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

INTERPRETIVE SUMMARY

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_t**) 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

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

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

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

Altogether, the economic importance of MCP and CF_t parameters in the dairy sector justifies for further investigation on the practical application at a wide-scale of milk FTIR spectroscopy to predict MCP and CF_t parameters, that could pioneer the entire goat cheese industry, at a farm, breeding and dairy plant levels. To this purpose, our objective was to i) investigate the potential of milk FTIR spectroscopy for the prediction of MCP and CF_t parameters in goats, and ii) quantify the effect of the farm variability on the prediction accuracy of MCP and CF_t parameters using individual Sarda goat milk samples.

MATERIALS AND METHODS

Farm Characteristics, Milk Sampling and Analyses

The study involved 452 Sarda goats reared in 14 farms (F01 to F14), distributed across the island of Sardinia (Italy). Sampled farms were officially registered in the flock book and recording system of provincial associations of goat breeders. Farms characteristics are summarized in Table 1. In brief, the extensive system consisted of family-managed farms, pasture feeding, natural mating, and milking on the return of goats from pasture; while the semi-extensive system was characterized by cultivated grasslands, control of estrus and kidding season.

Individual milk samples (100 mL/goat) were collected during the afternoon milking (one sampling day for each farm). Milk was sampled from the recorder jar under each stall in mechanical milking systems, and from the stainless steel graduated pails in the hand-milked systems, over the entire milking of each goat. Milk samples were then stored at 4°C and analyzed within 24 h after collection. For each individual milk sample, two measurements of MCP were performed using a lactodynamograph (Formagraph; Foss Electric A/S, Hillerod, Denmark) during a 30 min test analysis, following the procedure reported by Pazzola et al. (2018b). In brief, 10 mL of milk (in double) for each sample were heated to 35°C for 15 min, and then mixed with 200 µL of the rennet solution [Hansen Naturen Plus 215 (Pacovis Amrein AG, Bern, Switzerland), with 80±5% chymosin and 20±5% pepsin; 215 international milk clotting units/mL; diluted to 1.2% (wt/vol) in distilled water to

reach the final value of 0.0513 international milk clotting units/mL of milk]. Coagulation process occurred at 35°C. The MCP recorded were: 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).

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

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

where CF_t is curd firmness at time t (mm); CF_P is the asymptotical potential value of CF at an infinite time in absence of syneresis (mm); k_{CF} is the curd-firming instant rate constant (%/min); and RCT_{eq} is RCT estimated by CF_t equation on the basis of all data points (min). Values of the aforementioned traits out from the interval of the mean ±3 standard deviations (SD) were considered outliers and excluded from further analysis.

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

Somatic cell count (SCC) was determined by Fossomatic 5000 (Foss Electric A/S, Hillerod, Denmark) according to ISO-IDF standard (2006), and later transformed into the logarithmic somatic cell score [SCS = $\log_2(SCC \times 10^{-5}) + 3$; (Ali and Shook, 1980)]. Total bacterial count was determined using a BactoScan FC150 analyzer (Foss Electric A/S, Hillerod, Denmark) according to ISO-IDF standard (2004), and transformed into the logarithmic bacterial count [LBC = $\log_{10}$ (total bacterial count/1,000)].

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 mean±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 μ is the overall mean, x_{ij} are the FTIR wavelengths, β_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*

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

RESULTS AND DISCUSSION

Prediction Accuracy of Goat Milk Coagulation Traits

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

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

Regarding SCV predictions (Table 2), RCT and RCT_{eq} showed the highest R^2_{CAL} (0.64 and 0.61, respectively), followed by CF_P (R^2_{CAL} = 0.50). The remaining traits had R^2_{CAL} < 0.50, while the lowest was observed for k_{CF} (0.37). In general, results in the CAL set were comparable to those reported in ovine milk (Ferragina et al., 2017), in particular for the traits directly related to curd firmness (a_{30} and CF_P). In the VAL set, the R^2_{VAL} was lower and the RMSE was higher, albeit with much higher SD for both parameters compared to CAL, while the ranking among traits was analogous to the CAL. Since this was the first study investigating the effect of farm on the prediction accuracy of MCP and CF_t parameters in goat milk via FTIR spectroscopy, comparison with literature was restricted. However, a recent study (unpublished data) assessing the goat breed (four breeds considered) effect on the prediction of MCP and CF_t parameters via FTIR spectroscopy, by using a random 5-fold CV procedure, reported R^2_{VAL} from 0.42 to 0.68 for MCP (RCT and a_{60}, respectively) and from 0.14 to 0.60 for CF_t parameters (syneresis rate and CF_P, respectively). The study also 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_t parameters. This
230 variability was evident observing the high SD of both R^2_{VAL} and $RMSE_{VAL}$ (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_{80}), our expectation was to increase
236 R^2_{VAL}, 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^2_{VAL}
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_{20}, a_{30}, RCT_{eq}, k_{CF} and CF_P, respectively, with also similar SD to the SCV (data not shown).
240 However, although the minimum R^2_{VAL} was again close to 0, the maximum obtained R^2_{VAL} values
241 were increased (0.87, 0.73, 0.73, 0.85, 0.65 and 0.79 for RCT, k_{20}, a_{30}, RCT_{eq}, k_{CF} and CF_P,
242 respectively); representing an increase of ~20% for k_{20}, ~16% for RCT, a_{30} and k_{CF}, ~14% for CF_P,
243 with the minimum (~0.06%) for the RCT_{eq}. On average, R^2_{VAL} 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^2_{VAL} 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^2_{VAL} values, relative to the remaining farms across the traits (e.g., F02 vs.
249 F12), iii) different R^2_{VAL} patterns, showing either high or low R^2_{VAL} (e.g., F01 and F10 comparing
250 k_{CF} to all the rest of the traits), iv) general low predictability of k_{CF} trait with three farms (F01, F04
251 and F08) showing R^2_{VAL} close to 0, v) similar variation patterns across farms of RCT and RCT_{eq}
252 traits, and interestingly, vi) farm F12 showed R^2_{VAL} 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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459

460 **TABLES AND FIGURES**

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

	Management system[1]	
	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[2])	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 ±SD:		
Fat, %	5.01±0.98	5.33±1.32
Protein, %	3.97±0.52	3.87±0.51
SCS[2]	6.58±1.64	6.75±1.68
LBC[3]	1.80±0.91	1.71±0.86

462 [1]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; [2]asl = above sea level.
465 [2]SCS = $\log_2$ (SCC × 10^{-5}) + 3.
466 [3]LBC = logarithmic total bacterial count = $\log_{10}$ (total bacterial count/1,000).
467

Table 2. Descriptive statistics and repeatability (REP) of traditional milk coagulation properties (MCP) and curd firmness over time (CF_t) model parameters and results from Stratified Cross-Validation (SCV) calibrations using mid-infrared spectra of individual goat milk samples.

Item[1]	Descriptive Statistics[2]				Prediction Statistics[3]								
					Calibration				Validation				
	N	Mean	SD	REP	N	SD_{CAL}	R^2_{CAL}	$RMSE_{CAL}$	SD_{VAL}	R^2_{VAL}	$R^2_{interval}$	$RMSE_{VAL}$	$RMSE_{VAL}^{interval}$
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_{20}, 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_{30}, 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_t parameters													
RCT_{eq}, 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_{CF}, %/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_p, 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

[1]Traditional milk coagulation properties: 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;

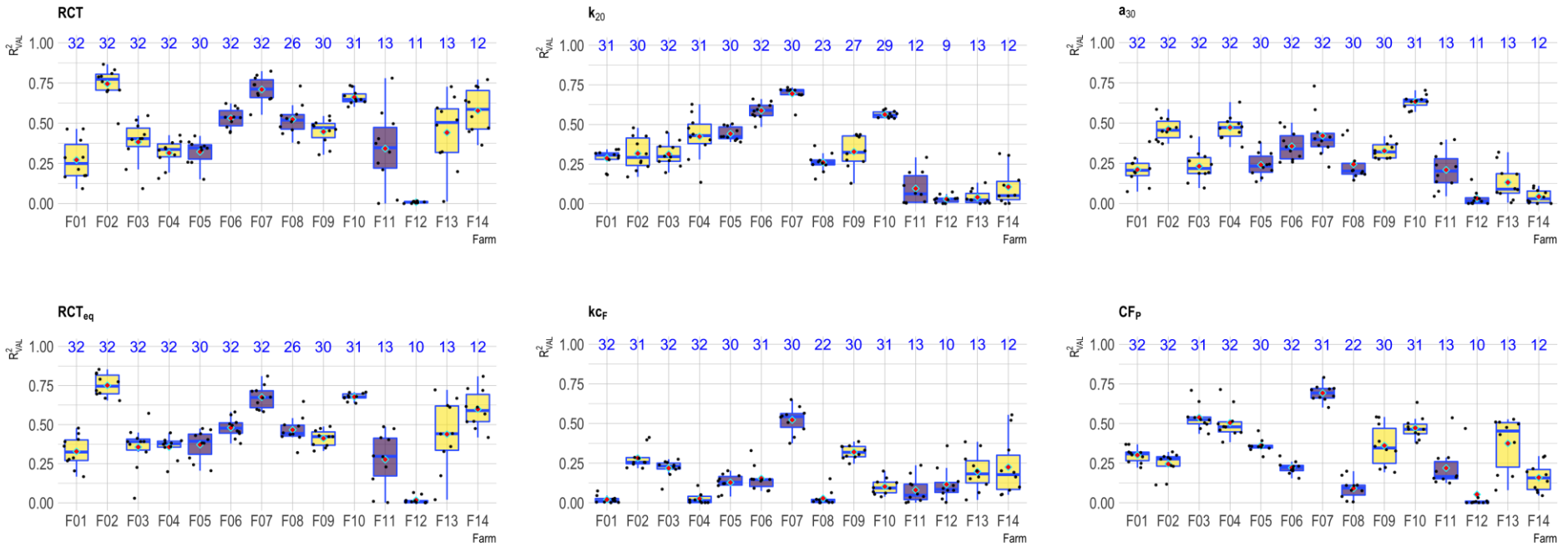
[2]Repeatability (REP), % = $\frac{\sigma^2_{Farm} + \sigma^2_{Animal} + \sigma^2_{Pendulum}}{\sigma^2_{Farm} + \sigma^2_{Animal} + \sigma^2_{Pendulum} + \sigma^2_e} \times 100$;

[3] Average ± SD from the SCV calibrations. For R^2_{VAL} and $RMSE^2_{VAL}$ also intervals of validations were included. Results were averaged over the 14 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[1] using mid-infrared

478 spectra of individual goat milk samples in the second stratified cross-validation scenario (SCV_{80})[2].



[1]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;

[2]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.

479