reduction of the neutrophils/lymphocytes ratio (Merlot, 2004). In cows, it has been shown that stress caused by transportation increases, in vitro, the migration capacity of neutrophils, which would indicate that they possess a greater capacity to reach the extravascular areas (Yagi et al., 2004). Consequently, we can consider the possibility that a certain degree of stress could increase the SCC in milk. There is very little information available on this aspect in goats. In this specie, some authors suggest that certain apparently stressful situations such as ruminal acidosis, vaccination against enterotoxemia (Lerondelle et al., 1992) and milking (Karzis et al., 2004; Salama et al., 2003) increase the SCC. However, other authors found that the stress induced by 45 minutes of transportation (McDougall et al., 2002) or by application of ACTH (Gaiato et al., 2012) did not affect SCC.

Our hypothesis is that a sufficiently intense stress could give rise to an increase in SCC in the milk obtained at the following milking, although this increase could be different depending on the parity number or mammary gland health status. The aim of the study was to test this hypothesis using goats with different parities (primiparous and multiparous) and mammary health status (with or without intramammary infection- IMI) and to challenge them with a short and acute stress. Milk yield and composition were also determined, and blood cortisol concentration was recorded as a physiological indicator of stress level suffered by the animals (Romero et al., 2015).

2. Material and methods

2.1. Experimental Design

The experiment was carried out at the farm of the Universitat Politècnica de València, using Murciano-Granadina dairy goats which were milked once daily (a more frequent practice than twice daily milking in the farms of this goat breed in our geographical area) at 8:30h. Annual health checks performed by official veterinary services showed that the farm was free from brucellosis, tuberculosis, *Mycoplasma agalactiae* and caprine arthritis-encephalitis virus.

A total of 40 goats (14, 6 and 20 of parity 1, 2 and ≥3, respectively) in their 4th month of lactation and housed into three pens (one for primiparous and two for multiparous) were used. Of these animals 23 had healthy udders, 11 unilateral IMI (2, 1 and 8 of parity 1, 2 and ≥3, respectively) and 6 bilateral IMI (3 and 3 of parity 2 and ≥3, respectively). All 23 halves udder with IMI showed subclinical infections. One infection was caused by Gram-negative bacilli (coliform) and all the rest by coagulase-negative staphylococci (*S. simulans*, n=5; *S. epidermidis*, n=4; *S. xylosus*, n=3; *S. caprae*, n=6; *Staphylococcus* spp., n=4). The experiment was carried out along 9 consecutive days (-4, -3, -2, -1, 0, 1, 2 , 3 and 4). On day -2 the animals were classified into 2 balanced groups (n=20 each) according to parity number, udder health status, SCC and milk production. Each group was assigned at random to control (CON) or stress (STR) treatment (Table 1). At 12:30 h of day 0, after milking, goats from group STR were moved to a 70 m^2 unfamiliar outdoor pen, situated 50 meters far away from the farm, and exposing them to visual and auditory stimulus from a barking dog for 20 minutes. One worker stayed in the pen, holding the dog's collar by the leash to avoid the dog coming into contact with the goats. Thereafter, goats came back inside the building, to their respective pens for blood sampling, and were kept together with CON goats during the rest of the

106   experiment. Variables were recorded in all goats (n=40). Milk production, composition, SCC

107   by mammary gland and whole udder were monitored daily on each goat during the 9 days of

108   experiment. Blood cortisol was recorded on each goat daily for 7 consecutive days (days -3, -

109   2, -1, 0, 1, 2 and 3). Four bacteriological analyses per mammary gland were performed on

110   each goat on days -4, -3, 1 and 3. No presence of abnormal features in mammary secretion

111   (clots, flakes, tints) was recorded during the experiment.

112

113   *2.2. Goat management and feeding*

114   Goats were machine milked once daily (08:30 h) in a routine including machine

115   stripping and dipping of the teats in iodine after teatcup removal. The milking parlor (2 × 12)

116   had 6 clusters (Almatic cluster G50, Delaval Agri, Tumba, Sweden) and a milk pipeline at 1.0

117   m above the platform (midlevel). Milking parameters were set at a rate of 90 pulsations per

118   minute, a vacuum level of 40 kPa, and a 60% pulsation ratio. All goats were permanently

119   stabled (available surface = 1.5 m^2/goat; feeder = 0.4 m/goat) and received the same feed

120   offered per head (as-fed; commercial concentrate for lactating goats = 1.2 kg/d; alfalfa hay =

121   1.0 kg/d; citrus pulp = 2.0 kg/d; ad libitum barley straw). Water was freely available in the

122   pens.

123

124   *2.3. Measured variables*

125   Total daily milk (machine milk plus machine stripping milk) from each animal was

126   recorded using 3.5-L jars, graduated in 50-mL divisions (Esneder Ref. 90001, Industrias

127   Berango S.L., Urduliz; Spain).

128   Samples for udder SCC and milk composition analyses were taken from the total milk

129   extracted from each animal in 50-mL polypropylene flasks with a hermetic seal. To determine

130   the SCC per gland, 40 mL of milk were collected from each teat separately, by manual

131 milking before teatcup attachment. All samples, with azidiol as preservative (0.01g of sodium

132 azide/100 ml), were kept refrigerated (4°C) between 24 and 36 h until analysis in the

133 laboratory. The SCC was analyzed with a Fossomatic 5000 (Foss Electric A/S, Hillerød,

134 Denmark). Milk composition (fat, crude protein, lactose and dry matter) was determined by

135 mid-infrared spectroscopy using a MilkoScan FT120 (Foss Electric A/S).

136   To obtain half udder samples for bacteriological analysis, teats were carefully cleaned

137 with 70% ethanol and the first 3 streams of foremilk were discarded. Approximately 5 mL of

138 milk were collected aseptically from each mammary gland. Samples were kept at 4°C for a

139 maximum of 12 h until bacteriological analysis. Ten microliters of each sample were sowed

140 on blood agar plates (5% washed sheep erythrocytes; Biomerieux, Lyon, France). Plates were

141 incubated aerobically at 37°C and examined at 24 h, 48 h, and 7 d. Cultures with 5 or more

142 identical colonies were considered positive for IMI. Bacterial groups were identified

143 according to National Mastitis Council recommendations (NMC, 2017). Identification of

144 staphylococci was performed using commercial micro methods (API® STAPH; BioMèriexu,

145 Lyon, France).

146   For cortisol analysis, 3.5 mL of blood samples from the jugular vein of each animal

147 were taken daily, always at 13:00 h, with plastic syringes. Samples were transferred to 5 mL

148 glass tubes containing 57 µl of 15% EDTA solution (BD Vacutainer K3; Becton, Dickinson

149 and Company, Franklin Lakes, NJ, USA). Samples were centrifuged immediately after

150 collection at 1500 g for 20 minutes. The blood plasma was distributed into Eppendorf tubes

151 (Eppendorf Iberica SLU, Madrid, Spain) that were frozen and stored at -40º C until analysis.

152 The concentration of cortisol in plasma was analyzed in duplicate at the Animal Physiology

153 Department of the Veterinary Faculty of Complutense University of Madrid (Spain) by the

154 Enzyme Immuno Assay technique (Munro and Lasley, 1988). Cortisol was extracted from

155 plasma using 2 ml of diethyl ether. The assay sensitivity was 0.03 ng/ml; the intra- and inter-

156 assay coefficients of variation were 5.7 and 8.9%, respectively.

157

158 *2.4. Definition of a Transient Elevation of SCC*

159   Only those SCC elevations which fulfilled the following characteristics were

160 considered "Transient Elevation" of SCC (TE$_{SCC}$) of non-infectious origin: 1) In healthy

161 udders, the SCC of each mammary gland underwent an important rise, of at least 2.5 times

162 compared to the day before; in udders with unilateral IMI, this SCC rise took place in both the

163 healthy half udder and the infected half udder. 2) SCC of udder milk samples also had the

164 same rise described above, reaching values of at least 1,000,000 cells/ml (700,000 cells/ml in

165 primiparous). 3) After one or several days (normally 1 to 4 days), the SCC of each mammary

166 gland and udder milk sample returned to similar values to those preceding the increase. Goats

167 with bilateral IMI were not considered to identify TE$_{SCC}$ of non-infectious origin.

168

169 *2.4. Statistical Analysis*

170 SCC of udder milk samples was analyzed using a repeated measures statistical model with

171 the following effects: Group (CON and STR), Parity (1+2 and ≥3), day (-4 to 4), goat (as

172 random; n=1 to 40) and interactions Group x Day, Group x Parity and Group x Parity x Day.

173 Cortisol, milk yield and composition variables were analyzed with same model but including

174 a covariate (milk yield and composition: for each goat, average for days -4 and -3; cortisol:

175 for each goat, result of day -3). SCC and cortisol data were log transformed (Ali and Shook,

176 1980) to normalize their distribution. The possible interaction between the stress and IMI on

177 SCC was studied with the half udders of parity ≥3 (13 healthy half udders and 7 IMI half

178 udders in both CON and STR groups). So, SCC of these 40 half udders were analyzed, in log,

179 using a repeated measures statistical model with the effects of Group (CON and STR), half

180   udder Health Status (IMI or healthy), Day (-4 to 4), half udder (as random; n=1 to 40) and

181   interactions Group x Day, Group x Health Status and Group x Day x Health Status. All these

182   statistical analyses were performed according to Littell et al. (1998) using the PROC MIXED

183   of the SAS Statistical Package (SAS Institute, 2008).

184   To study the relationship of SCC increase with the other variables, regression (Proc

185   REG of SAS) and correlation (Proc CORR of SAS) analyses were performed. Proc FREQ

186   was used to compare frequency of ET_{SCC}.

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188

189 **3. Results**

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191   Plasma cortisol was significantly affected by factors of Group ($P < 0.05$), Day ($P <$

192   0.001), Group x Day interaction ($P < 0.001$) and Covariate ($P < 0.01$); the others factors

193   considered in the statistical model were not significant ($P > 0.05$). The evolution of plasma

194   cortisol during the experiment, in log10, is represented in Fig. 1 for Parity 1+2 goats (Fig. 1a),

195   Parity $\geq$ 3 goats (Fig. 1b) and all goats (Fig. 1c). In the three cases, on day 0 log cortisol was

196   significantly ($P < 0.001$) higher in STR goats compared to CON goats (Fig. 1c: 1.3 ± 0.07 vs

197   0.5 ± 0.07 ng/ml; $P < 0.001$), but differences between both groups were not significant for the

198   remaining days. In addition, we can point out that, in STR goats, the average cortisol values

199   on day 0 was higher in Parity$\geq$ 3 goats than in Parity 1+2 goats (1.4 ± 0.09 vs 1.1 ± 0.09

200   ng/ml; $P < 0.05$).

201   LogSCC in udder milk was only affected significantly by the factors of Day ($P <$

202   0.001) and Parity ($P < 0.01$). as well as the interactions Group x Day ($P < 0.001$) and Group x

203   Day x Parity ($P < 0.001$). This triple interaction is related with the different evolution of

204   logSCC along days of the experiment according the Group and Parity factors. So, in Parity

1+2 goats the stress did not increase the SCC in STR group compared with CON group (Fig.

2a and 3a). However, in Parity $\geq$ 3 goats, logSCC of STR group showed an significant

increase on day 1 compared to CON group (log SCC= 6.50 $\pm$ 0.165 vs 5.90 $\pm$ 0.165 cells/ml,

respectively; $P < 0.05$; Fig. 2b). The SCC increase in STR was 6-fold with respect to the

values prior to the stress (3.1 million cells/ml and 0.49 million cells/ml, for geometric means

of SCC on days 1 and 0, respectively; Fig. 3b). With respect to the Parity factor, logSCC was

lower in Parity 1+2 goats than in Parity $\geq$ 3 goats (5.34 $\pm$ 0.109 vs 5.87 $\pm$ 0.109 cells/ml,

respectively; $P < 0.01$).

During the experiment, no Parity 1+2 goats in the CON and STR groups presented a

TE$_{SCC}$, according to the definition specified in Materials and Methods. In Parity $\geq$3 goats, we

identified a significantly ($P < 0.05$) more frequency of TE$_{SCC}$ in STR group (7 of 8 goats with

healthy or unilateral IMI udders) than in CON group (2 of 9 goats). In group STR all TE$_{SCC}$

appeared on day 1 and lasted for 1 day (2 goats of parity=3), 2 days (2 goats of parity=3 and

4) and 3 days (3 goats of parity $\geq$ 4), before returning to the previous values. In the CON

group, the 2 cases of TE$_{SCC}$ appeared on day -1 and lasted for 2 days (2 goats of parity= 3 and

4).

At mammary gland level (only Parity $\geq$3 goats), logSCC was affected significantly by

the factors Day ($P < 0.001$) and half udder Health Status (healthy: 5.48 $\pm$ 0.050; infected: 6.18

$\pm$ 0.068 cells/ml; $P < 0.001$), as well as the interactions Group x Day ($P < 0.001$) and Group x

Day x half udder Health Status ($P < 0.01$). On day 1, logSCC of parity $\geq$3 goats was

significantly higher in STR group with respect to CON group (log SCC= 6.50 $\pm$ 0.074 vs 5.84

$\pm$ 0.074 cells/ml, respectively; $P < 0.001$; Figure 4c), and this increase was observed in both

healthy mammary glands (Figure 4a) and infected mammary glands (Figure 4b). On day 2,

SCC continued to be significantly higher in STR group with respect to the CON group when

considering only healthy half udders ($P < 0.05$; Fig. 4a) or all the half udders ($P < 0.01$;

230   Figure 4c). Moreover, we can highlight that stress caused the SCC of healthy mammary

231   glands to increase until reaching similar values to the infected mammary glands of non-

232   stressed goats (log SCC: 6.23 ± 0.120 vs 6.25 ± 0.088 cels/ml; $P > 0.05$). The increase in SCC

233   due to stress depended on the previous values, but the trend was different depending on the

234   definition of this increase. If increase is expressed as difference (SCC day 1 – SCC day 0),

235   this was higher in the mammary glands that already set out with a high SCC (Fig. 5a; $r^2=0.70$;

236    $P < 0.001$). On the contrary, if the increase in SCC is expressed as a ratio (SCC day 1/SCC

237   day 0), this tended to diminish in the mammary glands with higher counts beforehand (Fig.

238   5b; $r^2=0.23$; $P < 0.05$).

239   With respect to milk yield and composition, statistical analysis results showed that, in

240   each of these variables, its covariate was significant ($P < 0.001$) while the Group and Parity

241   factors and Group x Day and Group x Parity interactions were not significant ($P > 0.05$). The

242   Day factor only affected to milk yield and protein significantly ($P < 0.001$ in both cases).

243   Finally, the triple interaction Group x Parity x Day was significant only for the milk yield

244   ($P<0.001$) and lactose ($P < 0.05$) variables. Table 2 presents the milk yield and composition

245   results on day 1 (first milking after the stress was applied), according to Group and Parity. We

246   can see that stressed parity ≥3 goats presented lower milk production and lower lactose

247   content compared to CON goats of parity ≥3; these differences were small (drop of 11.4% in

248   milk yield and 2.9% in lactose) but statistically significant ($P < 0.01$; Table 2). In contrast, in

249   goats of parity 1+2, these two variables did not present significant differences between CON

250   and STR group goats (Table 2). On day 1, fat, protein and dry matter variables did not differ

251   significantly between STR and CON groups (Table 2). On the other days of the experiment,

252   milk yield and composition variables did not differ significantly between the two groups

253   studied, both in the goats of Parity 1+2 and in Parity ≥3 and considering all goats.

254   Finally, considering only STR goats of parity ≥3, the increase in SCC at udder level on

255   day 1 had no significant correlation with plasma cortisol (day 0) or with milk yield decrease

256   (day 1). Moreover, in these goats the correlation between milk yield decrease on day 1 and

257   plasma cortisol (day 0) was not significant either.

258

259   **4. Discussion**

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261   This work has experimentally demonstrates, for the first time to our knowledge, that

262   stress can cause a marked elevation of SCC in lactating goats of parity ≥3. This increase is

263   transitory with a duration of 1 to 3 days, which is similar to the effect of estrus in goats

264   (Mehdid et al., 2013). Although the increase of SCC presented a great individual variability

265   (from 2.5-fold to 35-fold higher than the SCC values previous to stress), the average was

266   higher than the increase produced by estrus (6-fold increase in this work and 3.5 and 4-fold

267   for the estrus; Mehdid et al., 2013; Moroni et al., 2007). The fact that all the TEscc in the STR

268   group were concentrated on day 1 suggests that they were caused by the stress suffered by the

269   animals on day 0. In the CON group, we also identified two cases of TE$_{SCC}$ (both on day -1),

270   so it is possible that they were caused by estrus. In any case, both non-infection factors, stress

271   and estrus, raised the SCC sufficiently enough to interfere in its use as an indirect detection

272   method for IMI or, in bulk tank milk, as a commercial milk quality parameter.

273   Another notable aspect is that the increase in SCC due to stress was only found in

274   Parity ≥ 3 goats, but not in the younger animals. This is a difference compared to the effect of

275   estrus, as this factor also produces an augmentation of SCC in primiparous goats (Mehdid et

276   al., 2013). Romero et al. (2015) did not find that acute stress for 5 minutes increased the SCC,

277   but these authors only used primiparous IMI-free goats, which would coincide with what we

278   observed in the present work. Other authors did not find that the stress induced by 45 minutes

279 of transportation (McDougall et al., 2002) or by application of ACTH (Gaiato et al., 2012)

280 affected SCC, but in both studies the distribution of goats according to parity number was not

281 described and, therefore, we cannot know if it differs with our results. In ewes aged from 4 to

282 6 years subjected to stress (isolation test for 10 minutes), Caroprese et al. (2010) observed that

283 SCC was higher in high-cortisol ewes than in low-cortisol ewes, but the differences were

284 small (geometric means of 0.426 and 0.223 million cells/ml, respectively). Regarding the

285 cortisol records in our experiment, we must specify that in order to rule out any possible

286 circadian variation, the blood samples were taken at the same time each day, at 13:00 h, that

287 is, at a time of day when the cortisol levels are low in non-stressed goats (Kokkonen et al.,

288 2001; Romero et al., 2015). Two hypotheses could be formulated to explain the different

289 behavior of the goats with three or more lactations compared to the younger goats. The first

290 hypothesis would be that the younger goats suffered less stress (despite the fact that all

291 received the same treatment), as the average for cortisol was lower in parity 1+2 than parity

292 ≥3 goats; this low stress would be insufficient to trigger an increase of leukocytes in the blood

293 and/or increased leukocyte transfer to the mammary gland and milk. However, two aspects

294 prompt us to question this initial hypothesis: a) three goats of parity 1+2 and three goats of

295 parity ≥3 had similar cortisol values (22-30 ng/ml) but only the latter shown an increase of

296 SCC.; b) in stressed goats of parity ≥3, no significant correlation was observed between

297 cortisol (on day 0) and the increase in SCC (on day 1). The second hypothesis would be that

298 as goats grow older or undergo more lactations, anatomical-physiological changes are

299 generated in the mammary gland that would allow a greater transfer of leukocytes from blood

300 to milk. This hypothesis is coherent with the fact that in healthy udders the SCC is higher in

301 parity ≥3 goats than in primiparous goats (De Cremoux et al., 1996; Leitner et al., 2007;

302 Luengo et al., 2004).

303   Stress raised the SCC in both healthy and infected mammary glands in parity ≥3 goats.

304   In 77% of the healthy glands (10 of 13) SCC values higher than 1 million cells/ml were

305   reached, i.e., counts higher than the single threshold suggested by some authors for the

306   detection of IMI in goats (Contreras et al., 1996; Haenlein, 2002; Bergonier et al., 2003).

307   Moreover, We can also highlight that the increase in SCC due to stress (as difference: SCC

308   day 1 – SCC day 0) tended to increase as the cell counts presented by the mammary glands

309   rose. This suggests that the impact of stress on the SCC of bulk milk will be greater in herds

310   that present a high percentage of goats ≥3 parities, and the higher the prevalence of IMI, as

311   SCC increases with parity and presence of IMI (Raynal-Ljutovac et al., 2007). SCC of bulk

312   milk is used as an indicator of the health status of the herd and, in addition, many industries

313   use it to set the price of goat milk to be paid to farmers (Pirisi et al., 2007). So, given that

314   stress and estrus give rise to transitory elevations of SCC in goats, the interpretation of SCC at

315   goat level (IMI detection) or bulk tank level should never be based on a single milk sample,

316   but rather in several samples taken at intervals of at least one week.

317   Stress also caused lower milk production in goats of ≥3 parities. This falloff in

318   production was minor (near to 11%) and similar to that found due to estrus effect (decrease of

319   13%; Mehdid et al., 2013), and is not enough to explain the increase in SCC. The fact that in

320   these animals the stress also slightly lowered the lactose content in the milk could be related

321   with the drop in the milk production, as lactose is the primary osmotic regulator of milk

322   volume (Baumgard et al., 2017). The effect of stress on lactose is in agreement with Sano et

323   al. (1985), as these authors found that a severe heat stress decreased the mammary glucose

324   uptake and the lactose concentration in goat milk . The fact that stress in the parity 1+2 goats

325   did not affect the milk yield and composition agree with Romero et al. (2015) who found the

326   same result when IMI-free primiparous goats suffered an acute stress for 5 minutes. Gaiato et

327   al. (2012) found no alteration of quantitative and composition of milk produced in goats

328 punctually stressed via application of ACTH, but in this study the distribution of goats

329 according to parity number was not described. In ewes, Caroprese et al. (2010) also found that

330 a short-term acute stress had no effect on the secretion of milk components. In any case, the

331 fact that goats have a great ability to adapt to harsh environments (Silanikove, 2000) could

332 explain the limited impact of the stress on the milk yield and composition.

333

334 **5. Conclusions**

335 In this work we have demonstrated that an acute and punctual stress caused a

336 considerable rise of SCC in goats ≥3 parities. This increase was transitory, as the animals

337 recovered the pre-stress counts in a period of 1 to 3 days, and took place both in healthy and

338 infected mammary glands. In these goats, the milk production was also slightly reduced (drop

339 of 11%), along with lactose. In the younger goats, the stress studied did not affect the SCC

340 and milk yield and composition. We conclude that, in goats, stress is a non-infection factor

341 that can interfere with the use of SCC as an indirect method for detection of IMI or, in bulk

342 tank milk, as a commercial milk quality parameter.

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