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Goat farm variability affects milk Fourier-transform infrared spectra used for predicting coagulation properties

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## INTERPRETIVE SUMMARY

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**Goat farm variability affects milk Fourier-transform infrared spectra used for predicting coagulation properties.** *By Dadousis et al. page 000.* Fourier-transform infrared spectroscopy (FTIR) is widely used to predict milk protein and fat content in cattle and small ruminants, while its usefulness in various production, health and environmental traits is under continuous research. Driven by the large amount of goat milk destined for cheese production, in this study we investigated the potential of FTIR to predict milk coagulation and curd firmness (cheese related) traits in goats. Our results evidenced important farm variability that should be taken into account when developing FTIR prediction equations for milk coagulation traits in goats.

PREDICTION OF COAGULATION TRAITS IN SARDA GOAT MILK

**Goat farm variability affects milk Fourier-transform infrared spectra used for predicting coagulation properties.**

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## ABSTRACT

Driven by the large amount of goat milk destined for cheese production, and to pioneer the goat cheese industry, the objective of this study was to assess the effect of farm in predicting goat milk coagulation and curd firmness traits via Fourier-transform infrared spectroscopy (FTIR). Spectra from 452 Sarda goats belonging to 14 farms in Central and South- East Sardinia (Italy) were collected. A Bayesian linear regression model was used, estimating all spectral wavelengths' effects simultaneously. Three traditional milk coagulation properties [rennet coagulation time (RCT, min), time to curd firmness of 20 mm ( $k_{20}$ , min) and curd firmness 30 min after rennet addition ( $a_{30}$ , mm)] and three modeled over time curd firmness measures [( $RCT_{eq}$ : RCT estimated according to curd firmness change over time);  $k_{CF}$ : instant curd firming rate constant and  $CF_P$ : asymptotical curd firmness] were considered. A stratified cross-validation (SCV) was assigned evaluating each farm separately (validation set; VAL) and keeping all the rest farms to train (calibration set; CAL) the statistical model. Moreover, a SCV where 20% of the goats, randomly taken (ten replicates per farm), from the VAL farm entered the CAL set, was also considered ( $SCV_{80}$ ). To assess model performance, coefficient of determination ( $R^2_{VAL}$ ) and the root mean squared error of validation were recorded. The  $R^2_{VAL}$  varied between 0.14 to 0.45 ( $k_{CF}$  and  $RCT_{eq}$ , respectively), albeit the standard deviation was approximating half of the mean, for all the traits. Although, average results of the two SCV procedures were similar, in  $SCV_{80}$  the maximum  $R^2_{VAL}$  increased at about 15% across traits, with the highest being observed for  $k_{20}$  (20%) and the lowest for  $RCT_{eq}$  (6%). Further investigation evidenced important variability among farms, with  $R^2_{VAL}$  for some of them being close to 0. Our work outlined the importance of taking into account the effect of farm when developing FTIR prediction equations for coagulation and curd firmness traits in goats.

**Key words:** goat, coagulation, curd firmness, farm, infrared spectra

## INTRODUCTION

A large proportion of world goat milk is destined to cheese production, especially in those countries included in the Mediterranean basin (FAOSTAT, 2018). This region is characterized by adverse weather and environmental conditions, in which autochthonous goat breeds are well adapted and usually managed **in** extensive or semi-extensive management types (Di Trana et al., 2015; Stella et al., 2018). It has been shown that the farming system represents a very large source of variation (ranging between 16 to 70% of the total variability) in milk composition and milk processing characteristics, such as the coagulation properties (Pazzola et al., 2018b). These values are greater compared to those of bovine (between 9 to 16%; Bittante et al., 2015) and ovine (from 16 to 43%; Vacca et al., 2015) farming methods. Indeed, a great variability of goat farming has been reported (Usai et al., 2006). The importance of the type of farming system relates with the destination of the milk produced and the genetics of the animals (Pazzola et al., 2018b). For instance, harsh environments and extreme extensive management are more suitable for indigenous breeds (Di Trana et al., 2015), able to produce a milk **characterized by** better composition (e.g., high milk fat and protein), and technological characteristics than **that from** cosmopolitan breeds (Čermak et al., 2013; Paschino et al., 2020).

Among the milk technological characteristics, traditional milk coagulation properties (**MCP**) are widely used to describe the complex process of cheese-making. Moreover, the extension of MCP through the calibration of the curd firmness as a function of time (**CF<sub>t</sub>**) provides a more complete overview of the coagulation process (Bittante, 2011). There is an extensive and well-documented literature on the importance and relevance of MCP, mainly in cattle (Bittante et al., 2012; Stocco et al., 2017; Nilsson et al., 2019), but also in sheep (Caballero-Villalobos et al., 2018; Cipolat-Gotet et al., 2018) and to a less extent in goats (Vacca et al., 2020). In addition, MCP show heritability estimates between 0.15 – 0.27 in cattle (Dadousis et al., 2016) and 0.09 – 0.19 in sheep (Bittante et al., 2017). Hence, directional selection on desirable MCP characteristics is applicable. This could be of particular interest in goats, especially for those breeds (e.g., Alpine, Toggenburg) characterized by

73 weak or non-expressing alleles (e.g., F, N allele) of  $\alpha_{s1}$ -casein, associated with unfavorable  
74 coagulation process (Maga et al., 2009; Devold et al., 2011). However, high **MCP analysis** costs and  
75 logistics pose restriction for their wide-scale application.

76 Nowadays, a potential solution to overcome those limitations can be derived via Fourier-  
77 transform infrared (**FTIR**) spectroscopy. Indeed, there is an increasing interest in the dairy sector on  
78 the usefulness of FTIR information for the prediction of a variety of phenotypes (Tiplady et al., 2019),  
79 either directly measurable in milk (e.g., fatty acids; Soyeurt et al., 2006) or related to the milk  
80 processing characteristics (e.g., cheese-making traits, MCP; Ferragina et al., 2013; Visentin et al.,  
81 2017) and the animal condition (e.g., energy efficiency, lameness; McParland and Berry, 2016;  
82 Bonfatti et al., 2020). In dairy cattle, recent advanced research made applicable MCP predictions via  
83 FTIR spectroscopy in the milk payment system of some Protected Designation of Origin (PDO)  
84 cheese consortia to reward or penalize dairy farmers (e.g., Trentigrana PDO cheese; Benedet et al.,  
85 2018). In the case of small ruminants, the practical use of the FTIR predictions along the dairy chain  
86 is still lacking. Although there is ongoing research in sheep on the use of FTIR spectroscopy for the  
87 prediction of MCP and  $CF_t$  parameters (Correddu et al., 2016; Ferragina et al., 2017), up to present,  
88 there are no data available in goats.

89 An important factor to consider when developing prediction equations via milk FTIR spectra  
90 is the structure of the data, especially for traits not directly measurable in milk (e.g., technological  
91 traits, animal health, environment). In bovine milk, it has been shown that a random cross-validation  
92 (**CV**) might overestimate the prediction accuracy of methane emission traits (Wang and Bovenhuis,  
93 2019). Rather, a stratified CV, where for example each farm is evaluated separately, might provide a  
94 more realistic model assessment (Wang and Bovenhuis, 2019). In previous studies, great variability  
95 was observed in different goat farming systems (Usai et al., 2006) and in MCP and  $CF_t$  parameters  
96 among individual farms (Pazzola et al., 2018; Vacca et al., 2018). Hence, the type of goat farm is a  
97 factor that should be assessed and its effect quantified on FTIR prediction models for MCP and  $CF_t$   
98 parameters.



125 reach the final value of 0.0513 international milk clotting units/mL of milk]. Coagulation process  
126 occurred at 35°C. The MCP recorded were: rennet coagulation time (RCT, min), time to curd  
127 firmness of 20 mm (k<sub>20</sub>, min) and curd firmness 30 min after rennet addition (a<sub>30</sub>, mm).

128 During lactodynamographic analysis, the Formagraph instrument records every 15 s the width  
129 (mm) of the oscillatory graph designed by the pendula immersed in the milk samples after rennet  
130 addition. Consequently, 120 curd firmness (CF) observations are recorded for each individual milk  
131 sample. The 30 min test analysis allowed to use the following 3-parameter model (Bittante, 2011):

$$132 \quad CF_t = CF_P \times (1 - e^{-k_{CF}(t-RCT_{eq})})$$

133 where CF<sub>t</sub> is curd firmness at time t (mm); CF<sub>P</sub> is the asymptotical potential value of CF at an  
134 infinite time in absence of syneresis (mm); k<sub>CF</sub> is the curd-firming instant rate constant (%/min); and  
135 RCT<sub>eq</sub> is RCT estimated by CF<sub>t</sub> equation on the basis of all data points (min). Values of the  
136 aforementioned traits out from the interval of the mean ±3 standard deviations (SD) were considered  
137 outliers and excluded from further analysis.

138 For each milk sample, a FTIR spectrophotometer (MilkoScan FT6000; Foss, Hillerød,  
139 Denmark) was used to assess milk composition (fat and protein; ISO-IDF 2013), and to collect the  
140 spectrum over the range from wavenumber 5,011 to 925 × cm<sup>-1</sup>. Spectra were stored as absorbance  
141 (A) using the transformation A = log(1/T), where T is the transmission. Two spectral acquisitions  
142 were performed for each sample, and the results were averaged before data analysis.

143 Somatic cell count (SCC) was determined by Fossomatic 5000 (Foss Electric A/S, Hillerød,  
144 Denmark) according to ISO-IDF standard (2006), and later transformed into the logarithmic somatic  
145 cell score [SCS = log<sub>2</sub>(SCC × 10<sup>-5</sup>) + 3; (Ali and Shook, 1980)]. Total bacterial count was determined  
146 using a BactoScan FC150 analyzer (Foss Electric A/S, Hillerød, Denmark) according to ISO-IDF  
147 standard (2004), and transformed into the logarithmic bacterial count [LBC = log<sub>10</sub> (total bacterial  
148 count/1,000)].

149

150 *Statistical Analysis and FTIR Spectra*



151 *Modeling and Repeatability of Coagulation Traits*

152 Files containing the 120 CF values for each milk sample were processed fitting a curvilinear  
153 regression with the PROC NLIN procedure (SAS Institute Inc., Cary, NC). The parameters of each  
154 individual equation were estimated employing the Marquardt iterative method (350 iterations and  
155  $10^{-5}$  level of convergence).

156 To estimate the coefficient of repeatability (%), MCP and  $CF_t$  parameters (2 replicates per  
157 goat), were analyzed using a MIXED procedure (SAS Institute Inc., Cary, NC) that included the  
158 random effects of farm, animal, pendulum (measuring unit of the Formagraph instrument) and the  
159 residual. The coefficient of repeatability (**REP**, %) for MCP and  $CF_t$  parameters was then calculated  
160 as the ratio of the sum of the variances of the random effects of farm, animal and pendulum to the  
161 total variance.

162 *Spectra Editing and Chemometric Model*

163 Prior to spectra analysis, the absorbance values of every wavelength in the FTIR spectra of  
164 the milk samples, were centered and standardized to a null mean and a unit sample variance. To detect  
165 outliers, Mahalanobis distances were calculated by means of the Mahalanobis function implemented  
166 in the R software (R Core Team, 2013). No samples were discarded because all the spectra presented  
167 a distance value lower than the  $\text{mean} \pm 3$  standard deviations. The spectra were not subjected to any  
168 other mathematical pretreatment.

169 A Bayesian linear regression was used to predict the RCT,  $k_{20}$ ,  $a_{30}$ ,  $RCT_{eq}$ ,  $k_{CF}$  and  $CF_P$ . All  
170 phenotypes were regressed to 1,060 spectra under the following model:  $y = \mu + \sum_{j=1}^{1,060} x_{ij}\beta_j + e_i$ ,  
171 where  $\mu$  is the overall mean,  $x_{ij}$  are the FTIR wavelengths,  $\beta_j$  are the regression coefficients and  $e_i$   
172 the residual with  $iid \sim N(0, \sigma_e^2)$ . The BayesB model implemented in the *BGLR* R package was  
173 adopted (de los Campos and Perez-Rodriguez, 2014) as described in Ferragina et al. (2017).

174 *Stratified Cross-Validation Procedures*

175 A stratified external cross-validation (**SCV**) scheme was used to assess model's predictive  
176 ability, where one farm at a time consisted of the validation set (**VAL**). Goats from the remaining  
177 farms were consisted of the calibration (**CAL**) set. The procedure was repeated 14 times, such that  
178 all farms were evaluated. In addition, to assess the importance of shared variability between CAL and  
179 VAL, a SCV where 20% of the goats from one farm to be validated was included in CAL, and the  
180 VAL set consisted of the remaining 80% of the goats from the evaluated farm, was considered  
181 (referred to as **SCV<sub>80</sub>** hereafter). To account for individual sampling variability, the 20% of the goats  
182 was sampled at random and the procedure was repeated 10 times per farm. Results from SCV were  
183 averaged across the 14 farms and, in the **SCV<sub>80</sub>**, over the ten replicates per farm. For all calibrations,  
184 model performance was measured using the coefficient of determination (**R<sup>2</sup>**), the root mean squared  
185 error (**RMSE**), and the SD of both CAL and VAL sets.

186

## 187 **RESULTS AND DISCUSSION**

### 188 *Prediction Accuracy of Goat Milk Coagulation Traits*

189 Descriptive statistics and prediction results of the SCV are presented in Table 2. Mean values  
190 were consistent with those reported in the Sarda goat milk literature (Pazzola et al., 2018a).  
191 Repeatability of coagulation traits ranged from 98% (for RCT and RCT<sub>eq</sub>) to 84% (for k<sub>CF</sub> and CF<sub>P</sub>).  
192 The CF measurements (a<sub>30</sub> and CF<sub>P</sub> traits) are generally characterized by a reduced instrumental  
193 repeatability and reproducibility in later time after rennet addition, which is more profound after  
194 gelation (Ferragina et al., 2017). Compared to other species, repeatability values of goat RCT, RCT<sub>eq</sub>  
195 and CF<sub>P</sub> traits were similar to that of bovine (Stocco et al., 2017) and ovine (Ferragina et al., 2017).  
196 Goat milk is generally characterized by slower increase of curd firmness, weaker casein network  
197 forming after gelation, and earlier syneresis compared to bovine and ovine milk (Inglingstad et al.,  
198 2014; Pazzola et al., 2018b; Roy et al., 2020). Because of these characteristics of the goat coagulation  
199 process and, because the traditional lactodynamograph set up for analysis of bovine milk was  
200 designed to explore primarily the coagulation and the first part of curd-firming process, not syneresis,

201 a slight decrease of repeatability of CF measurements after RCT is expected. For this reason REP is  
202 commonly very high for the first traits measured (e.g., RCT and RCT<sub>eq</sub>) and tends to decrease over  
203 time both in the case of traditional and modeled coagulation traits (Stocco et al., 2015). This  
204 phenomenon is explained by the fact that, during the test, the variation related to the curd-firming and  
205 syneresis tends to accumulate over time. In the present study, only a<sub>30</sub> showed higher REP value than  
206 those reported for bovine (Stocco et al., 2017) and ovine milk (Ferragina et al., 2017). This could be  
207 due to the fact that milk from Sarda goats of the present study is characterized by very good milk  
208 quality (e.g., high fat and protein contents; Table 1) and coagulative aptitude, faster gelation and curd-  
209 firming, and firmer coagulum than other dairy goat breeds (e.g., Alpine, Saanen; Vacca et al., 2018).  
210 Among the factors influencing the reliability of the FTIR predictions, the goodness (repeatability and  
211 accuracy) of the reference values is very important (Caredda et al., 2016). Indeed, it is interesting to  
212 notice that the prediction accuracy decreased with progressed coagulation (e.g., higher for RCT and  
213 lower for a<sub>30</sub>), along with decreasing REP values (Table 2).

214       Regarding SCV predictions (Table 2), RCT and RCT<sub>eq</sub> showed the highest R<sup>2</sup><sub>CAL</sub> (0.64 and  
215 0.61, respectively), followed by CF<sub>P</sub> (R<sup>2</sup><sub>CAL</sub> = 0.50). The remaining traits had R<sup>2</sup><sub>CAL</sub> < 0.50, while the  
216 lowest was observed for k<sub>CF</sub> (0.37). In general, results in the CAL set were comparable to those  
217 reported in ovine milk (Ferragina et al., 2017), in particular for the traits directly related to curd  
218 firmness (a<sub>30</sub> and CF<sub>P</sub>). In the VAL set, the R<sup>2</sup><sub>VAL</sub> was lower and the RMSE was higher, albeit with  
219 much higher SD for both parameters compared to CAL, while the ranking among traits was analogous  
220 to the CAL. Since this was the first study investigating the effect of farm on the prediction accuracy  
221 of MCP and CF<sub>t</sub> parameters in goat milk via FTIR spectroscopy, comparison with literature was  
222 restricted. However, a recent study (unpublished data) assessing the goat breed (four breeds  
223 considered) effect on the prediction of MCP and CF<sub>t</sub> parameters via FTIR spectroscopy, by using a  
224 random 5-fold CV procedure, reported R<sup>2</sup><sub>VAL</sub> from 0.42 to 0.68 for MCP (RCT and a<sub>60</sub>, respectively)  
225 and from 0.14 to 0.60 for CF<sub>t</sub> parameters (syneresis rate and CF<sub>P</sub>, respectively). The study also  
226 confirmed decreased prediction accuracies in a SCV scenario (using three breeds as CAL, and the

227 remaining breed as VAL set), suggesting the importance of considering the breed of goats while  
228 developing FTIR calibrations. Similar to those results, our study showed the importance of  
229 considering the differences among farms on the prediction accuracy of MCP and CF<sub>t</sub> parameters. This  
230 variability was evident observing the high SD of both R<sup>2</sup><sub>VAL</sub> and RMSE<sub>VAL</sub> (Table 2), higher  
231 compared with a previous study on the same traits and statistical methodology in sheep (Ferragina et  
232 al., 2017).

233

### 234 *Effect of Farm Variability on the Prediction Accuracy of Coagulation Traits*

235 By including 20% of the VAL farm in the TRN set (SCV<sub>80</sub>), our expectation was to increase  
236 R<sup>2</sup><sub>VAL</sub>, since important variation was included in the model training, and also because, **by using** this  
237 approach, CAL and VAL dataset are not completely independent (Figure 1). On average, R<sup>2</sup><sub>VAL</sub>  
238 remained the same as the SCV procedure, and was of 0.45, 0.32, 0.29, 0.44, 0.17 and 0.33 for RCT,  
239 k<sub>20</sub>, a<sub>30</sub>, RCT<sub>eq</sub>, k<sub>CF</sub> and CF<sub>P</sub>, respectively, with also similar SD to the SCV (data not shown).  
240 However, although the minimum R<sup>2</sup><sub>VAL</sub> was again close to 0, the maximum obtained R<sup>2</sup><sub>VAL</sub> values  
241 were increased (0.87, 0.73, 0.73, 0.85, 0.65 and 0.79 for RCT, k<sub>20</sub>, a<sub>30</sub>, RCT<sub>eq</sub>, k<sub>CF</sub> and CF<sub>P</sub>,  
242 respectively); representing an increase of ~20% for k<sub>20</sub>, ~16% for RCT, a<sub>30</sub> and k<sub>CF</sub>, ~14% for CF<sub>P</sub>,  
243 with the minimum (~0.06%) for the RCT<sub>eq</sub>. On average, R<sup>2</sup><sub>VAL</sub> results for each coagulation trait  
244 among farms presented in Figure 1 were analogous to the SCV, albeit with no repetitions per farm in  
245 that case. A considerable R<sup>2</sup><sub>VAL</sub> variation among farms was observed (Figure 1). Interaction between  
246 farm and trait was also present. More precisely, across the traits, we observed: i) farms with either  
247 low or high variability of prediction model performance (e.g., F02 and F11 for RCT, respectively),  
248 ii) consistent high or low R<sup>2</sup><sub>VAL</sub> values, relative to the remaining farms across the traits (e.g., F02 vs.  
249 F12), iii) different R<sup>2</sup><sub>VAL</sub> patterns, showing either high or low R<sup>2</sup><sub>VAL</sub> (e.g., F01 and F10 comparing  
250 k<sub>CF</sub> to all the rest of the traits), iv) **general low predictability of k<sub>CF</sub> trait** with three farms (F01, F04  
251 and F08) showing R<sup>2</sup><sub>VAL</sub> close to 0, v) **similar variation patterns across farms of RCT and RCT<sub>eq</sub>**  
252 **traits**, and **interestingly, vi)** farm F12 showed R<sup>2</sup><sub>VAL</sub> close to 0 across all traits. Obviously, the overall

253 model performance presented in Table 2 and Figure 1 was improved (data not shown) when excluding  
254 this specific farm (F12). It is important to consider that the region where milk samples were collected  
255 has been characterized for decades by extensive and semi-extensive goat farming management, highly  
256 variable among areas of the island (Usai et al., 2006). As aforementioned, the variability of farms  
257 affects both composition and coagulation ability of goat milk (Vacca et al., 2018; Pazzola et al.,  
258 2018b). Hence, variability of  $R^2_{VAL}$  among farms was, up to an extent, expected. In particular, two of  
259 the farms (F11, F12) are located in a high altitude and adverse-environmental-conditions area. Those  
260 factors, together with the lower hygienic control practiced by the farmers over the goats (the flocks  
261 are let free to graze without supervision in extensive farms), represent a source of milk quality  
262 variation (Pazzola et al., 2018b), that further influences the processing characteristics. For example,  
263 changes occurring at milk composition and coagulation level often caused by bacterial or somatic cell  
264 counts are well documented in goats (Barrón-Bravo et al., 2013; Stocco et al., 2019). In addition, the  
265 high genetic variability characterizing the Sarda breed (Dettori et al., 2015; Pazzola et al., 2018a),  
266 and other non-genetic factors (e.g., parity, days in milk), might have caused the large differences in  
267 the  $R^2_{VAL}$  values among farms. It is important to consider that, usually, the CV cross-validation  
268 procedure is used to evaluate the performance of prediction equations, where data are split randomly  
269 into a CAL and a VAL set. However, it has been demonstrated that, when there are dependence  
270 structures in the data, CV may overestimate prediction accuracies (Roberts et al., 2017). In particular,  
271 Qin et al. (2016) indicated that random CV underestimates the error of the prediction equation when  
272 traits to be predicted are analyzed in batches, in which there are systematic differences among them.  
273 In our case, because of the differences among farms within farming systems (Table 1), we chose to  
274 build calibration equations directly at a farm level, in order to take into account the differences in  
275 milk coagulation traits (and therefore in the milk spectra) arising from the differences among farms.  
276 Wang and Bovenhuis (2019) investigated the feasibility of bovine milk IR spectra to predict methane  
277 emissions by comparing random and block CV (using farms as blocks) procedures. They showed  
278  $R^2_{VAL}$  values of 0.49 and 0.01, respectively for random and block CV. They suggested that the

279 difference in the prediction accuracy between the two procedures could have been due to the  
280 confounding effect of farm and date of milk IR collection, and especially to the breath sensors used  
281 to measure methane emissions, which largely differed among farms.

282

283

## CONCLUSIONS

284 Overall, our work evidenced the feasibility of using FTIR spectroscopy to predict MCP and  
285  $CF_t$  parameters in goat milk. Despite this, a great variability was observed among farms and traits.  
286 The generally low  $R^2_{VAL}$  do not justify for practical application, at present, of the predicted  
287 coagulation traits. However, among traits, RCT and  $RCT_{eq}$  showed the highest accuracies, while  $k_{CF}$   
288 was on the opposite line. Moreover, our results demonstrated the importance of farm variability in  
289 relation to coagulation traits, that should be considered while developing FTIR calibrations, in order  
290 to not incur in misleading accuracies. Future studies with other farming systems, statistical models,  
291 and with increased sample size are expected to show improvements in the model performance. A  
292 further investigation on the predictive performance of FTIR on individual cheese yield traits would  
293 be interesting.

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## REFERENCES

- 303  
304 Barrón-Bravo, O. G., A. J. Gutiérrez-Chávez, C. A. Ángel-Sahagún, H. H. Montaldo, L. Shepard, and  
305 M. Valencia-Posadas. 2013. Losses in milk yield, fat and protein contents according to  
306 different levels of somatic cell count in dairy goats. *Small Rumin. Res.* 113:421-431.
- 307 Benedet, A., C. L. Manuelian, M. Penasa, M. Cassandro, F. Righi, M. Sternieri, P. Galimberti, A.V.  
308 Zambrini, and M. De Marchi. 2018. Factors associated with herd bulk milk composition and  
309 technological traits in the Italian dairy industry *J. Dairy Sci.* 101:934-943.
- 310 Bittante, G. 2011. Modeling rennet coagulation time and curd firmness of milk. *J. Dairy Sci.* 94:5821-  
311 5832.
- 312 Bittante, G., C. Cipolat-Gotet, F. Malchiodi, E. Sturaro, F. Tagliapietra, S. Schiavon, and A.  
313 Cecchinato. 2015. Effect of dairy farming system, herd, season, parity and days in milk on  
314 modeling of the coagulation, curd firming and syneresis of bovine milk. *J. Dairy Sci.* 98:2759-  
315 2774.
- 316 Bittante, G., C. Cipolat-Gotet, M. Pazzola, M. L. Dettori, G. M. Vacca, and A. Cecchinato. 2017.  
317 Genetic analysis of coagulation properties, curd firming modeling, milk yield, composition  
318 and acidity in Sarda dairy sheep. *J. Dairy Sci.* 100:385-394.

319 Bittante, G., M. Penasa, and A. Cecchinato. 2012. Invited review: Genetics and modeling of milk  
320 coagulation properties. *J. Dairy Sci.* 95:6843-6870.

321 Bonfatti, V., P. N. Ho, and J. E. Pryce. 2020. Usefulness of milk mid-infrared spectroscopy for  
322 predicting lameness score in dairy cows. *J. Dairy Sci.* 103:2534-2544.

323 Caballero-Villalobos, J., A. Figueroa, K. Xibrraku, E. Angón, J.M. Perea, and A. Garzón. 2018.  
324 Multivariate analysis of the milk coagulation process in ovine breeds from Spain. *J. Dairy Sci.*  
325 101:10733-10742.

326 Caredda, M., M. Addis, I. Ibba, R. Leardi, M. F. Scintu, G. Piredda, and G. Sanna. 2016. Prediction  
327 of fatty acid in sheep milk by midinfrared spectrometry with a selection of wavelengths by  
328 genetic algorithms. *LWT Food Sci. Technol.* 65:503-510.

329 Čermak, B., V. Kral, J. Frelich, L. Boháčova, B. Vondrašková, J. Špička, E. Samkova, M.  
330 Podsedniček, A. Węglarz, J. Makulska, and P. Zapletal. 2013. Quality of goat pasture in less-  
331 favoured areas (LFA) of the Czech Republic and its effect on fatty acid content of goat milk  
332 and cheese. *Anim. Sci. Pap. Rep.* 31:331-346.

333 Cipolat-Gotet, C., M. Pazzola, A. Ferragina, A. Cecchinato, M.L. Dettori, and G.M. Vacca. 2018.  
334 Technical note: Improving modeling of coagulation, curd firming, and syneresis of sheep  
335 milk. *J. Dairy Sci.* 101:5832-5837.

336 Correddu, F., M. Cellesi, J. Serdino, M. G. Manca, M. Contu, M. C. Dimauro, I. Ibba, and N. P. P.  
337 Macciotta. 2019. Genetic parameters of milk fatty acid profile in sheep: Comparison between  
338 gas chromatographic measurements and Fourier-transform IR spectroscopy predictions.  
339 *Animal* 13:469-476.

340 Dadousis, C., S. Biffani, C. Cipolat-Gotet, E. L. Nicolazzi, A. Rossoni, E. Santus, G. Bittante, and A.  
341 Cecchinato. 2016. Genome-wide association of coagulation properties, curd firmness  
342 modeling, protein percentage, and acidity in milk from Brown Swiss cows. *J. Dairy Sci.*  
343 99:3654-3666.



344 de los Campos, G., and P. Perez Rodriguez. 2015. BGLR: Bayesian Generalized Linear Regression.  
345 R package version 1.0.4. Accessed May 25, 2019. [http://CRAN.R-](http://CRAN.R-project.org/package=BGLR)  
346 [project.org/package=BGLR](http://CRAN.R-project.org/package=BGLR).

347 Dettori, M. L., M. Pazzola, P. Paschino, M. G. Pira, and G. M. Vacca. 2015. Variability of the caprine  
348 whey protein genes and their association with milk yield, composition and renneting  
349 properties in the Sarda breed. 1. The LALBA gene. *J. Dairy Res.* 82:434-441.

350 Devold, T. G., R. Nordbø, T. Langsrud, C. Svenning, M. J. Brovold, E. S. Sørensen, B. Christensen,  
351 T. Ådnøy, and G. E. Vegarud. 2011. Extreme frequencies of the  $\alpha$ s1-casein “null” variant in  
352 milk from Norwegian dairy goats - Implications for milk composition, micellar size and  
353 renneting properties. *Dairy Sci. Technol.* 91:39-51.

354 Di Trana, A., L. Sepe, P. Di Gregorio, M. A. Di Napoli, D. Giorgio, A. R. Caputa, and S. Claps. 2015.  
355 The role of local sheep and goat breeds and their products as a tool for sustainability and  
356 safeguard of the Mediterranean environment. Pages 77-112 in *The Sustainability of Agro-*  
357 *Food and Natural Resources Systems in the Mediterranean Basin*. Springer International  
358 Publishing AG, Cham, Switzerland.

359 FAOSTAT (Food and Agriculture Organization of the United Nations Statistics Division). 2018.  
360 Statistical Database of the Food and Agriculture Organization of the United Nations. Accessed  
361 July 20, 2018. <http://www.fao.org/faostat/en/#data/QL>.

362 Ferragina, A., C. Cipolat-Gotet, A. Cecchinato, and G. Bittante. 2013. The use of Fourier-transform  
363 infrared spectroscopy to predict cheese yield and nutrient recovery or whey loss traits from  
364 unprocessed bovine milk samples. *J. Dairy Sci.* 96:7980-7990.

365 Ferragina, A., C. Cipolat-Gotet, A. Cecchinato, M. Pazzola, M. L. Dettori, G. M. Vacca, and G.  
366 Bittante. 2017. Prediction and repeatability of milk coagulation properties and curd-firming  
367 modeling parameters of ovine milk using Fourier-transform infrared spectroscopy and  
368 Bayesian models. *J. Dairy Sci.* 100:3526-3538.

369 Inglingstad, R. A., H. Steinshamn, B. S. Dagnachew, B. Valenti, A. Criscione, E. O. Rukke, T. G.  
370 Devold, S. B. Skeie, and G. E. Vegarud. 2014. Grazing season and forage type influence goat  
371 milk composition and rennet coagulation properties. *J. Dairy Sci.* 97:3800-3814.

372 ISO-IDF 2004. International Organization for Standardization and International Dairy Federation.  
373 Milk: Quantitative determination of bacteriological quality - Guidance for establishing and  
374 verifying a conversion relationship between routine method results and anchor method results.  
375 International Standard ISO 21187 and IDF 196:2004. 2004. ISO, Geneva, Switzerland, and  
376 IDF, Brussels, Belgium.

377 ISO-IDF 2006. International Organization for Standardization and International Dairy Federation.  
378 Milk: Enumeration of somatic cells - Part 2: Guidance on the operation of fluoro-opto-  
379 electronic counters. International Standard ISO 13366-2 and IDF 148-2:2006. 2006. ISO,  
380 Geneva, Switzerland, and IDF, Brussels, Belgium.

381 ISO-IDF 2013. International Organization for Standardization and International Dairy Federation.  
382 Milk and liquid milk products: Determination of fat, protein, casein, lactose and pH content.  
383 International Standard ISO 9622 and IDF 141:2013. 2013. ISO, Geneva, Switzerland, and  
384 IDF, Brussels, Belgium.

385 Maga, E. A., P. Daftari, D. Kültz, and M. C. T. Penedo. 2009. Prevalence of  $\alpha$ s1-casein genotypes in  
386 American dairy goats. *J. Anim. Sci.* 87:3464-3469.

387 McParland, S., and D. P. Berry. 2016. The potential of Fourier transform infrared spectroscopy of  
388 milk samples to predict energy intake and efficiency in dairy cows. *J. Dairy Sci.* 99:4056-  
389 4070.

390 Nilsson, K., H. Stålhammar, M. Stenholdt Hansen, H. Lindmark-Månsson, S. Duchemin, F. Fikse,  
391 D.J. de Koning, M. Paulsson, and M. Glantz. 2019. Characterisation of non-coagulating milk  
392 and effects of milk composition and physical properties on rennet-induced coagulation in  
393 Swedish Red Dairy Cattle. *Int. Dairy J.* 95:50-57.

394 Paschino, P., G. Stocco, M. L. Dettori, M. Pazzola, M. L. Marongiu, C. E. Pilo, C. Cipolat-Gotet, and  
395 G. M. Vacca. 2020. Characterization of milk composition, coagulation properties and cheese-  
396 making ability of goats reared in extensive farms. *J. Dairy Sci.* 103:5830-5843.

397 Pazzola, M., M. L. Dettori, and G. M. Vacca. 2018a. The Sarda goat, A resource for the extensive  
398 exploitation in the Mediterranean environment. In *Sustainable Goat Production in Adverse*  
399 *Environments: Volume II*. J. Simoes and C. Gutierrez, ed. Springer, Cham, Switzerland.

400 Pazzola, M., G. Stocco, P. Paschino, M. L. Dettori, C. Cipolat-Gotet, G. Bittante, and G.M. Vacca.  
401 2018b. Modeling of coagulation, curd firming, and syneresis of goat milk from 6 breeds. *J.*  
402 *Dairy Sci.* 101:7027-7039.

403 Qin, L. X., H. C. Huang, and C. B. Begg. 2016. Cautionary note on using cross-validation for  
404 molecular classification. *J. Clin. Oncol.* 34:3931-3938.

405 R Core Team. 2013. R: A language and environment for statistical computing. [http://www.R-](http://www.R-project.org/)  
406 [project.org/](http://www.R-project.org/).

407 Roberts, D. R., V. Bahn, S. Ciuti, M. S. Boyce, J. Elith, G. Guillera-Aroita, S. Hauenstein, J. J.  
408 Lahoz-Monfort, B. Schröder, W. Thuiller, D. I. Warton, B. A. Wintle, F. Hartig, and C. F.  
409 Dormann. 2017. Cross-validation strategies for data with temporal, spatial, hierarchical, or  
410 phylogenetic structure. *Ecography.* 40:913-929.

411 Roy, D., A. Ye, P. J. Moughan, and H. Singh. 2020. Gelation of milks of different species (dairy  
412 cattle, goat, sheep, red deer, and water buffalo) using glucono- $\delta$ -lactone and pepsin. *J. Dairy*  
413 *Sci.* 103:5844-5862.

414 Soyeurt, H., P. Dardenne, F. Dehareng, G. Lognay, D. Veselko, M. Marlier, C. Bertozzi, P. Mayeres,  
415 and N. Gengler. 2006. Estimating fatty acid content in cow milk using mid-infrared  
416 spectrometry. *J. Dairy Sci.* 89:3690-3695

417 Stella, A., E. L. Nicolazzi, C. P. Van Tassell, M. F. Rothschild, L. Colli, B.D. Rosen, T.S. Sonstegard,  
418 P. Crepaldi, G. Tosser-Klopp, S. Joost, M. Amills, P. Ajmone-Marsan, F. Bertolini, P.  
419 Boettcher, R. Boyle Onzima, D. Bradley, D. Buja, M.E. Cano Pereira, A. Carta, G. Catillo, L.

420 Colli, P. Crepaldi, A. Crisà, M. Del Corvo, K. Daly, C. Droegemueller, S. Duruz, A. Elbeltagi,  
421 A. Esmailizadeh, O. Faco, T. Figueiredo Cardoso, C. Flury, J.F. Garcia, B. Guldbandsen, A.  
422 Haile, J. Hallsteinn Hallsson, M. Heaton, V. Hunnicke Nielsen, H. Huson, S. Joost, J. Kijas,  
423 J.A. Lenstra, G. Marras, M. Milanese, C. Minhui, M. Moaeen-ud-Din, R. Morry O'Donnell,  
424 O. Moses Danlami, J. Mwacharo, E.L. Nicolazzi, I. Palhière, F. Pilla, M. Poli, J. Reecy, B.A.  
425 Rischkowsky, E. Rochat, B. Rosen, M. Rothschild, R. Rupp, B. Sayre, B. Servin, K. Silva, T.  
426 Sonstegard, G. Spangler, A. Stella, R. Steri, A. Talenti, F. Tortereau, G. Tosser-Klopp, E.  
427 Vajana, C.P. Van Tassell, W. Zhang, and the AdaptMap Consortium. 2018. AdaptMap:  
428 exploring goat diversity and adaptation. *Genet. Sel. Evol.* 50:61.

429 Stocco, G., C. Cipolat-Gotet, T. Bobbo, A. Cecchinato, and G. Bittante. 2017. Breed of cow and herd  
430 productivity affect milk composition and modeling of coagulation, curd firming, and  
431 syneresis. *J. Dairy Sci.* 100:129-145.

432 Stocco, G., C. Cipolat-Gotet, A. Cecchinato, L. Calamari, and G. Bittante. 2015. Milk skimming,  
433 heating, acidification, lysozyme, and rennet affect the pattern, repeatability, and predictability  
434 of milk coagulation properties and of curd-firming model parameters: A case study of Grana  
435 Padano. *J. Dairy Sci.* 98:5052-5067.

436 Stocco, G., M. Pazzola, M. L. Dettori, C. Cipolat-Gotet, A. Summer, and G. M. Vacca. 2019. The  
437 effect of udder health indicators on composition and coagulation traits of goat milk. *Int. Dairy*  
438 *J.* 98:9-16.

439 Tiplady, K.M., R.G. Sherlock, M.D. Littlejohn, J.E. Pryce, S.R. Davis, D.J. Garrick, R.J. Spelman,  
440 and B.L. Harris. 2019. Strategies for noise reduction and standardization of milk mid-infrared  
441 spectra from dairy cattle. *J. Dairy Sci.* 102:6357-6372.

442 Usai, M. G., S. Casu, G. Molle, M. Decandia, S. Ligios, and A. Carta. 2006. Using cluster analysis  
443 to characterize the goat farming system in Sardinia. *Livest. Sci.* 104:63-76.

444 Vacca, G. M., M. Pazzola, M. L. Dettori, E. Pira, F. Malchiodi, C. Cipolat-Gotet, A. Cecchinato, and  
445 G. Bittante. 2015. Modeling of coagulation, curd firming and syneresis of milk from Sarda. *J.*  
446 *Dairy Sci.* 98:2245-2259.

447 Vacca, G. M., G. Stocco, M. L. Dettori, E. Pira, G. Bittante, and M. Pazzola. 2018. Milk yield, quality  
448 and coagulation properties of 6 breeds of goats: Environmental and individual variability. *J.*  
449 *Dairy Sci.* 101:7236-7247.

450 Vacca, G. M., G. Stocco, M. L. Dettori, G. Bittante, and M. Pazzola. 2020. Goat cheese yield and  
451 recovery of fat, protein, and total solids in curd are affected by milk coagulation properties. *J.*  
452 *Dairy Sci.* 103:1352-1365.

453 Visentin, G., A. McDermott, S. McParland, D. P. Berry, O. A. Kenny, A. Brodkorb, M. A. Fenelon,  
454 and M. De Marchi. 2015. Prediction of bovine milk technological traits from mid-infrared  
455 spectroscopy analysis in dairy cows. *J. Dairy Sci.* 98:6620-6629.

456 Wang, Q., and H. Bovenhuis. 2019. Validation strategy can result in an overoptimistic view of the  
457 ability of milk infrared spectra to predict methane emission of dairy cattle. *J. Dairy Sci.*  
458 102:6288-6295.

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## TABLES AND FIGURES

461 **Table 1.** Characteristics of sampled farms (N = 14).

	Management system <sup>1</sup>	
	Extensive	Semi-extensive
Farms, no.	6	8
Goats, no.	183	269
Flock size, no. of farms:		
Small (< 100 goats)	1	1
Medium (100-200 goats)	3	5
Large (> 200 goats)	2	2
Altitude, no. of farms:		
Plain (< 200 m asl <sup>2</sup> )	3	2
Hill (200-500 m asl)	2	4
Mountain (> 500 m asl)	1	2
Milking, no. of farms:		
Mechanical	3	4
Hand-milked	3	4
Milk quality, mean $\pm$ SD:		
Fat, %	5.01 $\pm$ 0.98	5.33 $\pm$ 1.32
Protein, %	3.97 $\pm$ 0.52	3.87 $\pm$ 0.51
SCS <sup>2</sup>	6.58 $\pm$ 1.64	6.75 $\pm$ 1.68
LBC <sup>3</sup>	1.80 $\pm$ 0.91	1.71 $\pm$ 0.86

462 <sup>1</sup>Management system: extensive: family-managed farms, feeding at pasture, natural mating, milking  
463 when goats are back from pasture; semi-extensive system: cultivated grasslands, control of estrus and  
464 kidding season; <sup>2</sup>asl = above sea level.

465 <sup>2</sup>SCS =  $\log_2(\text{SCC} \times 10^{-5}) + 3$ .

466 <sup>3</sup>LBC = logarithmic total bacterial count =  $\log_{10}(\text{total bacterial count}/1,000)$ .

467

468 **Table 2.** Descriptive statistics and repeatability (REP) of traditional milk coagulation properties (MCP) and curd firmness over time (CF<sub>t</sub>) model  
 469 parameters and results from Stratified Cross-Validation (SCV) calibrations using mid-infrared spectra of individual goat milk samples.

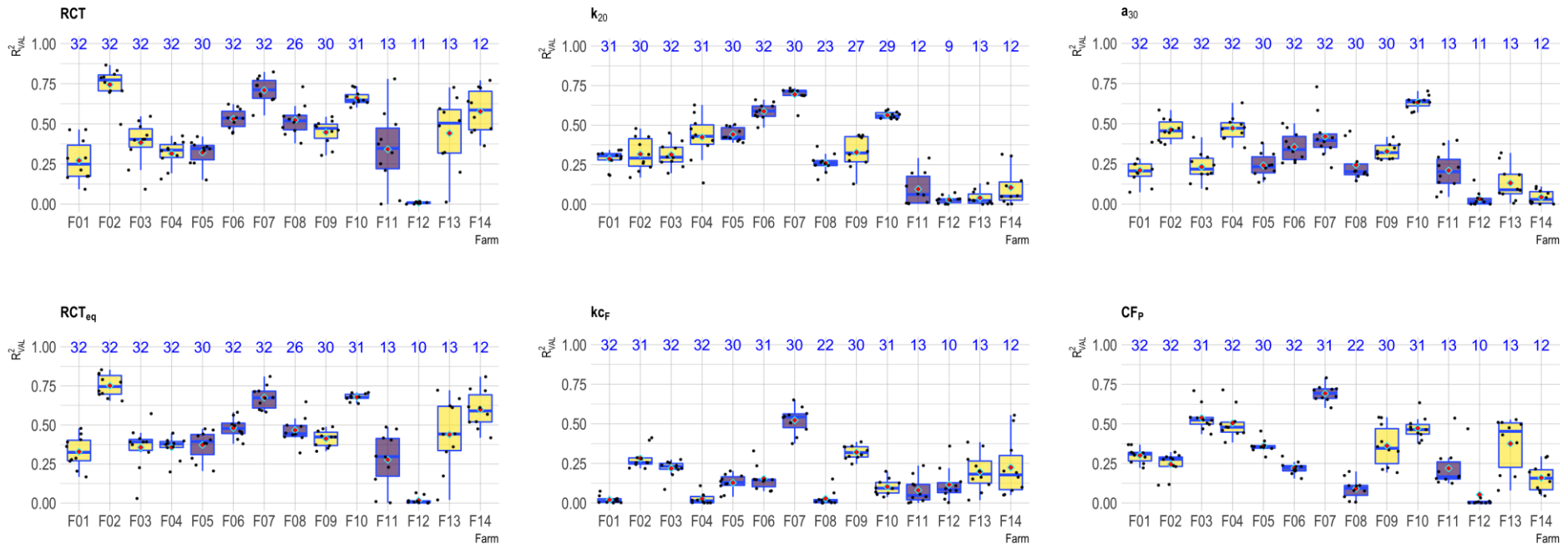
Item <sup>1</sup>	Descriptive Statistics <sup>2</sup>				Prediction Statistics <sup>3</sup>								
					Calibration				Validation				
	N	Mean	SD	REP	N	SD <sub>CAL</sub>	R <sup>2</sup> <sub>CAL</sub>	RMSE <sub>CAL</sub>	SD <sub>VAL</sub>	R <sup>2</sup> <sub>VAL</sub>	R <sup>2</sup> <sub>interval</sub>	RMSE <sub>VAL</sub>	RMSE <sub>VAL</sub> <sup>interval</sup>
Traditional MCP													
RCT, min	892	12.9	4.42	97.7	416	4.49±0.18	0.64±0.04	2.69±0.16	3.95±0.92	0.42±0.21	0.00-0.75	3.69±1.24	2.23-6.29
k <sub>20</sub> , min	839	3.5	1.12	85.6	397	1.12±0.01	0.49±0.05	0.80±0.04	0.99±0.26	0.29±0.21	0.00-0.61	0.91±0.16	0.72-1.27
a <sub>30</sub> , mm	901	37.5	11.0	87.2	422	11.0±0.34	0.47±0.03	8.07±0.33	10.2±3.27	0.27±0.17	0.02-0.63	9.63±3.49	6.47-17.83
CF <sub>t</sub> parameters													
RCT <sub>eq</sub> , min	892	13.6	4.06	97.7	415	4.06±0.14	0.61±0.05	2.55±0.15	3.65±0.80	0.45±0.20	0.00-0.80	3.30±1.04	2.10-5.32
k <sub>CF</sub> , %/min	867	22.9	8.46	84.2	408	8.21±0.17	0.37±0.07	6.60±0.37	7.91±2.00	0.14±0.16	0.00-0.56	8.28±2.16	5.01-12.69
CF <sub>p</sub> , mm	873	42.7	9.31	83.9	409	8.91±0.15	0.50±0.01	6.31±0.14	7.98±1.68	0.32±0.19	0.00-0.69	7.19±1.84	4.90-11.04

470 <sup>1</sup>Traditional milk coagulation properties: RCT = rennet coagulation time; k<sub>20</sub> = curd firming time; a<sub>30</sub> = curd firmness 30 min after rennet addition.  
 471 CF<sub>t</sub> model parameters according to 3-parameter model: RCT<sub>eq</sub> = RCT estimated according to curd firm change over time modeling; k<sub>CF</sub> = instant curd  
 472 firming rate constant; CF<sub>p</sub> = asymptotical curd firmness;

473 <sup>2</sup>Repeatability (REP), % =  $\frac{\sigma_{Farm}^2 + \sigma_{Animal}^2 + \sigma_{Pendulum}^2}{\sigma_{Farm}^2 + \sigma_{Animal}^2 + \sigma_{Pendulum}^2 + \sigma_e^2} \times 100$ ;

474 <sup>3</sup> Average ± SD from the SCV calibrations. For R<sup>2</sup><sub>VAL</sub> and RMSE<sup>2</sup><sub>VAL</sub> also intervals of validations were included. Results were averaged over the 14  
 475 runs (one per farm).

476 **Figure 1.** Coefficient of determination of validation ( $R^2_{VAL}$ ) results per farm (F01 to F14; purple boxes refer to extensive farms; yellow boxes refer  
 477 to semi-extensive farms) of traditional milk coagulation properties (MCP) and curd firmness over time ( $CF_t$ ) model parameters<sup>1</sup> using mid-infrared  
 478 spectra of individual goat milk samples in the second stratified cross-validation scenario ( $SCV_{80}$ )<sup>2</sup>.



<sup>1</sup>Traditional MCP: RCT = rennet coagulation time;  $k_{20}$  = curd-firming time;  $a_{30}$  = curd firmness 30 min after rennet addition.  $CF_t$  model parameters according to 3-parameter model:  $RCT_{eq}$  = RCT estimated according to curd firm change over time modeling;  $k_{cF}$  = instant curd firming rate constant;  $CF_p$  = asymptotical curd firmness;

<sup>2</sup>Each farm was evaluated separately with 20% of the farm included in the calibration set. The procedure was repeated ten times per farm (black dots);

Vertical lines within each boxplot represent the median, and red rhombus is the mean of the ten replicates per farm;

Blue numbers on top refer to the number of goats in validation per farm.