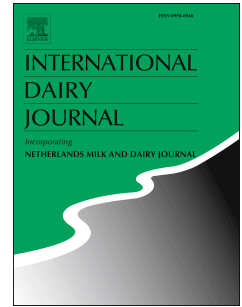# Accepted Manuscript

Effects of different genotypes at the CSN3 and LGB loci on milk and cheese-making characteristics of the bovine *Cinisara* breed

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1 **Effects of different genotypes at the CSN3 and LGB loci on milk and cheese-making**

2 **characteristics of the bovine *Cinisara* breed**

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27 ABSTRACT

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29 The *Cinisara* is an autochthonous Sicilian bovine breed reared mainly for production of

30 Caciocavallo Palermitano, a typical stretched-curd cheese. The distribution of A and B alleles at

31 both the β-lactoglobulin (LGB) and κ-casein (CSN3) loci in *Cinisara* cows and their influence on

32 milk traits and cheese yield and composition were analysed. The LGB alleles are associated with

33 significantly different effects on whey protein level (lower for BB genotype, $P<0.01$), and casein

34 index (higher value for BB genotype, $P<0.001$), while CSN3 alleles were associated with

35 significantly different effects on milk yield and coagulation properties; the BB genotype showed

36 higher values for milk yield ($P<0.01$) and curd firmness (a_{30} $P<0.01$ and a2r $P<0.01$) and lower

37 values for coagulation and curd firming time ($P<0.01$) than the AA genotype. Cheese made with

38 LGB BB milk showed higher percentage protein recovery ($P<0.01$); cheese made with CSN3 BB

39 milk showed higher percentage fat recovery ($P<0.05$).

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1.   Introduction

The protection of autochthonous genetic resources is a strategic tool to maintain the history and the culture of particular habitats, the ecological systems, and the local products that often show specific organoleptic and nutritional characteristics. All autochthonous breeds, thanks to natural selection, are adapted to their specific environments; autochthonous breeds are also more disease-resistant and are able to survive, reproduce and produce in harsh environmental conditions in which other more productive breeds fail to capitalise on their genetic potential. As a consequence, autochthonous breeds are an important tool for conservation of animal biodiversity (Ciotola et al., 2009). In this context, the production of Caciocavallo Palermitano cheese, made with *Cinisara* milk, can assist local economic development and contribute to the protection of indigenous genetic resources, since it is characterised by a strong link with the autochthonous breed and the territory.

The *Cinisara* breed is a dual-purpose bovine population reared in Sicily. The main distribution areas consist of the agricultural marginal areas in the province of Palermo, with an average herd size of 25 animals (DAD-IS database; FAO, 2016). The breed is characterised by medium size, black colour and a strong resistance to the summer high temperatures typical of the region; these cows produce about 3700 kg of milk per lactation (AIA, 2014). Since the number of individuals (about 5000 heads) is low, the *Cinisara* breed belongs to the "Italian Registrar for native cattle with a limited diffusion" (*Registro Anagrafico delle razze bovine autoctone a limitata diffusione*).

The importance of *Cinisara* breed, which is a Slow Food presidium, relies on its capability to exploit difficult environments and areas otherwise unsuitable for other breeds, and on the production of Caciocavallo Palermitano cheese, which is aged for up to one year and is available during all four seasons. According to Bonanno et al. (2013), extensive farming systems with cows fed at pasture are beneficial for physical, chemical, and sensory characteristics of this cheese,

66   production of which is based on traditional techniques involving the use of wooden tools and

67   addition of lamb or kid rennet.

68   Of course, dairy production is also affected by milk protein variants. The description of the

69   first milk protein polymorphism can be traced back to 1955, when Aschaffenburg and Drewry

70   (1955) discovered the two most frequent β-lactoglobulin (LGB) alleles, A and B. At present, 11

71   alleles are known at this locus (Caroli, Chessa, & Erhardt, 2009; Farrell et al., 2004; Gallinat et al.,

72   2013), which is located on chromosome BTA11 (Hayes & Petit, 1993). Later studies showed that

73   LGB alleles greatly influence the β-lactoglobulin content of milk, with a reduced synthesis for cows

74   with the LGB B allele compared with LGB A allele of about 25%. Furthermore, LGB BB milk

75   shows a higher amount of total casein, from +0.08% to +0.13%, as well as a lower level of whey

76   proteins (–12%). These differences are associated with a notable positive effect of the LGB B allele

77   on cheese-making parameters (Di Stasio & Mariani, 2000; Heck et al., 2009).

78   The polymorphism at the κ-casein locus (CSN3) was the last to be discovered. This locus is

79   located on chromosome BTA6 in a 250-kb stretch where the four casein genes (CSN1S1, CSN2,

80   CSN1S2, and CSN3) are clustered (Ferretti, Leone, & Sgaramella, 1990; Threadgill & Womack,

81   1990). At present, at least 12 variants are known at the CSN3 locus, with A and B alleles being the

82   most frequent (Caroli et al., 2009). Several studies have reported conflicting results on the effects of

83   these two alleles on some dairy traits. Nearly all studies reported that the CSN3 B allele is

84   associated with higher levels of total protein and higher amounts and proportions of caseins in milk

85   compared with CSN3 A allele (for a review see Di Stasio & Mariani, 2000). On the other hand, no

86   effect of the CSN3 alleles was observed on protein and casein content in milk by Graml,

87   Buchberger, Klostermeyer, and Pirchner (1985) and Nilsen et al. (2009). Furthermore, the CSN3 B

88   allele has been associated with a higher cheese yield in cheeses such as Cheddar, Mozzarella,

89   Parmigiano-Reggiano, Svecia, and Gouda (Buchberger & Dovč, 2000). As far as milk coagulation

90   properties, several studies consistently associated the CSN3 B allele with a more uniform micellar

91 pattern and consequently a lower coagulation time and greater curd firmness (Bittante, Penasa, &

92 Cecchinato, 2012; Di Stasio & Mariani, 2000). In contrast, the CSN3 E allele is associated with

93 unfavourable milk coagulation properties (Caroli, Bolla, Budelli, Barbieri, & Leone, 2000; Jõudu et

94 al., 2009; Kübarsepp et al., 2006), probably due to the presence of micelles with a low percentage of

95 κ-casein (Ikonen, Ojala, & Syväoja, 1997).

96 The aim of this study was to characterise *Cinisara* breed for LGB and CSN3 loci and, for

97 the first time in this breed, analyse the influence of these loci on milk traits, cheese yield and

98 composition.

99

100 **2. Materials and methods**

101

102 *2.1. Animals and Management*

103

104 A total of 326 *Cinisara* lactating cows, from 15 farms located in the typical production area

105 of *Caciocavallo Palermitano* cheese (Sicily, Italy), were analysed. In these farms, animals were

106 reared in an extensive system and, during the spring period from mid-March to late May, fed mainly

107 with natural pasture.

108

109 *2.2. DNA Analysis*

110

111 Blood samples were obtained from the coccygeal vein using Vacutainer (Becton, Dickinson

112 and Company, Franklin Lakes, NJ, USA) tubes containing potassium ethylene diamine tetra-acetic

113 acid (K-EDTA). DNA from 200 μL of whole blood was extracted using NucleoSpin Blood

114 QuickPure (Macherey Nangel, Germany) following guideline procedures.

5

115   A 397 bp fragment of the CSN3 gene was amplified from 326 *Cinisara* DNA samples using

116 the following primers: forward C1 5'CCAAGCCCAGCCAACTACCA3'; reverse C2:

117 5'AGCCCATTTCGCCTTCTCTGTAA3'.

118   A 422 bp fragment of the LGB gene was amplified from the same DNA samples using the

119 following primers: forward L1 5'TCTCCCTGGCTCCATCTGACTTC3'; reverse L2:

120 5'GGAAGCAGGTGGCACGGCAGT3'.

121   PCR reactions for both CSN3 and LGB amplification were carried out in a final volume of

122 50 μL containing 200 ng DNA, 1X PCR buffer, 3 mᴍ MgCl2, 400 μᴍ dNTPs, 20 pmol each

123 primer, and 2.5 U Taq DNA polymerase (Promega, USA).

124   The PCR reactions were accomplished as follow: denaturation for 45 sec at 95 °C;

125 annealing/extension for 1 min at 70 °C, for 35 cycles for both amplifications. The CSN3 PCR

126 products were digested separately with *Hin*dIII and *Hae*III restriction endonucleases to distinguish

127 A and B alleles and A and E alleles, respectively. The LGB PCR products were digested with *Hph*I

128 restriction endonuclease to identify A and B alleles. The PCR-RFLP products were analysed on 2%

129 agarose gels stained with ethidium bromide.

130

131 *2.3.   Milk and cheese, sampling and analysis*

132

133   Three hundred and seven individual milk samples, collected during the morning milking,

134 were analysed for fat, lactose and somatic cell count by an infrared method (Combi-foss 6000, Foss

135 Electric, Hillerød, Denmark). The pH and titratable acidity were measured by a pH meter (HI 9025;

136 Hanna Instruments Inc., Ann Arbor, MI, USA) and as Soxhlet-Henkel degrees (°SH 50 mL^{-1}),

137 respectively. Total nitrogen (TN), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) were

138 determined by standard FIL-IDF procedures (FIL-IDF, 1964, 1993) according to milk nitrogen

139 fractions indicated by Aschaffenburg and Drewry (1959). From these nitrogen fractions, total

140 protein (TN×6.38), casein {[(TN-(NCN×0.994)]×6.38}, whey protein [(NCN-NPN)×6.38] and

6

141 casein index [casein/(TN×6.38)] were calculated. Individual milk samples were evaluated for

142 clotting parameters by measuring clotting time (r, min), curd-firming time (k_{20}, min), curd firmness

143 (a_{30}, mm) and curd firmness after 3 times the clotting time (a_{2r}, mm) with a Formagraph instrument

144 (Foss Electric).

145 A laboratory micro cheese-making process was developed to manufacture pressed-curd type

146 cheeses from each of 307 individual milk samples. The main equipment consisted of a heater fitted

147 with thermostat and a four water baths equipped with a digital temperature controllers and pumps

148 for water mixing to ensure homogeneous temperature. Every water bath could contain 6 vats

149 (capacity 2000 mL); thus, the apparatus allowed processing of 24 samples simultaneously. The milk

150 samples collected from each farm were processed during the same cheese-making trial, and so 15

151 batches of cheeses were manufactured, each corresponding to the farm of origin. Each refrigerated

152 (4 °C) sample of raw and whole milk (1700 mL), contained in a pyrex glass beaker, was heated in

153 the water bath for about 30 min to reach 37 °C. After addition of 8.75 mL calf liquid rennet

154 (1:15,000, 80 ± 5% chymosin, and 20 ± 5% pepsin; Chr. Hansen, Parma, Italy) diluted in distilled

155 water (1.6:100), milk was maintained at 37 °C for 1 h until coagulation. The curd was then broken

156 using a glass stick until it was reduced into small cubes like rice grains. After cooking at 80 °C for 4

157 min in the water bath, the curd was removed from the beaker and pressed with hands into a

158 cylindrical, perforated plastic mould of 10 cm diameter to drain the whey, and turned every 3 min to

159 facilitate draining. After 15 min, each mould was held in the water bath at 60 °C for 1 h. Then the

160 cheese was placed on a flat surface for draining, weighed after 1 h, and transferred to a cellar for 7

161 days at a temperature of 16 °C and a relative humidity of 80%. For each sample, cheese yield at 1 h

162 and 7 days and water loss between days 1–7 were measured.

163 Individual cheese samples at 7 days were analysed for chemical composition and physical

164 characteristics. Cheeses were evaluated, using standard FIL-IDF methods, for dry matter (IDF,

165 1982), protein (IDF, 1964a), fat (IDF, 1986) and ash (IDF, 1964b) content. Fat and protein recovery

166 in cheese were calculated as percentage of fat and protein in the cheese with respect to the fat and

7

167 protein content in the corresponding milk. Cheese pH was measured directly with a pH-meter

168 equipped with a spear electrode FC 200 (HI 9025; Hanna Instruments Inc.).

169

170 *2.4. Statistical analysis.*

171

172   Statistical analysis was performed using the GLM procedure of SYSTAT 13 (SYSTAT

173 2009, Software Inc., Chicago, IL, USA) and analysis included CSN3 genotype (AA, AB, BB), LGB

174 genotype (AA, AB, BB), days in milk classes (DIM: 0–101, 102–203, 204–305), farm (FM: 1–15)

175 and the interaction CSN3 × LGB as fixed factors. The differences between means were tested with

176 Fisher's LSD test. Significance was declared at $P \leq 0.05$, and tendencies were declared at

177 $0.05 < P \leq 0.10$. Pearson's correlation coefficients and Bonferroni probabilities were calculated

178 between the parameters measured in this study.

179

180 **3. Results and discussion**

181

182 *3.1. CSN3 and LGB genotyping*

183

184   A total of 326 *Cinisara* cows were typed at the CSN3 and LGB loci; genotype and allele

185 frequencies observed at both loci are shown in Table 1, together with those reported by Chiofalo,

186 Micari, and Sturniolo (1981) and Guastella, Marletta, Bordonaro, and D'Urso (2006). Only two

187 animals with the CSN3 AE genotype were identified and are not considered in Table 1 and for the

188 following analyses. This is the first time that carriers of CSN3 E allele were reported in the *Cinisara*

189 breed. The identification of carriers of the CSN3 E allele should be monitored by breeders to avoid

190 an increase in the frequency of this allele, which is associated with poor milk coagulation properties

191 (Caroli et al., 2000). According to data shown in Table 1, genotype distributions at both loci are in

192 Hardy-Weinberg equilibrium (Falconer & Mackay, 1996). Furthermore, comparison with data of

8

193   Chiofalo et al. (1981) and Guastella et al. (2006) shows that, in the last 34 years, LGB allele

194   frequencies did not change, whereas the CSN3 B allele frequency was reduced by about 11% in the

195   *Cinisara* breed.

196

197   *3.2.   Effects of CSN3 and LGB loci on milk traits*

198

199   The least-squares means and standard errors for milk and cheese analysed traits are given in

200   Table 2, and indicate that genotypes at the CSN3 locus significantly affect milk yield. In particular,

201   CSN3 BB and AB cows produce a significantly higher quantity of milk than do CSN3 AA cows

202   (i.e., 12.6 and 12.0 versus 9.9 kg day^{-1}, respectively). These results show that, to increase milk

203   yield, the actual decreasing trend of the CSN3 B allele frequency, in the Cinisara breed, should be

204   reversed. Furthermore, LGB AA cows show a tendency ($P<0.10$) to produce more milk than LGB

205   BB and AB cows (i.e., 12.8 versus 10.8 and 10.9 kg day^{-1}, respectively). These results, together

206   with those of several authors who observed both significant and null effects of the different CSN3

207   and/or LGB genotypes on milk yield (Bonfatti, Di Martino, Cecchinato, Vicario, & Carnier, 2010;

208   Deb et al., 2014; Gonyon et al., 1987; Ikonen, Ojala, & Ruottinen, 1999; Lin et al., 1989; Van

209   Eenennaam & Medrano, 1991), can be explained by linkage disequilibrium between alleles of the

210   analysed loci and quantitative trait loci (QTLs) affecting milk yield. In fact, analysis of the

211   chromosomal distribution of the QTLs affecting the variability of bovine productions

212   (www.animalgenome.org and references therein) shows that both the CSN3 (BTA6) and LGB

213   (BTA11) loci are embedded in genomic regions where different authors have identified QTLs with

214   effects on milk yield in different breeds.

215   As a consequence, dominance effects in the two heterozygous genotypes and the strongly

216   significant interaction (epistasis) between these two loci for milk yield (Table 2) could be ascribed

217   to QTLs rather than to CSN3 and LGB loci. In particular, analysis of data in Table 2 shows that

218   dominance effects are +0.75 kg day^{-1} for CSN3 and –0.9 kg day^{-1} for LGB, whereas data obtained

9

219 from cows clustered according to the two genotypes (not shown) reveal that the maximum value of

220 epistatic interaction (+0.78 kg day^{-1}, corresponding to a 7.5% higher daily milk production) is

221 observed in the homozygous CSN3 BB × LGB AA genotypes.

222   LGB genotypes significantly affect non-casein nitrogen level ($P<0.01$), whey protein level

223 ($P<0.01$), and casein index ($P<0.001$). In particular, the LGB BB genotype was associated with

224 significant lower non-casein nitrogen and whey protein levels, and higher casein index. In the

225 *Cinisara* breed, we observed the same results as were consistently reported by several authors in

226 different breeds (for a review see Di Stasio & Mariani, 2000). The effects of the LGB BB genotypes

227 on whey protein content, and consequently on casein index, were known since the first

228 identification of the polymorphism at the LGB locus (Aschaffenburg & Drewry, 1955), and could

229 be due to nucleotide differences in the promoter regions associated with differential LGB allelic

230 expression (Martin, Szymanowska, Zwierzchowski, & Leroux, 2002).

231   Analyses of clotting parameters (Table 2) showed that CSN3 BB milk has shorter

232 coagulation and curd firming times and stronger curd firmness (a_{30}) compared with CSN3 AA and

233 AB milk. Again, the results that were observed for these parameters in the *Cinisara* breed agree

234 with those consistently reported by several authors (for a review see Bittante et al., 2012) and could

235 be determined by an effect of the CSN3 polymorphism on the stabilisation and size of casein

236 micelles (Walsh et al., 1998). In particular, the CSN3 BB genotype is associated with a larger total

237 micellar surface, which facilitates the action of rennet (Di Stasio & Mariani, 2000; Mariani et al.,

238 1976).

239

240 *3.3.   Effects of CSN3 and LGB loci on cheese-making parameters*

241

242   In Table 3, the effects of CSN3 and LGB genotypes on cheese yield and composition are

243 reported. In contrast to what might be expected according to the observed effects of CSN3

244 genotypes on clotting parameters and LGB genotypes on casein index, we did not observe any

10

245 effects of genotypes at both loci on cheese yield. Similar results were reported by Glantz, Lindmark

246 Månsson, Stålhammar, and Paulsson (2011). Furthermore, Bonfatti et al. (2011) found that bulk

247 milk with high CSN3 B content gave higher cheese yield compared with milk with a lower

248 proportion of CSN3 B. In this study, milk from cows with CSN3 BB and LGB BB genotypes gave

249 a slightly higher cheese yield, and the differences with the other genotypes did not reach significant

250 levels.

251   According to data shown in Table 3, genotypes CSN3 BB and AB are significantly

252 associated with greater fat recovery percentage in cheese. In addition, CSN3 BB genotype is

253 associated, as a tendency ($P \leq 0.10$), with a lower cheese water loss during the first 7 days after

254 manufacture. This result could be due to the better milk-clotting parameters of the CSN3 BB and

255 AB milk, which could be responsible for an increase in fat entrapment (Choi & Ng-Kwai-Hang,

256 2002; Mariani et al., 1976; Walsh et., 1998) and water retention during the cheese-making process.

257 In this regard, Alipanah and Kalashnikova (2007) also observed a higher fat recovery in the curd for

258 milk produced by cows with CSN3 BB and AB genotypes. Moreover, the LGB BB genotype is

259 significantly associated with a higher protein recovery in cheese (Table 3) as a consequence of the

260 effect of this genotype on casein index (Di Stasio & Mariani, 2000) and on casein retention in curd

261 (Hallén, Lundén, Allmere, & Andrén, 2010). These results are in accordance with those of Bittante,

262 Cipolat-Gotet, and Cecchinato (2013) who found that protein and, to a lesser extent, fat recovery in

263 curd have higher heritabilities than those of fat and protein content in milk. Nevertheless, in this

264 study, the higher fat and protein recoveries in cheeses from CSN3 BB and AB genotypes, and the

265 LGB BB genotype, respectively, showed only weak and not significant effects in increasing cheese

266 yield. However, on the whole, the cheese yield expressed as DM percentage was moderately

267 correlated with both fat (r = 0.45; $P<0.001$) and protein recoveries (r = 0.30; $P<0.001$), as already

268 observed by Bittante et al. (2013). Finally, since cheese yield and the recovery of milk components

269 are strongly influenced by cheese-making technology (Cipolat-Gotet, Cecchinato, De Marchi, &

270 Bittante, 2013; Jakob & Puhan, 1992), and we used a micro cheese-making procedure, further

11

analyses are necessary to evaluate if similar results can be observed for the production of

Caciocavallo Palermitano cheese, which requires a different cheese-making procedure and is

normally subjected to a longer period of ripening.

4. Conclusions

In this paper, the effects of CSN3 and LGB genotypes on dairy traits measured in the

Cinisara breed are reported for the first time. As expected, and in accordance with the results

observed by several other authors in different breeds, CSN3 genotypes affected milk-clotting

parameters and LGB genotypes affected whey protein percentage and casein index. In addition,

effects of the different genotypes at both loci on milk yield of the *Cinisara* breed were observed. In

this case, linkage disequilibrium between alleles at both loci and QTLs affecting milk yield could

explain the observed results, including interaction effects, with the consequence that the CSN3 and

LGB loci can be considered as genetic markers for this trait. Finally, no significant effects of the

genotypes at these loci on milk total casein content and cheese yield were observed. The higher

percentages of milk fat and protein retention, observed in cheeses from CSN3 BB and LGB BB,

respectively, are worth being further investigated with respect to the cheese-making processes used

to obtain the typical dairy products.

Acknowledgements

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Table 1

Genotype distributions and allele frequencies at the CSN3 and LGB loci of the bovine *Cinisara* breed.[a]

Locus	N	Genotypes			Allele frequencies		References
		AA	AB	BB	A	B	
CSN3	324	76	144	104	0.460	0.540	This study
	248	58	79	111	0.393	0.607	Chiofalo et al. (1981)
	156	29	85	42	0.458	0.542	Guastella et al. (2006)
LGB	326	20	97	209	0.210	0.790	This study
	248	14	82	152	0.222	0.778	Chiofalo et al. (1981)
	156	6	58	92	0.224	0.776	Guastella et al. (2006)

[a] For this study, CSN3 $\chi^2 = 3.56$, $P>0.05$; LGB $\chi^2 = 3.50$, $P>0.05$.

Table 2

Least square mean and standard error (SE) of milk traits and clotting parameters of genotypes at κ-casein (CSN3) and β-lactoglobulin (LGB) loci of *Cinisara*

breed.

Item	Genotype CSN3			Genotype LGB			Significance		
	AA	AB	BB	AA	AB	BB	CSN3	LGB	CSN3 × LGB
Milk traits									
Animals	72	133	102	20	95	192			
Days in milk	154±6	148±5	147±5	148±8	150±4	151±3	ns	ns	ns
Milk yield (kg day^{-1})	9.9±0.57^B	12.0±0.18^A	12.6±0.57^A	12.8±0.83$^{\&}$	10.9±0.40$^{\$}$	10.8±0.30$^{\$}$	**	†	**
Somatic cells count (log$_{10}$ mL^{-1})	5.26±0.09	5.04±0.08	5.07±0.09	5.05±0.14	5.23±0.07	5.05±0.05	ns	ns	ns
pH	6.5±0.03	6.5±0.02	6.5±0.03	6.5±0.04	6.5±0.02	6.5±0.01	ns	ns	ns
Titratable acidity (°SH 50 mL^{-1})	3.9±0.14	4.1±0.11	3.8±0.14	3.8±0.20	4.0±0.10	4.1±0.07	ns	ns	ns
Fat (%)	3.39±0.14	3.41±0.12	3.33±0.14	3.17±0.20	3.49±0.10	3.48±0.07	ns	ns	ns
Lactose (%)	4.99±0.04	5.00±0.03	5.02±0.04	5.02±0.06	4.98±0.03	5.02±0.02	ns	ns	ns
Total nitrogen (%)	0.594±0.010	0.593±0.010	0.584±0.010	0.590±0.020	0.590±0.010	0.591±0.010	ns	ns	ns
Non-casein nitrogen (%)	0.141±0.004	0.140±0.003	0.136±0.004	0.145±0.006^A	0.141±0.003^A	0.132±0.002^B	ns	**	ns
Protein (%)	3.79±0.06	3.78±0.05	3.73±0.06	3.77±0.09	3.76±0.04	3.77±0.03	ns	ns	ns
Casein (%)	2.89±0.06	2.89±0.05	2.86±0.06	2.85±0.08	2.87±0.04	2.93±0.03	ns	ns	ns
Whey protein (%)	0.70±0.02	0.69±0.02	0.67±0.02	0.72±0.04^A	0.70±0.02^A	0.64±0.01^B	ns	**	ns
Casein index (%)	0.764±0.006	0.765±0.005	0.767±0.006	0.755±0.008^B	0.763±0.004^B	0.778±0.003^A	ns	***	ns
Clotting parameters									
Coagulation time (r; min)	18.8±0.69^A	18.1±0.59^A	16.0±0.70^B	17.6±0.99	18.0±0.49	17.3±0.37	**	ns	ns
Curd firming time (k$_{20}$; min)	4.3±0.33^{Aa}	3.3±0.28^{Ab}	2.7±0.34^B	3.7±0.48	3.4±0.24	3.1±0.18	**	ns	ns
Curd firmness (a$_{30}$; mm)	29.7±1.97^B	35.9±1.68^B	39.3±1.99^A	33.7± 2.87	34.7±1.40	36.5±1.07	**	ns	ns
Curd firmness (a$_{2r}$; mm)	35.1±1.33^B	41.6±1.19^A	42.8±1.34^A	39.5± 1.93	39.0±0.94	41.0±0.72	***	ns	ns

[a] Means within a row with different superscript upper and lower case letters and with symbols differ at *P*≤0.01, *P*≤0.05 and *P*≤0.10, respectively. For

significance columns: ***, *P*≤0.001; **, *P*≤0.01; †, *P*≤0.10; ns, not significant

Table 3

Least square mean and standard error (SE) of cheese composition of genotypes at κ-casein (CSN3) and β-lactoglobulin (LGB) loci of

Cinisara breed.

Item	Genotype CSN3			Genotype LGB			Significance		
	AA	AB	BB	AA	AB	BB	CSN3	LGB	CSN3 × LGB
Cheese yield at 1 h (%)	12.59±0.33	12.60±0.27	12.75±0.33	12.21±0.47	12.81±0.23	12.92±0.17	ns	ns	ns
Cheese yield at 7 d (%)	11.07±0.29	11.16±0.24	11.37±0.29	10.75±0.42	11.38±0.20	11.47±0.15	ns	ns	ns
Cheese water loss 1–7 days (%)	12.2±0.60$^\&$	11.2±0.50$^{\&\$}$	10.8±0.60$^\$$	11.81±0.86	11.19±0.42	11.21±0.31	†	ns	ns
Dry matter (DM) at 7 d (%)	53.49±0.67	54.13±0.56	52.96±0.67	53.15±0.97	53.62±0.47	53.82±0.35	ns	ns	*
Cheese yield 7 d (% DM)	5.88±0.16	6.00±0.13	6.00±0.16	5.70±0.23	6.04±0.11	6.14±0.08	ns	ns	ns
Protein (% DM)	49.62±0.90	49.75±0.75	49.53±0.90	50.51±1.30	49.06±0.62	49.34±0.46	ns	ns	ns
Fat (% DM)	35.29±1.23	36.74±1.03	35.48±1.23	35.29±1.78	36.28±0.86	35.96±0.63	ns	ns	ns
Ash (% DM)	5.14±0.11	5.20±0.09	5.38±0.11	5.42±0.16	5.16±0.07	5.15±0.05	ns	ns	†
Fat recovery in cheese (%)	61.09±1.63^b	65.90±1.36^a	64.90±1.63^a	64.77±2.36	63.34±1.13	63.79±0.84	*	ns	ns
Protein recovery in cheese (%)	80.26±0.67	79.86±0.56	80.49±0.67	78.96±0.97^B	79.97±0.47^B	81.70±0.35^A	ns	**	ns
pH at 7 d	6.03±0.09	6.16±0.07	6.17±0.08	6.17±0.10	6.09±0.07	6.11±0.07	ns	ns	ns

[a] Means within a row with different superscript upper and lower case letters and with symbols differ at $P \leq 0.01$, $P \leq 0.05$ and $P \leq 0.10$, respectively. For

significance columns: **, $P \leq 0.01$; *, $P \leq 0.05$; †, $P \leq 0.10$; ns, not significant.