



## Leveraging milk mid-infrared spectroscopy to authenticate animal welfare, farming practices, and dairy systems of Parmigiano Reggiano cheese

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### ABSTRACT

Increasing consumer concerns underscore the importance of verifying the practices and origins of food, especially certified premium products. The aim of this study was to evaluate the ability of Fourier-transform mid-infrared (FT-MIR) spectroscopy to authenticate animal welfare parameters, farming practices, and dairy systems. Data on farm characteristics were obtained from the Parmigiano Reggiano Consortium in northern Italy. Animal welfare data were collected by trained veterinarians using the assessment protocol developed by the Italian National Reference Center for Animal Welfare (CReNBA), and bulk milk test-day data were obtained from the laboratory of the Breeders Association of the Emilia Romagna Region. A merged final dataset of 12,083 bulk FT-MIR spectra records from 949 farms was created. Using a nonhierarchical clustering approach, the farms were classified into 5 dairy systems: 2 traditional systems comprising farms located in either the Apennines or the Po Plain; 2 modern systems, one that used TMR and one did not; and one traditional dairy system comprising farms rearing local breeds. To evaluate the ability of bulk milk to capture differences in farming systems, we conducted an ANOVA on milk composition. The linear models included the following effects: season, dairy system, farm, and the interaction between dairy system and season. The effect of the dairy system was significant for all milk composition traits. A 10-iteration linear discriminant analysis was used to evaluate the discriminative ability of the spectra in classifying farming practices and dairy systems. The average results of the

area under the receiver operating characteristic curve revealed good authentication performance for genetic type (0.98), housing system (0.91), and feeding system (0.89), and medium-low authentication performance for geographical area (0.70); poor results were obtained for the percentage of concentrate in the diet and animal welfare parameters (0.57–0.64). With regard to dairy systems, the best result was obtained when dairy systems were grouped into 2 simplified categories, traditional versus modern (0.89), instead of the 5 categories (0.87). The results of this study show that FT-MIR is a useful tool for authenticating farming practices and dairy systems, but not animal welfare as defined by CReNBA evaluation criteria. Our results show that infrared spectroscopy has the potential to authenticate dairy products and quality label certifications.

**Key words:** dairy cow, animal welfare, quality labels, Fourier-transform mid-infrared, discriminant analysis

### INTRODUCTION

European Protected Designation of Origin (PDO) certification is intended to meet consumer demand for high quality products distinguished by a specific production area and regulated production processes (Espejel et al., 2008). This certification has enabled the PDO dairy sector to create a connection between consumers and the various steps of the production chain. With more than 3.5 million wheels of cheese produced per year, Parmigiano Reggiano is one of the most consumed and widely exported Italian PDO cheeses (Lovarelli et al., 2022). This hard, slow-ripened, cooked cheese is produced according to strict regulations supervised by the PDO certification body and the Parmigiano Reggiano Consortium (PRC) (Cozzi et al., 2019). Consumers are now showing a growing interest in understanding the details

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The list of standard abbreviations for JDS is available at [adsa.org/jds-abbreviations-25](https://adsa.org/jds-abbreviations-25). Nonstandard abbreviations are available in the Notes.

of milk production systems, in particular the geographical area of origin (Becchi et al., 2023), the breeds used (Romanzin et al., 2015), animal welfare standards (de Graaf et al., 2016), and feeding practices (Moscovici Joubran et al., 2021). This has led the PRC to create additional distinctions in production origin, such as cheeses produced from single breed milk or from herds reared in mountainous areas. In addition, since 2004, the Italian Ministry of Health (<http://www.salute.gov.it>) has been collaborating with the Experimental Zooprophyllactic Institute of Lombardy and Emilia Romagna (IZSLER) to develop and implement, through the Italian National Reference Center for Animal Welfare (CReNBA), an official protocol to assess animal welfare (Bertocchi et al., 2018). Therefore, routine verification and authentication of animal welfare and farming practices (i.e., use of antibiotics, animal feeding or reduction of greenhouse gas emissions), performed by CReNBA trained veterinarians, has become a key objective in the dairy industry (Olynk and Ortega, 2013). For these purposes, farm information needs to be gathered continuously, quickly and cost effectively. Because milk collection takes place daily, it could be a suitable source of information to meet these conditions. Mid-infrared spectroscopy (MIR) has the potential to be a reliable tool for the authentication of farming practices, not only in terms of the above-mentioned criteria for data collection, but also because MIR has been widely used in the dairy industry for several decades (Bergamaschi et al., 2020; Prache et al., 2020). Mid-infrared spectroscopy is a nonperturbative and label-free technique that employs the interaction between infrared light and the molecular bonds of a sample to extract biological information (Baker et al., 2014; Mota et al., 2024). Its ability to distinguish between different milk samples is made possible by the variations in their biochemical compounds (Gross and Bruckmaier, 2019). Mid-infrared spectroscopy has been found to be effective in determining the composition of milk (ICAR, 2016), detecting adulteration (Santos et al., 2013), identifying the animal species of origin (Souhasou et al., 2018), determining freshness (Su et al., 2024), and predicting blood biomarkers for evaluating animal health and welfare at the individual level (Giannuzzi et al., 2023; Mota et al., 2023). Nonetheless, the potential of MIR to authenticate farming practices (Kamadal and Karoui, 2015; Soyeurt, 2023), and particularly animal welfare parameters (Arnould et al., 2013; Bahadi et al., 2021), using bulk milk has been relatively underexplored. Encouraging results so far include the authentication of feed restrictions in PDO cheese (Coppa et al., 2021), feeding systems (Valenti et al., 2013; Capuano et al., 2014), and farming systems (Bergamaschi et al., 2020). Because of their known effect on milk composition, production characteristics such as geographical

origin, feeding system, and breeds used are expected to have the potential for authentication by MIR. However, it appears that better results are obtained by combining several farming practices rather than analyzing them individually (Coppa et al., 2021). Combining production characteristics allows for the detection of greater differences in milk fingerprinting, which could pave the way for the authentication of production systems. In addition, changes in milk composition are also attributable to factors related to animal welfare parameters. One of the most obvious is cow health (Arnould et al., 2013), as observed, for example, through mammary gland condition (Dufour et al., 2011), energy balance (Stoop et al., 2009; McParland et al., 2011), body condition (Roche et al., 2009), and metabolic disorders (Giannuzzi et al., 2023). Other animal welfare indicators, such as freedom of movement, access to water and feed (Bahadi et al., 2021; Golher et al., 2021), and even animal cleanliness (Sant'Anna and Paranhos da Costa, 2011), have also been associated with variability in milk composition, which means that milk constituents could be used as indicators of animal welfare (Giannuzzi et al., 2024a,b). It also means that MIR could, in turn, also identify animal welfare parameters (Bahadi et al., 2021).

The objectives of this study, therefore, were to evaluate the effectiveness of Fourier-transform MIR (FT-MIR) technology to authenticate (1) animal welfare parameters, (2) farming characteristics and practices, and (3) dairy systems classified through cluster analysis in terms of common farming practices and farm characteristics.

## MATERIALS AND METHODS

### Data Structure

For the present study, data on farms affiliated with the PRC (northern Italy) were collected from various sources. The PRC provided data on farm practices and characteristics, including structure, size, and management, from 983 farms across various areas, including 128,411 cows of Holstein Friesian, Brown Swiss, Reggiana, and Modenese breeds. For the same farms, animal welfare evaluation was conducted by CReNBA. The farms were surveyed in 2022 and 2023. Milk yield and composition data were obtained from the official Italian milk recording system and comprised 22,010 records from 1,508 farms compiled between January 2022 and September 2023. Bulk milk spectral data were obtained from the laboratory of the Breeders Association of the Emilia Romagna Region (ARAER) in Reggio Emilia, Italy; these consisted of 20,363 records from 1,485 farms collected over the same period. The data from these various sources were merged to create the final dataset, which consisted of 12,083 records

from 949 farms (Supplemental Figure S1; see Notes), with an average of 12.73 ( $\pm 2.75$ , SD) sample spectra records per farm.

### Animal Welfare Evaluation Data

The CReNBA scoring system for dairy cattle evaluates various aspects of welfare using a protocol based on specific indicators, following the guidelines of the Ministry of Health and often aligned with the European Welfare Quality approach (Ventura et al., 2021). Veterinarians trained by CReNBA gather data using standardized checklists, ensuring consistency. Observations are made through direct inspection of the herd, sometimes involving individual assessments of selected animals to get a representative sample. The evaluation is an assessment of the farm as a whole and consists of 70 indicators divided in 3 different areas: Area A refers to farm management (**MGT**), area B to farm structure and equipment (**SAE**), and area C to animal-based measures (**ABM**), the latter including potential effects directly observable on the animals. The MGT score considered the number of farm workers and their level of expertise, the method of supplying feed and drinking water to the different livestock categories (lactating and dry cows, heifers, calves), the management of sick animals, and barn cleanliness. The SAE score was based on the characteristics of the barn (presence or absence of external paddocks, the spaces allocated for resting and feeding, availability of drinking troughs, and floor and bedding conditions), the presence and management of a specific area for diseased animals, the milking system maintenance, and the condition of the equipment for maintaining an optimal internal climate. The ABM score focused on the animals' nutritional status assessed by BCS, their cleanliness, the prevalence of diseases (e.g., lameness, mastitis, ketosis, abscesses, reproductive tract infections), and the mortality rate per animal category. The comprehensive checklist for livestock species and production systems is available at <https://www.classyfarm.it/>.

The results of this evaluation are conveyed through a welfare score, as described by Bertocchi et al. (2018). Briefly, the score for each area is calculated as the weighted sum of the scores for each component (observation) related to a particular area, with the final value ranging from 0% (the lowest level of animal welfare) to 100% (the highest level of animal welfare). In this study, the scores were discretized in 3 classes, following the methodology of Ginestreti et al. (2020). We used the mean  $\pm 0.5$  SD as a threshold for each area score, thus obtaining the following classes: low, for farms scoring 70% or less; intermediate, for those scoring between 70% to 80%; and high, for those scoring more than 80%.

### Farming Practices and Characteristics Data

Farm data were provided by the PRC and included the geographical area (using a 500 m above sea level threshold of altitude to classify areas as either the Po Plain or the Apennines), the number of animals (lactating cows, dry cows, replacement heifers) and their breeds (including Holstein Friesian, Brown Swiss, Reggiana, and Modenese), the type of animal housing (freestall vs. tiestall), and feed administration (TMR vs. separated feedstuffs with concentrates administered via automatic systems and forages ad libitum). In addition, information on the proportion of concentrate (%) in the diet of lactating cows was recorded for each farm. In this study, a 50% threshold was used to evaluate the predictive ability of concentrate proportion, categorizing its use into 2 groups: low ( $<50\%$ ) and high ( $\geq 50\%$ ). This threshold was selected to capture the largest differences in animal feeding and their potential effects on milk production. Three farms have been removed from the final dataset because of missing or incomplete information.

### FT-MIR Spectra Collection and Editing

The FT-MIR spectra were recorded and analyzed with a L140–14L (Foss A/S, Hillerød, Denmark). The data comprised infrared absorbance values at 1,060 wavenumbers ( $5,000$  to  $930 \times \text{cm}^{-1}$ ) from the short-wavelength to the long-wavelength infrared regions (D'Amico et al., 2009). The transmittance ( $T$ ) of the spectra was converted to absorbance ( $A$ ) using the equation  $A = \log(1/T)$ , after which the values for each spectral wavelength were standardized to a mean of 0 and a SD of 1. Quality was assessed by Mahalanobis distance, with spectra having a distance value greater than the mean  $\pm 3$  SD considered outliers (Whitfield et al., 1987). All analyses were conducted using the R software version 4.3.2 (R Core Team, 2023).

### Statistical Analysis

**Clustering.** The information related to geographical area, housing system, genetic type, feeding system, milk yield (**MY**; kg/cow per day), and herd size (number of cows) was used to perform a clustering analysis to classify farms into different dairy systems according to common farming practices. Specifically, we used k-means clustering (Yang and Sinaga, 2019), a nonhierarchical clustering technique, using the  $k$ -means function of the *Stats* R package (R Core Team, 2023). The selection of this technique was driven by the presence of both qualitative and quantitative information on the farms. The optimal  $k$  number of clusters was determined based on the within-cluster sum of square error (elbow method, Syakur et al., 2018; Nainggolan



et al., 2019), and confirmed by the Dunn index (Ben Ncir et al., 2021). For all the information included in the clustering, the frequencies of the qualitative traits and the descriptive statistics of the quantitative traits were carried out for each cluster identified.

**Analysis of Variance.** An ANOVA for functional longevity and milk composition traits was performed using a linear mixed model using the *lme4* R package (Bates et al., 2015), after removing outliers (mean  $\pm$  3 SD). The milk composition traits evaluated were protein (%), fat (%), lactose (%), urea (mg/dL), fat:protein ratio (FPR), and SCS (U). The model was fitted using the following formula:

$$y_{ijkl} = \mu + \text{Season}_i + \text{Dairy system}_j + (\text{Season} \times \text{Dairy system})_{ij} + \text{Herd}_k (\text{Dairy system})_j + e_{ijkl},$$

where  $y_{ijkl}$  is the response variable;  $\mu$  is the general mean;  $\text{Season}_i$  is the season ( $i$  = winter, December to February; spring, March to May; summer, June to August; or autumn, September to November),  $\text{Dairy system}_j$  is the dairy system ( $j$  = number of dairy systems identified from the clustering analysis);  $\text{Season}_i \times \text{Dairy system}_j$  is the effect of the interaction between season  $i$  and the dairy system  $j$ ;  $\text{Herd}_k$  is the random effect of the  $k$  farm ( $k$  = 1 to 949) nested within the  $\text{Dairy system}_j$ ; and  $e_{ijkl}$  is the random residual. With this model, the effect of dairy system was tested on the error line of herd effect, whereas the remaining effects were tested on the residual term.

In addition, for the proportion of concentrate (%) and the animal welfare parameters, measured in one test-day and restricted in one specific period, thus not affected by other nuisances (i.e., season), a simple general linear model was adopted using the following formula:

$$y_{ij} = \mu + \text{Dairy system}_i + e_{ij},$$

where  $y_{ij}$  is the response variable;  $\mu$  is the general mean;  $\text{Dairy system}_i$  is the dairy system ( $i$  = number of dairy systems identified from the clustering analysis); and  $e_{ij}$  is the random residual. For the 2 models, the differences between the LSM in each model were contrasted with Bonferroni correction and were declared significant at a threshold of  $P < 0.05$ .

**Predictive Model for Animal Welfare, Farming Practices, and Farm Characteristics.** A linear discriminant analysis (LDA) was performed to evaluate the discriminative ability of the spectra in the classification of the established animal welfare classes, farming practices and farm characteristics using the R packages *caret* (Kuhn, 2008) and *MASS* (Venables and Ripley, 2002). To ensure the robustness and consistency of the analysis, we performed 10 iterations on

**Table 1.** Frequency of animal welfare, farming practices, and characteristics by categories

Item	Frequency, % farms
Animal welfare scores, 0%–100%	
Management: area A	
High (>80%)	36.9
Intermediate (>70% to ≤80%)	35.5
Low (≤70%)	27.6
Farm structure and equipment: area B	
High (>80%)	11.2
Intermediate (>70% to ≤80%)	32.6
Low (≤70%)	56.3
Animal-based measures: area C	
High (>80%)	37.1
Intermediate (>70% to ≤80%)	51.4
Low (≤70%)	11.5
Geographical area	
Po Plain	73.9
Apennines	26.1
Housing system	
Freestall	67.9
Tiestall	32.1
Genetic type	
Specialized	97.3
Holstein Friesian, prevalent	42.8
Holstein Friesian	42.3
Brown Swiss, prevalent	11.2
Brown Swiss	1.1
Nonspecialized	2.7
Local breeds (Reggiana, Modenese)	2.7
Feeding system	
No TMR	56.9
TMR	43.1
Percentage of concentrate inclusion in the lactating cows' diet	
High (≥50% as fed)	17.6
Low (<50% as fed)	82.4

the LDA. In each iteration, the dataset was randomly split into a training set (comprising 75% of the data) for model calibration, and a testing set (comprising 25% of the data) to assess the model performance. Both sets included all the classes for the dependent variable in similar proportions using the *partition* function from *groupdata2* R package (Olsen, 2023). Independence between data sets was maintained by assigning all spectral samples from a given farm to either the training or the testing set. The analysis was performed with all samples as well as separately within each season of the year to avoid biases due to season. Animal welfare parameters (MGT, SAE, ABM) divided into the established classes (low: ≤70%, medium: >70% to ≤80%, and high: >80%), farming practices, and farm characteristics were set as dependent variables, and spectral wavelengths were set as independent variables. Model performance was assessed by evaluating its accuracy in predicting classes within the test sets. The first evaluation focused on the model's ability to identify different classes by analyzing the average percentage of correctly classified samples (CC%) for each class obtained

from the 10 iterations of the LDA. The CC% was calculated by dividing the number of correctly classified category  $X$  samples by the total number of category  $X$  samples in the reference data. In addition, to evaluate the overall model performance, the mean area under the receiver operating characteristic curve (**ROC-AUC**) was calculated from the 10 iterations of the LDA. The receiver operating characteristic (**ROC**) curve graphically represents the true positive rate versus the false positive rate for continuous values of a test measure in binary classification scenarios (Hoo et al., 2017). The ROC-AUC ranges from 0 to 1, where 1 indicates perfect classification, 0.5 indicates no discriminative power, and values below 0.5 indicate worse than random classification. To obtain the ROC for each LDA cycle, each class was considered “positive,” and the rest were treated as “negative” using the one-versus-all approach (Galar et al., 2011). The macro-average was then obtained by averaging the results of all groups using the R *multiROC* package (Wei et al., 2018).

**Predictive Model for Dairy Systems.** The ability of FT-MIR to authenticate the dairy system was evaluated by LDA, with the same methodology as described in the previous section applied to the individual farming practices. In addition, to better interpret and understand the classification results (predicted vs. true) produced by the predictive LDA model for discriminating the dairy system classes, a confusion matrix was constructed. This matrix was derived by averaging the class classification results from the 10 confusion matrix interactions obtained from the LDA model (one interaction per LDA cycle) using all samples (Heydarian et al., 2022).

## RESULTS

### Population Descriptive Statistics

The farms analyzed in this study had an average herd size of  $123 \pm 109$  cows, and an average MY of  $30 \pm 5.86$  kg/cow per day. The milk composition included  $3.39 \pm 0.15\%$  protein,  $3.75 \pm 0.34\%$  fat,  $4.86 \pm 0.20\%$  lactose,  $26.98 \pm 5.49$  mg/dL urea, and  $2.80 \pm 0.63$  SCS, with an FPR of  $1.11 \pm 0.09$ . The average functional longevity, defined as number of calvings, was  $2.42 \pm 0.34$  lactations/cow, and the diet included  $42.1 \pm 7.4\%$  concentrate. Regarding the animal welfare parameters, the average scores were  $75.7 \pm 9.6\%$  for MGT,  $68.1\% \pm 9.6\%$  for SAE, and  $77.5 \pm 6.2\%$  for ABM. The frequencies of the classes obtained from the survey on animal welfare, farming practices, and farm characteristics are presented in Table 1. These include animal welfare parameters (low, intermediate, and high), geographical area (2 classes: Po Plain vs. Apennines), housing system (2 classes: freestall vs. tiestall), genetic type (2 classes:

specialized breeds, Holstein Friesian and Brown Swiss, and the prevalence of these breeds vs. local breeds, Reggiana and Modenese), feeding system (2 classes: no TMR vs. TMR), and use of concentrates on the diet (low:  $<50\%$ , high:  $\geq 50\%$ ).

### Clustering Analysis Results

According to the elbow method and confirmed by the Dunn index, the  $k$ -means analysis was fixed to 5 clusters (Supplemental Figure S2; see Notes), as it maximized coherence within the clusters and differences between clusters. The results of the cluster analysis and the dairy system classification are presented in Table 2. The 5 clusters identified consisted of different numbers of farms from the total surveyed (949 farms). The 5 clusters comprised 195 (21%), 147 (15%), 26 (3%), 201 (21%), and 380 (40%) farms, respectively. The farms in the first cluster were located in the Apennines, most using tiestalls (71%), and rearing animals of a predominantly specialized genetic type fed mainly with single feedstuffs administered separately (no TMR, 90%). These farms had herds of  $60 \pm 36$  cows with a MY of  $26.5 \pm 4.6$  kg/cow per day. Given these characteristics, the cluster was named the “traditional Apennines” (TA) dairy system. The second cluster closely resembled the first for almost all descriptors, except that all the farms were located in the Po Plain; MY was  $26.3 \pm 4.4$  kg/cow per day, and the herd size was  $55 \pm 24$  cows. This cluster was therefore labeled the “traditional Po Plain” (TP) dairy system. The third cluster was the smallest and differed from TP in rearing predominantly nonspecialized genetic type (local breeds). The MY was  $18.3 \pm 4.4$  kg/cow per day, and the herd size was  $52 \pm 38$  cows. It was called the “traditional Po Plain with local breeds” (TPLB) dairy system. About 27% of the TPLB farms are in the Apennines area, and 31% use freestall housing. The last 2 clusters together represented 61% of all farms surveyed. These clusters had the larger herds, with  $120 \pm 73$  cows and  $190 \pm 137$  cows for the fourth and fifth clusters, respectively, and predominantly reared specialized genetic types in freestalls. In the fourth cluster, forages are administered separately from the concentrate, and MY was  $30.7 \pm 4.6$  kg/cow per day; it was labeled the “modern without TMR” (MWTMR) dairy system. The fifth cluster uses TMR, MY was  $33.2 \pm 4.4$  kg/cow per day, and it was labeled the “modern with TMR” (MTMR) dairy system. Additionally, to better evaluate the performance of FT-MIR in authenticating the dairy system, we tested its ability to differentiate between “macro-cluster,” namely traditional versus modern systems. For this purpose, traditional dairy systems were combined into a single “traditional” group, and modern systems were grouped separately into a “modern” group.

**Table 2.** Descriptive characteristics ( $\pm$ SD) of the cluster obtained from the k-means analysis and consequent dairy system classification; the percentage digits show the share of farms with the reported level of the trait

Trait	Cluster				
	1	2	3	4	5
Farms within cluster, n	195	147	26	201	380
Herd size, n	60 $\pm$ 36	55 $\pm$ 24	52 $\pm$ 38	120 $\pm$ 73	190 $\pm$ 137
Classification	Traditional Apennines	Traditional Po Plain	Traditional Po Plain with local breeds	Modern without TMR	Modern with TMR
Descriptor					
Geographical area, %	Apennines (100)	Po Plain (100)	Po Plain (73)	Po Plain (100)	Po Plain (88)
Housing system, %	Tied (71)	Tied (100)	Tied (69)	Free (100)	Free (100)
Genetic type	Predominantly specialized	Predominantly specialized	Predominantly nonspecialized	Predominantly specialized	Predominantly specialized
Feeding system, %	No TMR (90)	No TMR (93)	No TMR (100)	No TMR (100)	No TMR (100)
MY, <sup>1</sup> kg/cow per day	26.5 $\pm$ 4.6	26.3 $\pm$ 4.4	18.3 $\pm$ 4.4	30.7 $\pm$ 4.6	33.2 $\pm$ 4.4

<sup>1</sup>MY = milk yield.

### Analysis of Variance: Effects of Dairy System and Season

The results of the ANOVA for milk composition, functional longevity, percentage of concentrate in the lactating cows' diet, and animal welfare parameters are given in Table 3. Statistical significance was obtained for the effects of both season and dairy system across all investigated traits ( $P < 0.05$ ). The dairy system  $\times$  season interaction significantly affected 4 traits: protein (%), fat (%), FPR, and SCS (U). The LSM for the season effect showed the same pattern across traits: a decrease in LSM values from winter to spring, further decreasing in summer (the lowest value), and then increasing in autumn, except for SCS, which reached the highest values in summer (3.02) and autumn (3.06), and the lowest in winter (2.89) and spring (2.84; Supplemental Table S1, see Notes). Regarding the dairy system  $\times$  season interaction, some changes were observed in the ranking between dairy systems for the concentration of some traits, but the LSM values follow the same pattern throughout the seasons described before (Supplemental Figure S3, see Notes). The LSM for the effect of dairy system on milk composition, functional longevity, inclusion of concentrate (%) in the lactating cows' diet, and animal welfare parameters are presented in Figure 1. In the case of the milk composition, the LSM results across the traditional systems were statistically equivalent, except for SCS (U), which was significantly higher in TPLB than in all the other dairy systems ( $P < 0.05$ ). Functional longevity (lactations/cow) exhibited the same trend. In contrast, TPLB had the lowest LSM value of all the dairy systems with respect to the use of concentrates (%). The LSM results for the modern systems, however, were equivalent for only 2 milk composition traits, FPR and SCS. The MTMR dairy system had higher values than the other systems for lactose content (%), and the proportion of concentrate used in the diet (%). The MWTMR system yielded lower values than the other systems for 3 milk composition traits, protein (%), fat (%), and FPR. Among the animal welfare parameters (scores 0%–100%), the score for MGT was higher ( $P < 0.05$ ) in MTMR than in the other dairy systems. For the SAE parameter such as MGT, MTMR had the highest LSM, although the LSM results for the other dairy systems were less clear for this parameter, with each system having equivalent values to at least 2 of the other systems. The TP dairy system had the lowest LSM value for ABM, which had equal values in all the other systems.

### Animal Welfare, Farming Practices, and Farm Characteristics Authentication Performance

The results of the LDA classification for animal welfare parameters, farming practices, and farm char-

**Table 3.** Results (*F*-value and significance) from ANOVA of cluster and season as fixed effects and their interaction for milk composition, functional longevity, concentrate inclusion (%) in the lactating cows' diet, and animal welfare parameter traits<sup>1</sup>

Item <sup>2</sup>	N	P1	P99	<i>F</i> -value			RMSE <sup>3</sup>
				Season	Dairy system	Season × dairy system	
Milk composition							
Protein, %	12,010	3.06	3.77	439***	26***	4***	0.1
Fat, %	11,948	2.98	4.66	339***	19***	2*	0.21
FPR	11,878	0.88	1.37	88***	15***	2**	0.06
Lactose, %	11,617	4.25	5.27	290***	50***	2	0.16
Urea, mg/dL	11,752	16.48	38.85	20***	7***	1	5.28
SCS, U	12,024	1.48	4.37	115***	11**	6***	0.32
Functional longevity, lactations/cow	11,891	1.7	3.4	16***	36***	1	0.11
CON	949	20	55	—	76***	—	6.4
Animal welfare scores, 0%–100%							
MGT	949	50.6	93.6	—	12***	—	9.3
SAE	949	46.1	87.5	—	27***	—	9.13
ABM	949	60.2	88.0	—	16***	—	5.95

<sup>1</sup>Five levels of dairy system: traditional Apennines, traditional Po Plain, traditional Po Plain with local breeds, modern without TMR, modern with TMR; 4 levels of season: winter, spring, summer, autumn.

<sup>2</sup>FPR = fat:protein ratio; CON = concentrate proportion (%) in the lactating cows' diet; MGT = farm management (area A); SAE = structure and equipment (area B); ABM = animal-based measures (area C).

<sup>3</sup>RMSE = root mean square error.

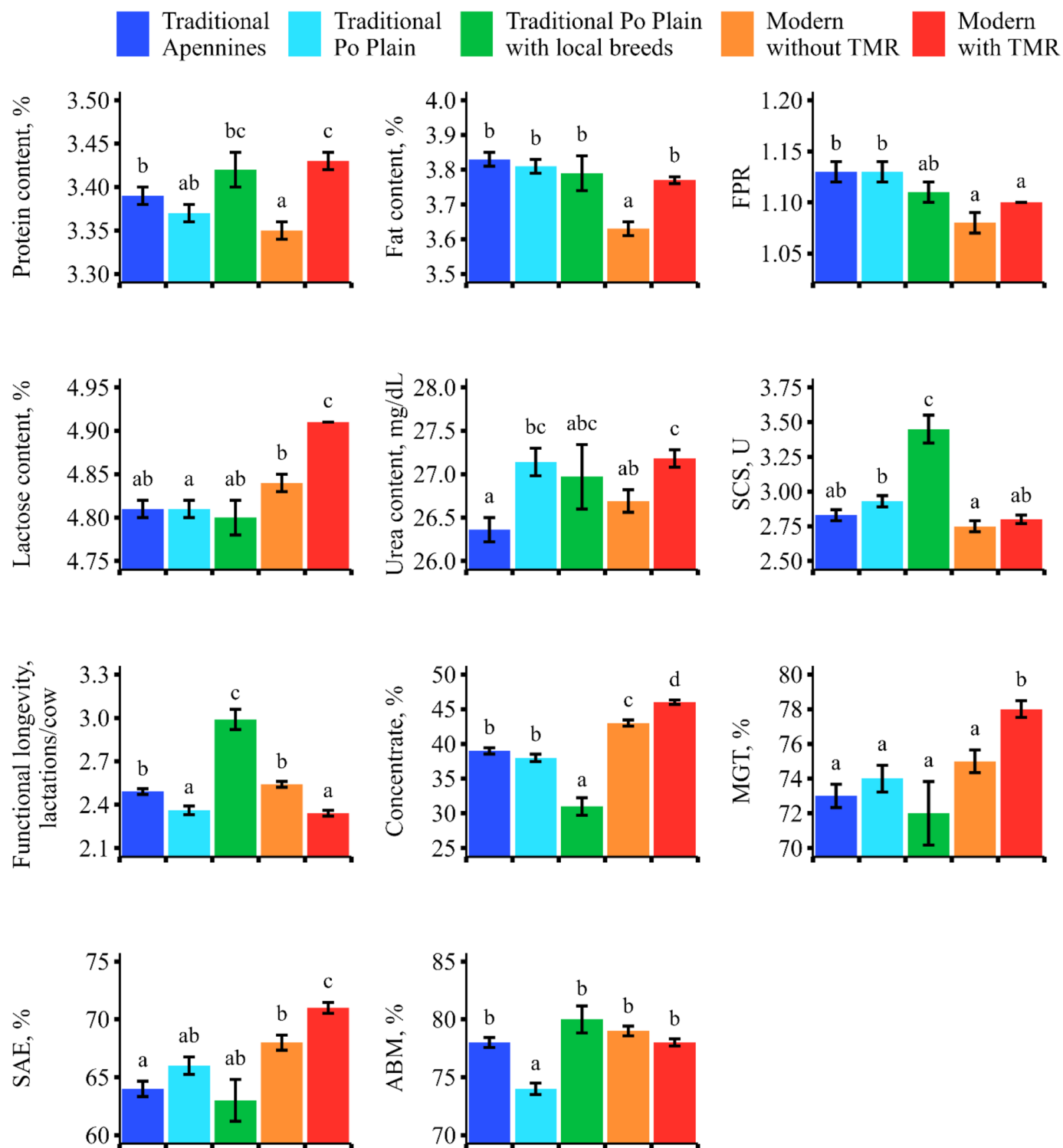
\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

acteristics are presented in Table 4. The genetic type, housing system, and feeding system obtained the highest ROC-AUC results. The genetic type obtained ROC-AUC results ranged from 0.95 to 0.99; regarding CC%, the specialized class showed consistently results across all analyses (99%), whereas the nonspecialized class (local breeds) displayed wide variability (interval: 55%–74%). In the case of the housing system, the ROC-AUC interval ranged from 0.89 to 0.93, and the 2 individual classes exhibited similar variability. The values for the freestall class ranged from 87% to 91%, whereas for the tiestall class, they ranged from 73% to 77%. For the feeding system, the ROC-AUC ranged from 0.87 to 0.91. The CC% results were balanced, with values ranging from 77% to 82% for the TMR class and from 82% to 85% for the no TMR class. The ROC-AUC results for geographical region and use of concentrates were lower than those for previous traits. For geographical region, they ranged regarding the individual classes, the CC% for the Po Plain ranged from 82% to 91%, whereas for the Apennines the results were lower and more variable, ranging from 24% to 51%. The ROC-AUC of concentrate use ranged from 0.58 to 0.64; this trait exhibited highly varied CC% values, the low class ranging from 86% to 98%, and the high class from 5% to 19%. The lowest results of all the traits were for animal welfare: The ROC-AUC results showed little variation, the interval ranging from 0.53 to 0.58, and the CC% values, with some exceptions, were generally below 50% for all classes and analysis scenarios.

### Dairy System Authentication Performance

The mean performance results obtained from the LDA model using the test dataset for the cluster and macro-cluster classifications are presented in Table 5. The ROC-AUC results for the cluster classification model ranged from 0.84 to 0.87 across the different analyses, covering all samples and within each season. The highest ROC-AUC result was obtained when using all samples and winter samples, the lowest using autumn samples. The MTMR and TPLB systems had the best results for CC% with values in the ranges of 74% to 81% and 54% to 76%, respectively. The average results of the confusion matrix using all samples (Table 6) showed that the model primarily misclassified MTMR samples as MWTMR (9% of the total MTMR reference samples) or as TA (7%). The TPLB samples were mainly misclassified as TA (12%), and the remaining systems combined accounted for just under 11% of misclassified TPLB samples. With regard to the other traditional systems, the CC% for TA ranged from 43% to 51% across different analyses, with the model primarily misclassifying this system as MTMR (22%), followed by TP (18%) and MWTMR (16%). The CC% for TP ranged from 40% to 50%, and was primarily misclassified as TA (34%), and the remaining samples were misclassified as one of the other dairy systems (about 26%). The CC% for the other modern system, MWTMR, ranged from 49% to 52%, with samples predominantly misclassified as MTMR (26%) and TA (14%). In the case of the macro-cluster classification





**Figure 1.** Least squares means of milk composition, functional longevity, concentrate inclusion (%) in the lactating cows' diet, and animal welfare parameter traits for the dairy system effect. The LSM with different letters (a–c) differ significantly ( $P < 0.05$ ). Error bars represent SE. MGT = management (area A); SAE = structure and equipment (area B); ABM = animal-based measures (area C).



**Table 4.** Authentication mean results obtained from the test set after running the LDA model 10 times using all samples, as well as separately within each year season for animal welfare parameters, farming practices, and characteristic traits<sup>1</sup>

Item <sup>2</sup>	Category <sup>3</sup>	All samples		Winter		Spring		Summer		Autumn	
		CC%	ROC-AUC	CC%	ROC-AUC	CC%	ROC-AUC	CC%	ROC-AUC	CC%	ROC-AUC
MGT	High	56	0.58	48	0.55	49	0.56	49	0.58	41	0.53
	Medium	35		34		36		39		32	
	Low	30		29		30		34		34	
SAE	High	2	0.57	11	0.55	6	0.53	8	0.54	14	0.56
	Medium	23		29		27		31		33	
	Low	83		69		73		69		63	
ABM	High	30	0.58	41	0.57	39	0.56	38	0.57	46	0.56
	Medium	72		57		63		59		53	
	Low	30		14		11		13		16	
GA	Apennines	24	0.70	42	0.75	34	0.71	41	0.74	51	0.75
	Po Plain	91		88		88		86		82	
HS	Freestall	91	0.91	90	0.92	91	0.93	90	0.90	87	0.89
	Tiestall	74		77		77		73		74	
GT	Specialized	99	0.98	99	0.99	99	0.98	99	0.97	99	0.95
	Nonspecialized	74		75		72		62		55	
FS	TMR	77	0.89	82	0.91	80	0.89	76	0.87	76	0.87
	No TMR	85		84		82		84		82	
CON	High	5	0.64	14	0.61	13	0.62	12	0.59	19	0.58
	Low	98		91		93		90		86	

<sup>1</sup>CC% = correct classify percentage; ROC-AUC = receiver operating characteristic area under the curve. ROC-AUC results are the macro-average of the obtained individual ROC-AUC curves (one vs. all approach).

<sup>2</sup>MGT = management (area A); SAE = structure and equipment (area B); ABM = animal-based measures (area C); GA = geographical area; HS = housing system; GT = genetic type; FS = feeding system; CON = concentrate proportion (%) in the lactating cows' diet.

<sup>3</sup>For MGT, SAE, and ABM, the categories are based on the score (0%–100%): low ( $\leq 70\%$ ), intermediate ( $>70\%$  to  $\leq 80\%$ ), and high ( $>80\%$ ). For CON, the high ( $\geq 50\%$ ) and low ( $<50\%$ ) categories are the proportion of concentrate inclusion in the lactating cows' diet.

(modern and traditional systems), the ROC-AUC showed minimal variability across the different analyses (range: 0.88 to 0.9). Taking each class separately, the “modern class” consistently achieved better results, ranging from 86% to 88%, while the results for the “traditional class” ranged from 73% to 78%, the highest performance for this class being in winter.

## DISCUSSION

### Discriminant Model Approach

Linear discriminant analysis is an effective model for food authentication (Granato et al., 2018; Jiménez-Carvelo et al., 2019), and has been successfully used with milk MIR spectra data to authenticate farming practices and dairy systems (Bergamaschi et al., 2020; Frizzarin et al., 2021). For the authentication of daily variables (e.g., cow diet composition), it is preferable to record milk spectra and reference data simultaneously (Coppa et al., 2021). However, if the aim is to authenticate quite stable practices over time (as in our study), several long-term milk spectra can be collected with only a few associated reference data points (Gori et al., 2012; Soyeurt et al., 2022). Models developed using this approach can integrate the minor annual variation in secondary practices on the dairy system and produce quite robust results as

observed on Soyeurt et al. (2022). In any case, seasonal variability in milk composition could introduce biases and affect the model's performance (Chen et al., 2014; Franceschi et al., 2019; Timlin et al., 2021), which must be checked to correctly interpret the results. In our trial, the overall performance of the model remained stable among seasons for farming practices and dairy systems. Consequently, there is no need to examine the seasonal pattern of the predictive model any further.

### Animal Welfare: Effect of Dairy System and Authentication Performance

Due to the complexity of identifying and measuring cow welfare (Roche et al., 2009), the chemical composition of milk has been proposed as an indicator of it (Giannuzzi et al., 2024b). This would therefore allow the authentication of animal welfare by infrared spectroscopy (Arnould et al., 2013; Bahadi et al., 2021). Some milk constituents have been proposed for this purpose, such as lactose content, which is associated with udder condition (Bobbo et al., 2016; Televičius et al., 2021), and milk urea content, which is an indicator of the livestock's feeding status. Low milk urea content may indicate a deficiency in protein intake, whereas an excessively high content may affect uterine, hormonal, and ovarian functions (Roy et al., 2011). The FPR has also

**Table 5.** Authentication mean results obtained from the test set after running the LDA model 10 times using all samples, as well as separately within each year season for cluster and macro-cluster dairy system classification<sup>1</sup>

Item	Category <sup>2</sup>	All samples		Winter		Spring		Summer		Autumn	
		CC%	ROC-AUC	CC%	ROC-AUC	CC%	ROC-AUC	CC%	ROC-AUC	CC%	ROC-AUC
Cluster	TA	44	0.87	51	0.87	43	0.86	49	0.86	48	0.84
	TP	40		43		41		50		40	
	TPLB	76		68		62		56		54	
	MWTMR	52		52		51		49		52	
	MTMR	81		82		81		78		74	
Macro-cluster <sup>3</sup>	Traditional	73	0.89	78	0.90	75	0.90	74	0.88	73	0.88
	Modern	88		87		88		85		86	

<sup>1</sup>CC% = correct classify percentage, ROC-AUC = receiver operating characteristic area under the curve. ROC-AUC results are the macro-average of the obtained individual ROC-AUCs curves (one vs. all approach).

<sup>2</sup>TA = traditional Apennines; TP = traditional Po Plain; TPLB = traditional Po Plain with local breeds; MWTMR = modern without TMR; MTMR = modern with TMR.

<sup>3</sup>The macro-cluster was created by merging individual cluster classes based on whether they belonged to a modern or traditional system.

been proposed as an indicator of the animal's susceptibility to metabolic diseases, such as acidosis and ketosis (Buttchereit et al., 2011; Guliński, 2021). Regarding the CReNBA evaluation, the LSM results showed that for MGT (the number of workers, worker training, and related activities) there were no significant differences between the dairy systems, except MTMR, which evidenced significantly higher scores than the others ( $P < 0.05$ ; Figure 1). This difference could be due to the better staff training, including veterinary support, associated with the larger herd size and intensive farm structure of MTMR (Lindena and Hess, 2022). Technological support and freestalls are also usually more common in modern and large farms, making tasks such as cleaning, feeding, and animal care more efficient (Bewley et al., 2017; Pouloupoulou et al., 2018; Edwards et al., 2020). In the case of SAE, which evaluated space, design, farm materials, and maintenance, MTMR again had the highest LSM score. However, there was greater confusion in the LSM values of this parameter in the other dairy systems because with SAE, as with MGT, the relationship between the factors evaluated and milk composition is fairly indirect (Meyer et al., 2004; Walker et al., 2004; Appuhamy et al., 2016). These factors (MGT and SAE) appear to be strongly influenced by financial investment (Fernandes et al., 2021; Hansen, 2023). In this study, we found that as the scores of these parameters increased, so did the average herd size, MY, and the use of freestalls and TMR. Among the different aspects assessed by CReNBA methodology (Bertocchi et al., 2018), the ABM is probably the parameter most clearly related to individual animal welfare (e.g., BCS, cleanliness, integument alterations, prevalence of lameness or mastitis, and so on) and consequently to possible relationships with milk composition (Sant'Anna and Paranhos da Costa, 2011; Arnould et al., 2013; Bahadi et al., 2021). Nevertheless, the LSM results for ABM revealed no differences between the different

dairy systems, except TP, which had significantly lower score than the others. To interpret this result, it is important to note certain considerations. For this parameter, we did not find the simultaneous increase in economic resources (e.g., herd size and MY) and CReNBA score, which initially suggested no relationship with the economic investment of the dairy system. The reason for this could be the housing system—TP uses exclusively tiestalls (100%), followed by TA and TPLB, which use tiestalls about 70% of the time—even though the CReNBA evaluation is theoretically based on the type of housing (Bertocchi et al., 2018). Nevertheless, we found that when the housing system was included as a factor in the ANOVA, the freestall class was assigned a higher score for ABM. It is also worth noting that ABM is the least specific of the 3 parameters related to housing type, differing only in freedom of movement and hoof health (Bertocchi et al., 2018). Without knowing the scores assigned to each checklist item, it is difficult to determine whether these differences are in fact critical. Given these uncertainties, these results must be treated with caution. As CReNBA is a herd level welfare evaluation, it is expected to reflect changes in bulk milk composition, as bulk milk quality relates to the herd as a whole, taking into account overall management and farm environment conditions (Velthuis and van Asseldonk, 2011). Although each CReNBA welfare parameter has several elements that could influence milk composition, the classes established for each parameter (low, medium, and high) did not appear to follow a defined pattern. Differences in milk composition do not appear to be associated with the classes of the different welfare parameters studied. Similar results were found by Ginestreti et al. (2020). The interactions between the items included in each CReNBA welfare parameter and their individual effects on milk composition are also unknown. Furthermore, the CReNBA evaluation counts the entire herd, including

**Table 6.** Confusion matrix mean results obtained from the test set after running the linear discriminant analysis (LDA) model 10 times using all samples for the cluster dairy system classification

Prediction	Reference				
	Traditional Apennines	Traditional Po Plain	Traditional Po Plain with local breeds	Modern without TMR	Modern with TMR
Traditional Apennines	249	153	11	90	82
Traditional Po Plain	104	185	3	38	27
Traditional Po Plain with local breeds	6	5	68	4	3
Modern without TMR	89	49	5	345	114
Modern with TMR	123	62	2	169	997

nonlactating cows (Bertocchi et al., 2018), which could further reduce the possible association with milk composition (Ginestreti et al., 2020). Additionally, intrinsic limitations of phenotypes derived from bulk tank milk include the dilution of individual milk information (Ginestreti et al., 2020), reduced variability (Visentin et al., 2015), and the nonlinear relationship between individual milk and bulk tank milk (De Lorenzi et al., 2021). These factors could contribute to the almost null authentication performance observed in this study for animal welfare traits using FT-MIR.

#### **Authentication of Farming Practices and Farm Characteristics by Infrared Spectroscopy**

The ability of infrared spectroscopy to authenticate farming practices is mainly due to the biochemical changes that such practices bring about, both directly and indirectly, in milk composition. This ability has been demonstrated for feeding practices (Valenti et al., 2013; Capuano et al., 2014), as feed is considered the most important factor affecting milk composition (Chilliard et al., 2007; Cabiddu et al., 2022). Among the feed-related practices studied, the best results were obtained for feeding system (TMR vs. no TMR; Table 4). This is confirmed by similar studies, where a high proportion of samples were correctly classified according to the feeding system (Gori et al., 2012; Frizzarin et al., 2021), although in both cases there were differences in diet between the systems. In any case, the use of TMR alone implies differences in milk composition. This is mainly because using TMR, which combines all dietary ingredients (e.g., concentrates, forages, minerals, other additives) into a single formulated ration rather than allowing cows to consume ingredients separately, ensures a nutritionally balanced intake in each bite (Schingoethe, 2017). Consequently, the use of TMR provides cows with a more precise and palatable diet, which reduces sorting behavior, improves nutrient assimilation, and reduces absorption variability (Bargo et al., 2002; Ferland et al., 2018). However, authentication based on a single feed criterion can lead to unreliable results (Coppa et

al., 2021), as observed in the present study when only the variable of concentrate inclusion (%) in the diet was included (ROC-AUC <0.64). Even when Coppa et al. (2021) used a different threshold to categorize the classes (28%), they still obtained similar poor results. Although genetic type is known to affect milk composition (Soyeurt et al., 2006; Stocco et al., 2017), as an isolated factor it lacks potential for authentication by infrared spectroscopy (Mouazen et al., 2009; Valenti et al., 2013). The successful authentication observed (ROC-AUC >0.95) is attributable to significant differences in the dairy systems in which the animals are housed and fed. Despite the potential advantages of local breeds over specialized cows in terms of functional and milk quality traits, there is still a significant disparity in MY (Gandini et al., 2007; Mancin et al., 2024). This difference in MY production, in turn, has a direct impact on the resource inputs and outputs that could influence the cow's diet (Sherf, 2000; Soini et al., 2012). Differences in resources are also well known in farm systems with different types of housing systems. Because the installation of freestalls requires significant investment, it is typically larger farms that use them, whereas smaller farms use tiestalls (Summer et al., 2014). For example, in this study, freestall farms had 3 times as many cows and a MY that was 5.8 kg/cow per day greater than tiestall farms. Housing system design is also responsible for significant differences in farm management and organization, as tiestalls required more working hours per cow, which affects the overall productivity of the farm (Poulopoulou et al., 2018). Other factors, such as environmental protection, space for the cow (for eating, sleeping, drinking), feeding design, or udder health, that are closely related to the housing system can affect milk composition (Summer et al., 2014; Bewley et al., 2017; Biasato et al., 2019), which could explain the good performance obtained (ROC-AUC >0.89). In the case of geographical area, the a priori difference in the composition of the milk is due to expected differences in available resources between the Apennine and the Po Plain systems. Historically, the former has been associated with small herds of native breeds grazing on local resources (Santini et al., 2013). However,

traditional farms in the hills and mountains are gradually shifting toward more intensive systems (Cocca et al., 2012; Sturaro et al., 2013), especially in mountainous areas with lower elevations (Cocca et al., 2012). This would reduce the hypothetically expected gap between production regions. For example, in this study, resources such as freestalls and TMR were used by 41% and 26% of the farms in the Apennines, whereas in the Po Plain, they were used by 77.5% and 49% of the farms, respectively. Furthermore, the average MY and the proportion of concentrates in the diet (%) were 27.3 kg/cow per day and 40% in the Apennines and 30.8 kg/cow per day and 43% in the Po Plain. Therefore, the medium-low average performance we observed (ROC-AUC of 0.70) was expected and was in line with the findings of Coppa et al. (2012) and Valenti et al. (2013). The putative characteristics of mountain milk (e.g., color, fatty acid profile) appear to be insufficient for discrimination by infrared spectroscopy, so it would be difficult to authenticate geographical area using this technique (Engel et al., 2007; Coppa et al., 2012). As the differences in management and resources between these areas decrease, it will become even more difficult to make positive predictions from infrared spectra (Cozzi et al., 2009).

### **Authentication of Dairy System by Infrared Spectroscopy**

The results of dairy system authentication by infrared spectroscopy depend on the overall variability in agricultural practices among the different systems, especially where more restrictive practices are concerned (Coppa et al., 2021). This could explain the variability in authentication performance observed in similar studies (Cozzi et al., 2009; Bergamaschi et al., 2020; Manuelian et al., 2021). The clustering method was used to create groups of dairy systems with the greatest differences in management and practices, potentially reflecting variations in milk composition. To determine the optimal number of clusters, we used the elbow method, which establishes a threshold based on the variance in the groups, beyond which adding one more group does not significantly improve the results (Bholowalia and Kumar, 2014). However, when analyzing a larger number of dairy systems, it is more likely that some of them will present similarities, leading to a loss of performance (Bergamaschi et al., 2020). For instance, in this study, close to 30% of TA and TPLB had freestall housing systems, the principal characteristic of the modern systems. At the same time, in the Apennine region, the typical TA area, 27% of farms were TPLB, and 12% MTMR. This could be one of the reasons for the confounding or minor effect of dairy system on milk composition. The LSM results did not show clear differences between the

systems, as observed in other studies where the dairy system had a weak effect on milk composition (Sturaro et al., 2013; Bittante et al., 2015). For example, there were no significant differences between the LSM for all milk composition traits in 2 of the 3 traditional systems, and for 4 of these traits, the 3 traditional systems had statistically equivalent values ( $P < 0.05$ ). Furthermore, it is likely that certain farming practices have a greater influence on milk composition than others (Manuelian et al., 2021). This may help to explain why MWTMR is equivalent to MTMR in only 2 composition traits, as they have the same characteristics, except for feeding system. The confusing effect of dairy system is mirrored in the predictive model, so that the CC% was good for only 2 classes (Table 5), as explained by the confusion matrix (Table 6). In contrast, the overall performance of the model was good (ROC-AUC  $>0.84$ ), which is because the ROC-AUC converts the multiple classification problem into a binary one (Galar et al., 2011). Because analyzing fewer classes results in greater significant differences in their effects on milk composition (Coppa et al., 2012), dairy systems are likely to be diverse, hence able to be authenticated (Cozzi et al., 2009). The LSM results confirmed this when we applied the same mixed model as for the cluster, but using the macro-cluster classification. All LSM show significant differences between the traditional and modern systems, allowing for the authentication of individual classes and of the total model (ROC-AUC  $>0.88$ ). This is in line with the findings of Bergamaschi et al. (2020), who obtained 73.5% accuracy in discriminating 3 classes, and 65% accuracy in discriminating 5 classes using FT-MIR.

### **CONCLUSIONS**

This study examined the feasibility of using FT-MIR to identify characteristics of dairy systems. The results show that it is possible to authenticate farming practices, and thus differentiate dairy systems. Because milk quality is already routinely assessed by FT-MIR within milk recording schemes on most dairy farms, this opens the way to routinely authenticating the practices and systems under which dairy products are produced. However, the discriminatory performance of FT-MIR appears suitable for routine use only when substantial differences in farming practices affect milk composition, thus improving the predictive ability of FT-MIR. Regarding the authentication of CReNBA animal welfare area using FT-MIR, this currently does not seem feasible, suggesting that the variations in herd animal welfare captured by different CReNBA indices are only weakly reflected in bulk milk. The results of this research could help dairy production chains target effective authentication techniques to differentiate Parmigiano Reggiano products.



## NOTES

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**Nonstandard abbreviations used:** ABM = animal-based measures; CC% = correctly classified samples; CON = concentrate proportion in the lactating cows' diet; CRENBA = Italian National Reference Center for Animal Welfare; FPR = fat:protein ratio; FT-MIR = Fourier-transform MIR; LDA = linear discriminant analysis; MGT = farm management; MIR = mid-infrared spectroscopy MTMR = modern with TMR; MWTMR = modern without TMR; MY = milk yield; PDO = Protected Designation of Origin; PRC = Parmigiano Reggiano Consortium; ROC = receiver operating characteristic; ROC-AUC = area under the receiver operating characteristic curve; SAE = structure and equipment; TA = traditional Apennines; TP = traditional Po Plain; TPLB = traditional Po Plain with local breeds.

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