



Effects of breed, farm intensiveness, and cow productivity level on cheese-making ability predicted using infrared spectral data at the population level

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ABSTRACT

Fourier-transform infrared (FTIR) spectra collected during milk recording schemes at population level can be used for predicting novel traits of interest for farm management, cows' genetic improvement, and milk payment systems. The aims of this study were as follows. (1) To predict cheese yield traits using FTIR spectra from routine milk recordings exploiting previously developed calibration equations. (2) To compare the predicted cheese-making abilities of different dairy and dual-purpose breeds. (3) To analyze the effects of herds' level of intensiveness (HL) and of the cow's level of productivity (CL). (4) To compare the patterns of predicted cheese yields with the patterns of milk composition in different breeds to discern the drivers of cheese-making efficiency. The major sources of variation of FTIR predictions of cheese yield ability (fresh cheese or cheese solids produced per unit milk) of individual milk samples were studied on 115,819 cows of 4 breeds (2 specialized dairy breeds, Holstein and Brown Swiss, and 2 dual-purpose breeds, Simmental and Alpine Grey) from 6,430 herds and exploiting 1,759,706 FTIR test-day spectra collected over 7 yr of milk sampling. Calibration equations used were previously developed on 1,264 individual laboratory model cheese procedures (cross-validation R^2 0.85 and 0.95 for fresh and solids cheese yields, respectively). The linear model used for statistical analysis included the effects of parity, lactation stage, year of calving, month of sampling, HL, CL, breed of cow, and the interactions breed \times HL and breed \times CL. The HL and CL stratifications (5 classes each) were based on average daily secretion of milk net energy per cow. All effects were highly significant ($P < 0.001$). The major conclusions were as follows.

(1) The FTIR-based prediction of cheese yield of milk goes beyond the knowledge of fat and protein content, partially explaining differences in cheese-making ability in different cows, breeds and herds. (2) Differences in cheese yields of different breeds are only partially explained by milk fat and protein composition, and less productive breeds are characterized by a higher milk nutrient content as well as a higher recovery of nutrients in the cheese. (3) High-intensive herds not only produce much more milk, but the milk has a higher nutrient content and a higher cheese yield, whereas within herds, compared with less productive cows, the more productive cows have a much greater milk yield, milk with a greater content of fat but not of protein, and a moderate improvement in cheese yield, differing little from expectations based on milk composition. Finally, (4) the effects of HL and CL on milk quality and cheese-making ability are similar but not identical in different breeds, the less productive ones having some advantage in terms of cheese-making ability. We can obtain FTIR-based prediction of cheese yield from individual milk samples retrospectively at population level, which seems to go beyond the simple knowledge of milk composition, incorporating information on nutrient retention ability in cheese, with possible advantages for management of farms, genetic improvement of dairy cows, and milk payment systems.

Key words: infrared predictions, FTIR, cheese yield, milk composition, breed \times environment interaction

INTRODUCTION

Cheese manufacture is the main use of milk worldwide (USDA, Foreign Agricultural Service, 2020), and in the European Union it accounts for almost 40% of milk produced (75% in Italy; Eurostat, 2020). Cheese yield (the ratio between the cheese produced and the milk processed) is therefore the most important economic attribute of milk (Emmons, 1993). Genetic improve-

Received March 22, 2021.

Accepted June 30, 2021.

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ment of cheese yield is not pursued directly, as taking routine measurements of this property using milk samples from individual cows is very expensive and time consuming (Hicks et al., 1981; Cipolat-Gotet et al., 2016b). Traditionally, attempts to improve cheese yield are made indirectly by including the fat and protein (or casein) contents of milk in selection indices (VanRaden, 2004; Miglior et al., 2005). It is implicitly assumed that the recovery rates of milk nutrients (fat and protein) in cheese are constant, and that the retention of water in the curd depends only on the fat and protein content. However, it has been clearly shown that the recovery of fat and protein in the curd during cheese-making is affected by many environmental factors (Law and Tamine, 2010) and also by the genetics of the lactating females (Othmane et al., 2002; Bittante et al., 2013; Dadousis et al., 2017a,b). Environmental factors and genetics affect also the retention of water in the curd not only during cheese-making, but also while the cheese is ripening, thus influencing the final yield of ripened cheese (Cipolat-Gotet et al., 2020).

In a previous study on cows of 6 dairy and dual-purpose breeds reared in multibreed herds (Stocco et al., 2018), we showed that the actual cheese yield, measured experimentally for individual cows using model cheese-making procedures, may differ from the theoretical cheese yield obtained from predictions based on milk composition (Emmons et al., 1990). Given the conceptual similarity to the residual DMI (the difference between a measured and an expected value), the ratio between the actual and the expected cheese yield could be interpreted as an estimation of the efficiency of cheese production. We also showed that cheese-making efficiency not only differs among different cows within the same herd and breed but is also significantly affected by the breed of cow and the level of productivity intensiveness of the herd (Stocco et al., 2018).

To translate this theoretical knowledge into practical use for the benefit of the dairy chain, it is important to find methods for predicting cheese yield that are not merely based on milk composition but are also able to capture other intrinsic factors affecting the actual cheese yield of milk. We tested the feasibility of using Fourier-transform infrared (**FTIR**) spectroscopy to predict cheese yield and nutrient recoveries in practice (Bittante et al., 2014; Ferragina et al., 2015). These and other FTIR predictions of cheese yield (Bonfatti et al., 2017; El Jabri et al., 2019) have been found to be heritable and can therefore be used to establish a selection program aimed at improving the cheese-making ability in different breeds (Cecchinato et al., 2015; Bonfatti et al., 2017; Sanchez et al., 2018).

What has not yet been fully clarified is whether FTIR-based predictions, unlike traditional cheese yield

formulas based on milk fat and protein contents, are able to capture these other intrinsic factors at the population level and whether they are able to reproduce the differences among different breeds and dairy systems found experimentally using laboratory model cheese-making procedures. The aims of this study, therefore, were as follows: (1) to predict cheese yield traits at the population level using FTIR from a large data set of milk infrared spectra collected during routine milk recordings, with special attention to circannual variations and to the effects of lactation stage; (2) to compare the predicted cheese-making abilities of different dairy and dual-purpose breeds; (3) to stratify different herds within breed according to the herd's level of intensiveness and different cows within herds according to the cow's level of productivity to analyze their effects on the predicted traits; and (4) to compare the patterns of predicted cheese yields with the patterns of milk composition in different breeds (interactions) to discern the drivers of cheese-making efficiency.

MATERIALS AND METHODS

Experimental Design

To meet the objectives of this study, we consulted the historical database of the Breeders Federation of Alto Adige/Südtirol (Associazione Provinciale delle Organizzazioni Zootecniche Altoatesine / Vereinigung der Südtiroler Tierzuchtverbände, Bolzano/Bozen, Italy) of the northeastern area of the province of Bolzano/Bozen in Italy. We extracted all the data pertaining to cows of the 4 most common breeds in the province. These were 2 specialized dairy breeds, Holstein (**HO**) and Brown Swiss (**BS**), and 2 dual-purpose breeds, Simmental (**SI**) and Alpine Grey (**AG**). Multibreed herds, that is, those with cows of 2 or more breeds (about one-third of the total), were treated as 2 or more single-breed herds.

The herds within each breed were assigned to 1 of 5 classes on the basis of the herd's intensiveness of production level (**HL**), defined on the basis of the corrected average daily milk energy output of the herd (**dMEO**, MJ/d), described later. Individual cows within each herd were also classified into 5 productivity levels (**CL**) according to their individual dMEO.

Milk Recording Data and Editing

The data we used were collected during milk recording sessions between January 2011 and December 2017. We extracted a total of 1,898,994 test-day records. Only herds with more than 5 cows, and cows with more than 5 records, were retained. Parity was classified as first, second, third, fourth, or fifth and over. The numbers

of herds, cows, lactations, and test days retained after editing and used for the data analyses are summarized in Table 1.

Test-day milk recording data included daily milk yield. Milk composition was determined from a sample (with preservative added) at the laboratory of the Federazione Latterie Alto Adige/Sennereiverband Südtirol (Bolzano/Bozen). The milk samples were analyzed with a MilkoScan FT+ 6000 (Foss A/S) on the basis of FTIR spectra using the calibration equations preinstalled by the manufacturer. All operations complied with ICAR (2016) guidelines. The milk components examined in this study were fat, protein, and lactose. For all milk traits, only data within the range of mean (μ) \pm 3 standard deviations (SD; σ) for each trait were retained.

Editing and Pre-Processing of Milk FTIR Spectra and Predictions of Cheese Yield Traits

The FTIR spectra used in the study were edited as follows: a principal component analysis was performed on the FTIR spectra, and Mahalanobis distances were calculated from the first 5 principal component scores. The probability level for the chi-squared distribution of each sample was then calculated, and samples with a probability of <0.01 were considered outliers and removed from the data set (Shah and Gemperline, 1989). To overcome the spectral variations, the absorbance values for every wave were centered to a null mean and standardized to a unit sample variance within year periods.

Two previously determined calibration equations, developed on the entire spectral interval, were used to predict the cheese yield of the milk samples from individual cows. These equations were obtained from a large study carried out on 85 herds managed under different dairy systems, which involved sampling 1,167 individual cows (2.0 L of milk per cow) once during

a calendar year and processing the milk samples into individual model cheeses (Ferragina et al., 2013).

The first equation predicted the cheese yield of the unprocessed milk sample in terms of the obtainable fresh curd, expressed as a percentage of the milk destined for cheese-making. This equation had a coefficient of determination (R^2) of cross-validation of 0.85, and a standard error (SE) of prediction, corrected for bias, of 0.97%. To account for the possibility of controlling water retention in the fresh cheese by modifying the cheese-making procedure, and to account for the possibility that the residual variability of this trait cannot be easily controlled, the second equation predicted cheese yield in terms of the obtainable DM of fresh cheese expressed as a percentage of the milk destined for cheese-making. This second equation had a larger R^2 of cross-validation (0.95) and a smaller SE of prediction (0.27). The corresponding ratio of prediction to deviation was 2.45 for the first equation and 4.24 for the second, corresponding to very good predictability and excellent predictability, respectively, according to the classification proposed by Viscarra Rossel et al. (2007).

Stratification by Herd Intensiveness Level and Cow Productivity Level

The herds were stratified into 5 HL levels, defined according to the average dMEO of all the lactating cows in the herd. The net energy content (NE_L) of the milk was estimated using the following equation proposed by the NRC (2001):

$$NE_L \text{ (Mcal/kg)} = 0.0929 \times \text{fat, \%} + 0.0547 \\ \times \text{protein, \%} + 0.0395 \times \text{lactose, \%},$$

where NE_L is the energy of one kilogram of milk. The NE_L values obtained were converted to MJ/kg and multiplied by the daily milk yield of each cow (kg/d) to obtain the individual dMEO of each cow (MJ/d). The

Table 1. Data available after editing by breed of cows¹

Item, N	Total	HO	BS	SI	AG
Years	7	7	7	7	7
Herds	6,430	1,298	2,212	1,800	1,120
Lactating cows	115,819	24,421	42,278	33,503	15,617
Lactations	291,129	57,081	103,353	87,855	42,840
First lactations	95,049	20,048	34,277	27,920	12,804
Second lactations	74,976	15,830	26,746	22,001	10,399
Third lactations	55,780	10,964	19,990	16,597	8,229
Fourth lactations	33,578	5,866	11,912	10,452	5,348
\geq Fifth lactations	31,746	4,373	10,428	10,885	6,060
Test days/FTIR spectra	1,759,706	355,662	656,694	509,858	237,492

¹HO = Holstein; BS = Brown Swiss; SI = dual-purpose Simmental; AG = Alpine Grey.

herds were classified according to their dMEO using a mixed model of the following form:

$$y_{ijklmnop} = \mu + P_i + D_j + YS_k + b_1B_l + b_2S_m + b_3A_n + b_4H_o + R_p + e_{ijklmnop},$$

where $y_{ijklmnop}$ is the dMEO for the test-day; μ is the general mean; P_i is the parity number ($i = 1, 2, 3, 4$, or ≥ 5); D_j is the category of DIM ($j = 12$ groups of 30 d each, with the last category open); YS_k is the combined effect of year-season ($k = 2011$ – 2017 ; seasons = April–September or October–March); B_l , S_m , A_n , and H_o are the percentages in the herd of Brown Swiss, Simmental, Alpine Grey, and Holstein cows, respectively; b_1 , b_2 , b_3 , and b_4 are the linear regression coefficients for B_l , S_m , A_n , and H_o , respectively; R_p is the random effect of herd ($p = 6,430$ herds); and $e_{ijklmnop}$ is the random experimental error. Herd and residuals were assumed to have a normal distribution with a mean of zero and variances of σ_h^2 and σ_e^2 , respectively. The herd solutions were used to classify them into 5 dMEO levels (HL 1–5). The 5 classes of HL were obtained as follows: first class (HL-1) with herds $< -1.5\sigma$; second class (HL-2) -1.5σ to -0.5σ ; third class (HL-3) -0.5σ to $+0.5\sigma$; fourth class (HL-4) $+0.5\sigma$ to $+1.5\sigma$; and fifth class (HL-5) $> +1.5\sigma$, where σ is the SD of the herd's least squares means (LSM) within breed.

To classify the individual cows within each breed and within the herds (CL), we used a mixed model of the following form:

$$y_{ijklmn} = \mu + P_i + D_j + YS_k + HL_l + A_m + e_{ijklmn},$$

where y_{ijklmn} is the dMEO for the test-day; μ is the general mean; P_i is the parity number ($i = 1, 2, 3, 4$, or ≥ 5); D_j is the category of DIM ($j = 12$ groups of 30 d each, with the last category open); YS_k is the combined effect of year-season ($k = 2011$ – 2017 ; seasons = April–September or October–March); HL_l is the herd intensiveness level ($l = 1$ – 5); A_m is the random effect of the animal ($m = 115,819$ cows); and e_{ijklmn} is the random experimental error. Animal and residuals were assumed to have a normal distribution with a mean of zero and variances of σ_a^2 and σ_e^2 , respectively. The cow solutions were used to classify them into 5 dMEO levels (CL A–E) as follows: first class (CL-A) with cows $< -1.5\sigma$; second class (CL-B) -1.5σ to -0.5σ ; third class (CL-C) -0.5σ to $+0.5\sigma$; fourth class (CL-D) $+0.5\sigma$ to $+1.5\sigma$; and fifth class (CL-E) $> +1.5\sigma$, where σ is the SD of animals within breed and herd. Table 2 summarizes the numbers of herds, cows, and test dates, and the average dMEO of each class within each breed. The number of

herds in the different strata of HL and CL were not much different from expectations on the basis of assumption of a normal distribution. The number of cows per herd for all breeds is increased moving from HL-1 to HL-2, because high-intensive herds tended to be larger than low-intensive ones. Thus, the number of cows (and test dates) increased with HL (but not with CL), with respect to “normality” expectations. Finally, the number of test dates per cow increased with CL (but not with HL), possibly because of an anticipated culling of low-producing cows.

Statistical Analyses

Milk yield, composition, and cheese-related traits were analyzed using a linear model of the following form:

$$y_{ijklmnop} = \mu + P_i + D_j + Y_k + M_l + HL_m + CL_n + B_o + HL_m \times B_o + CL_n \times B_o + e_{ijklmnop},$$

where $y_{ijklmnop}$ is the response of the trait (milk yield, fat, protein, lactose, fresh cheese, cheese solids); μ is the general mean; P_i is the parity number ($i = 1, 2, 3, 4$, or ≥ 5); D_j is the category of DIM ($j = 12$ groups of 30 d each, with the last category open); Y_k is the effect of year ($k = 2011$ – 2017); M_l is the effect of the month ($l = \text{January–December}$); HL_m is the herd intensiveness level ($m = 1$ – 5); CL_n is the cow productivity level ($n = 1$ – 5); B_o is the effect of breed ($o = \text{Brown Swiss, Simmental, Alpine Grey, or Holstein}$); $HL_m \times B_o$ is the effect of the interaction between herd intensiveness level m and breed o ; $CL_n \times B_o$ is the effect of the interaction between cow productivity level n and breed o ; and $e_{ijklmnop}$ is the random residual. The models were fitted using the *lm* and *aov* functions in R.

Least squares means (LSM) were estimated for each trait for the effects of month, DIM, breed, HL, and CL, and the interactions HL \times Breed and CL \times Breed. Orthogonal contrasts were estimated between the LSM of traits for the effect of breed as follows: (a) specialized dairy (HO and BS) versus dual-purpose breeds (SI and AG); (b) within specialized breed (HO vs. BS); and (c) within dual-purpose breed (SI vs. AG). All data editing and statistical analyses were conducted in the R environment (R Core Team, 2016).

RESULTS AND DISCUSSION

Main Sources of Variation in Milk Yield and Composition, and in Cheese Yield Traits

Table 3 summarizes the descriptive statistics and the results of the ANOVA conducted on the 6 traits con-

Table 2. Descriptive statistics of stratification of data according to the 5 classes of herd intensiveness level (HL) and the 5 classes of the cow productivity level (CL)¹

Item	HL					CL				
	HL-1	HL-2	HL-3	HL-4	HL-5	CL-A	CL-B	CL-C	CL-D	CL-E
Holstein										
Herds, N	70	282	483	328	135	571	1,060	1,089	800	428
Cows, N	844	3,761	7,913	8,038	3,865	1,474	5,811	9,823	5,675	1,638
Test dates, N	14,194	58,053	116,355	117,026	50,034	15,734	75,110	147,725	91,531	25,562
dMEO, MJ/d	63.5	72.7	83.0	91.8	101.3	65.1	74.4	82.8	90.9	99.2
Brown Swiss										
Herds, N	167	549	837	485	174	986	1,857	2,030	1,710	920
Cows, N	1,285	6,483	14,205	13,184	7,121	2,442	10,215	17,263	9,466	2,892
Test dates, N	19,290	100,439	223,236	206,689	107,040	24,288	134,551	276,500	168,880	52,475
dMEO, MJ/d	50.0	61.3	71.1	80.9	92.2	54.7	63.6	71.3	78.6	87.4
Simmental										
Herds, N	123	431	710	389	147	763	1,411	1,617	1,388	751
Cows, N	1,233	5,622	12,015	9,377	5,256	1,983	8,117	13,521	7,617	2,265
Test dates, N	17,884	87,406	184,863	140,948	78,757	20,422	107,218	213,134	129,958	39,126
dMEO, MJ/d	49.1	59.5	69.6	79.6	89.6	54.1	62.4	69.6	76.7	84.6
Alpine Grey										
Herds, N	39	284	541	227	29	447	859	968	809	450
Cows, N	207	2,064	7,748	4,654	944	933	3,879	6,132	3,569	1,104
Test dates, N	2,390	30,391	117,150	72,260	15,301	7,696	47,753	96,636	64,445	20,962
dMEO, MJ/d	31.3	39.8	48.1	56.7	64.6	34.6	41.9	48.2	54.4	61.4

¹The HL classes were designed within breed using the LSM of individual herds for daily milk energy output (dMEO, MJ/d). The CL were designed using the cow's residual dMEO within breed and herd. The 5 classes of HL (or CL) were obtained as follows: first class (HL-1 or CL-A) with herds (or cows) $< -1.5\sigma$; second class (HL-2 or CL-B) -1.5σ to -0.5σ ; third class (HL-3 or CL-C) -0.5σ to $+0.5\sigma$; fourth class (HL-4 or CL-D) $+0.5\sigma$ to $+1.5\sigma$; fifth class (HL-5 or CL-E) $> +1.5\sigma$, where σ is the SD of herds' LSM within breed (or cow SD of animals within breed and herd).

Table 3. Descriptive statistics and ANOVA (F -values) of milk yield, milk composition, and cheese yield¹

Item ²	df	Milk yield, kg/d	Milk composition, %			Cheese yield, %	
			Fat	Protein	Lactose	Fresh cheese ²	Solids ³
Descriptive statistics							
Mean	—	23.2	3.95	3.33	4.79	15.06	7.22
SD	—	7.4	0.50	0.29	0.18	2.05	0.83
ANOVA							
Year of calving	6	3,691	823	4,109	1,441	4,355	3,449
Month	11	996	550	2,235	1,806	214	384
Parity	4	47,847	94	6,023	49,986	1,549	1,161
Stage of lactation	11	138,265	3,013	21,924	14,868	52,075	59,310
Breed	3	372,766	47,210	108,402	12,905	3,467	4,144
Herd intensiveness class	4	246,246	9,050	31,458	4,150	14,221	16,904
Cow productivity class	4	131,360	4,608	55	1,372	1,536	1,531
Herd intensiveness by breed	12	214	248	607	57	57	64
Cow productivity by breed	12	170	53	84	42	15	15
RMSE ⁴	1,759,637	4.0	0.44	0.31	0.14	1.73	0.70

¹All effects are highly significant ($P < 0.001$); thus, P -values are not reported in table.

²Cheese yield of fresh cheese is the weight of the fresh cheese after salting, expressed as a percentage of the weight of the milk processed.

³Cheese yield of solids is the weight of the DM of the fresh cheese, expressed as a percentage of the weight of the milk processed.

⁴RMSE = root mean squared error.

sidered in this study. Due to the very large amount of data analyzed, all the effects included in the statistical model were highly significant for all traits ($P < 0.001$), but they are not necessarily all relevant to the dairy industry. The relative importance of the various factors for each trait can be evaluated from the size of the F -values in the table. It is worth noting that the different traits are ranked differently according to the sources of variation.

Year of production was a notable source of variation but was never among the most important for any of the traits considered. Milk yield exhibited an increasing pattern during the 7 yr of observation (21.2–22.5 kg/d), reflecting the positive genetic trend in the breeds and improvements in feeds and management. In the case of milk composition and predicted cheese yield traits, the yearly fluctuations (SD of LSM of the year effect: $\pm 0.28\%$) are probably more a reflection of the effects of climatic variations on the animals and the quantity and quality of forages produced. Seasonal variations were less important than yearly variations, with the exception of lactose content, but because some of the traits exhibited interesting patterns, this will be illustrated and discussed in the next section. As expected, parity affected the daily milk yield, which increased with the maturity of the cow (19.3 kg/d on average at first lactation, to 22.6 kg/d on average for the fifth+ lactation) but also had an appreciable decreasing effect on the lactose (4.86% on average at first lactation to 4.69% at fifth+ lactation) and protein contents (3.30–3.23%), and a much smaller effect on milk fat content. In parallel with the variations in nutrient contents, cheese yield

traits also decreased with increasing parity of the cow (15.0% on average at first lactation to 14.7% at fifth+ lactation).

For milk yield, fat, and protein contents, the second most important source of variation was HL, whereas CL was less important and differed little from the effect of lactation stage, with the exception of milk protein content, which was much less affected than fat content and milk yield by CL (Table 3). Lactose presented a completely different picture, being primarily affected by the cow's parity and then by lactation stage. Breed of cow, HL, and CL were much less important for this trait than for the other milk components.

Finally, the predicted cheese yield traits were influenced first by the cow's stage of lactation and then by HL. The effects of parity, breed, and CL on cheese yield were less important. The unexpected differences in the ranking of the sources of variation between milk composition and cheese yield are worth discussing in detail and will be dealt with in following sections.

For all traits, the interaction of HL and CL with the cow's breed was highly significant ($P < 0.01$) but quantitatively much less relevant than either the effect of breed or the effects of HL and CL. Nonetheless, these interactions will be discussed in detail, because they contribute to identifying different genetic and environmental drivers of cheese-making efficiency.

The results on the relative importance of different source of variation of milk composition traits and cheese yields cannot be compared with others, because the scientific literature does not contain any studies that quantify simultaneously the relative importance

of different sources of variation (year, season, parity, lactation stage, breed, dairy system, and cow productivity) for several traits.

The yearly variation of milk yield and the effects of parity followed the expected patterns, as also reported by other authors in same study area (Kühl et al., 2020), and, being not included in the objective of this study, are not shown and discussed in detail.

Circannual Variation in Milk Yield and Composition, and in Cheese Yield Traits

As depicted in Figure 1, the circannual pattern of the 6 traits considered all showed a sinusoidal pattern with a wave period of one calendar year. Daily milk yield reaches its zenith (crest of the wave) in the winter months (December–March), and its nadir (trough of the wave) in the summer months (July and August). This pattern has frequently been observed in the northern hemisphere (Heck et al., 2009), but the peak-to-peak amplitude was also found to be very small (less than 1 kg/d). The prevailing climate in the study area is humid-continental (warm summer subtype) according to the Köppen classification (Belda et al., 2014), and heat stress during summer is not frequent. Regarding feeding systems, the more intensive modern farms use total mixed rations with a roughly constant composition all year round, whereas the more traditional farms use hay and sometimes silages with compound feed. Traditional farms that transhume part or all of their herd to summer highland pastures are normally excluded from milk recording during the pasturing period (Bittante et al., 2015).

Milk fat content reaches its nadir in spring (May) and its zenith in autumn (October), whereas milk protein content reaches these values about one month earlier (April and September, respectively). In both cases, the peak-to-peak amplitude is about 0.1% (Figure 1). In the case of lactose content, the amplitude is much smaller (0.02%), and the nadir is reached contemporaneously with fat and protein (April–May), while the zenith is later, in December.

The patterns of the predicted cheese yield traits differ slightly from the typical sinusoid: they are rather flat from autumn to winter and in early spring, then rise to their peak, reaching the zenith in July when expressed as fresh cheese, and in August as cheese DM (Figure 1). We can speculate, from the fact that they reach their zenith about 2 mo earlier than fat and protein content, that the efficiency of milk nutrient recovery in cheese is not constant throughout the year but is instead slightly higher in summer than in autumn.

The peak-to-peak amplitude of cheese solids yield is also about 0.1%, which is lower than the value expected assuming an additive effect of milk fat and protein and after taking into account their average recovery rates in the curd (Emmons et al., 1990). The amplitude of fresh cheese yield is only slightly greater than that of cheese solids, meaning that water retention is probably not greatly affected by seasonal variations.

It is worth noting that the seasonal relationships between milk yield, milk nutrient concentrations, cheese yield and quality, and defects in cheeses are very complex. Several traditional cooperative dairies in the area produce a hard, long-ripened cheese (Trentingrana) in accordance with European Union regulations for Protected Designation of Origin certification (Legislative Decree July 20, 2006), and the bimonthly batches of cheese produced are monitored by the Consortium of Cooperative Dairies for sensory characteristics (on sampled wheels) and for quality classification of all wheels after 9 and 18 mo of ripening. Both the sensory evaluations and quality classifications are the basis of a quality-based payment system for the cheese. Monitoring over 10 yr has established that both the sensory descriptors and quality classifications of cheese follow a circannual pattern (Bittante et al., 2011a,b). The quality score, which summarizes the sensorial description, reaches its highest average value for cheeses produced during spring (Bittante et al., 2011b), when daily milk yield is high, and milk nutrient concentrations and cheese yield percentages are low (Figure 1), and its lowest values for cheese produced during summer. The cheese classification, based specifically on the presence or absence of cheese defects, showed that the cheeses produced from late spring to early summer had the highest incidence of top-quality wheels, and those produced in winter the lowest (Bittante et al., 2011a). This means that the cheese yield is related to but does not overlap with milk composition, whereas the quality of the cheese seems to depend mainly on other factors, among which milk and cheese microbiota may play a major role (Carafa et al., 2019).

Effects of Lactation Stage on Milk Yield and Composition, and on Cheese Yield Traits

Stage of lactation had a much greater effect than parity on the milk traits, with the exception of lactose (Table 3), and was the most important source of variation in predicted cheese yield traits. Figure 2 depicts the lactation curve for all 6 milk traits considered. The lactation curve for daily milk yield reached its maximum peak between the first and second month after

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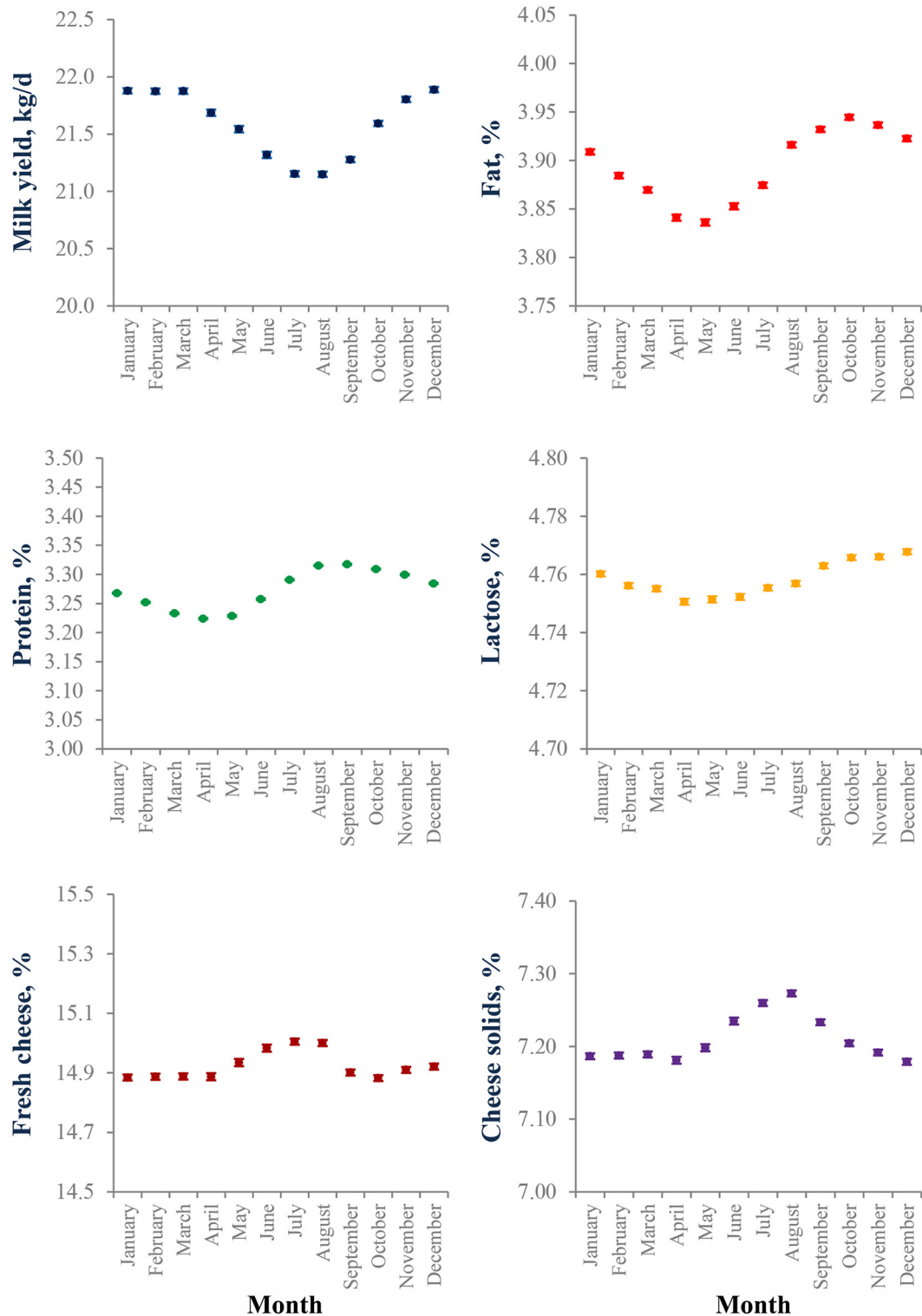


Figure 1. Circannual variation of milk yield and composition and cheese yield (LSM ± SE). Cheese yield of fresh cheese is the weight of the fresh cheese after salting, expressed as a percentage of the weight of the milk processed; cheese yield of DM is the weight of the DM of the fresh cheese, expressed as a percentage of the weight of the milk processed.

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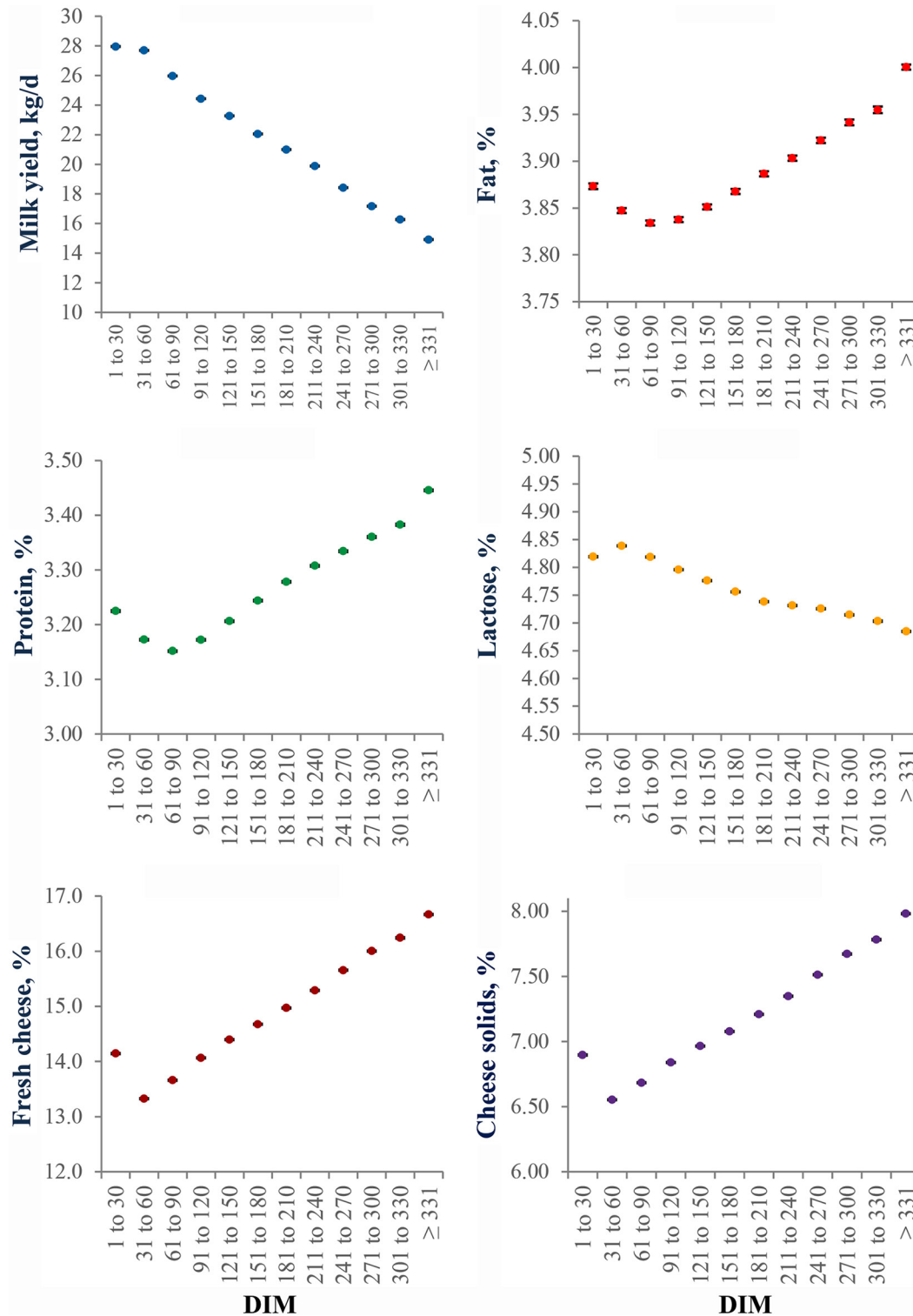


Figure 2. Effect of lactation stage (classes of DIM) on milk yield and composition and predicted cheese yields (LSM \pm SE). Cheese yield of fresh cheese is the weight of the fresh cheese after salting, expressed as a percentage of the weight of the milk processed; cheese yield of DIM is the weight of the DM of the fresh cheese, expressed as a percentage of the weight of the milk processed.

calving, and then declined almost linearly until a final value almost half that of the peak (“zenith curve”). In previous studies we found the peak to be higher and later for cows with higher productivity levels and reared in intensive farms (Stocco et al., 2017).

The lactation curve for milk fat and protein content had a reversed shape (“nadir curve”), reaching its minimum during the third month of lactation and increasing thereafter almost linearly until the end of lactation. The pattern of milk fat and protein does not therefore mirror that of milk yield, as it reaches its peak later. Contrary to fat and protein, lactose content had a “zenith curve,” as did daily milk yield, reaching its peak value during the second month of lactation and thereafter decreasing until the end.

The curves for the 2 predicted cheese yield traits had a shape more similar to the curves for milk fat and protein contents (“nadir curves”), but with some differences: The initial decrease is sharper and shorter, reaching minimum values during the second month of lactation; that is, one month earlier than milk components. Quantitatively, the maximum variation (from the nadir to the end of lactation) in milk fat content was about +0.17%, and in milk protein +0.30%. In the same interval, the predicted cheese solids yield increased by 1.5% and the predicted fresh cheese yield by +3.4%. The increase in the former trait is much larger than expected, given the variation in fat and protein (3 times), meaning that the rate of recovery of milk fat and protein in the cheese is not constant but rather increases during lactation, thus improving cheese-making efficiency. The large increase in fresh cheese yield (7 times the increase in fat and protein) clearly shows that the increased efficiency of fat and protein recovery in the curd is accompanied by a parallel increase in water retention.

Using laboratory cheese-making procedures with milk from individual cows (1,167 model cheeses produced from Brown Swiss cows), we found that protein recovery in the cheese increased during the first part of lactation (about +1% unit) and remained constant throughout the second part (Cipolat-Gotet et al., 2013). In contrast, fat recovery in the cheese decreased during the first half of lactation and increased during the second. Overall, the recovery of milk solids and energy decreased until the peak of lactation (between 1 and 2 mo after calving) and then increased until the end of lactation (+4% units for total solids, +1.5% for energy). Therefore, the improvement in cheese-making efficiency throughout lactation that we observed in this study when comparing the predicted cheese yield and milk composition is not an artifact of the FTIR predictions but reflects a phenomenon observed experimentally in the laboratory over more than 1,000 model

cheese-makings using milk from individual cows. In a recent study carried out with cows of 6 breeds (Holstein, Brown Swiss, Jersey, Simmental, Alpine Grey, and Rendena) reared in multibreed herds, we found a similar increase in milk total solids and energy recoveries in the cheese throughout lactation (513 individual model cheeses; Stocco et al., 2018), but we also found a significant interaction with the breed of the cow.

We have also found that the recovery of nutrients in cheese is not constant throughout lactation in other species. In buffaloes, in particular, we observed increases in the recovery of protein in the cheese of about 4 percentage units with advancing lactation, in total solids of 8 units, and in milk energy of 5 units, whereas the recovery of fat increased by only 1 unit (180 individual cheese-makings; Cipolat-Gotet et al., 2015). We also found a significant improvement in the recovery of total milk solids (+5% units) and energy (+3.5% units) in the manufacture of sheep’s milk cheese, but not of protein and fat (169 individual cheese-makings, Sarda sheep breed; Cipolat-Gotet et al., 2016a). In the case of goat milk, we observed much smaller variations throughout lactation, but here we used a simplified cheese-making procedure (560 individual cheese-makings, 6 breeds; Vacca et al., 2018).

It is not easy to test the process of making cheese using milk from cows at different stages of lactation on an industrial scale, but Kefford et al. (1995) mimicked industrial production of Cheddar cheese using bulk milk from cows at mid- or late-lactation. Those authors also observed a significant increase in milk total solids recovery in the cheese (+2% units) but no significant variations in fat and protein recoveries.

The main reason for this variation in cheese-making efficiency corrected for milk fat and protein contents is probably found in the proportions of the different milk protein fractions throughout lactation. In fact, in a recent study modeling changes throughout lactation in the detailed protein profiles of milk produced by cows of 6 breeds raised in the same geographical area as the present study, we found that different nitrogenous fractions are characterized by very different lactation curve shapes (Amalfitano et al., 2021). Amalfitano et al. (2019) showed that increasing the concentrations or proportions of α_{S1} -casein and κ -casein in milk clearly improves coagulation, curd firming, and syneresis properties, whereas increasing the concentrations or proportions of α_{S2} -casein and β -lactoglobulin causes a worsening of these properties. Using the laboratory model cheese-making procedure, Cipolat-Gotet et al. (2018) confirmed that the α_{S1} -CN concentration and proportion exerts a favorable effect on cheese yield and the recovery of both milk fat and protein in the curd, whereas α_{S2} -CN and β -LG exert an unfavorable effect

on cheese yield and nutrient recoveries. Both β -CN and κ -CN had positive effects on cheese yield but the opposite effect on milk nutrient recovery in the curd: β -CN improved the recovery of protein and worsened the recovery of fat, whereas κ -CN worsened the recovery of protein and improved the recovery of fat. As the major casein fractions α_{S1} -CN and β -CN expressed as proportions of total milk nitrogen $\times 6.38$ have almost opposite lactation patterns (the former “downward,” the latter “zenith”), and, as both have a favorable effect on cheese yield, they tend to compensate for each other. Also, α_{S2} -CN and κ -CN had opposite lactation patterns (the former “downward,” the latter “upward”), but, because α_{S2} -casein has an unfavorable effect and κ -casein a very favorable effect, both fractions contributed to explaining the increasing efficiency of cheese-making throughout lactation.

Effects of Breed on Milk Yield and Composition, and Cheese Yield Traits

The LSM of the effect of breed are summarized in Table 4. They confirm that the daily production of the specialized dairy breeds is higher than that of the dual-purpose breeds, and that within the former group daily production of HO is higher than BS, whereas in the latter group SI is higher than AG. It is worth noting that these differences are due not only to breed characteristics but also to the environment, facilities, management, handlers, feeding techniques, and hygiene practices, as the cows of these different breeds are often reared in monobreed herds. The inclusion of HL in the model does not correct the estimates of the breed effect for differences in farm intensiveness, because the herds were stratified according to HL within each breed—that is, the HL classes are specific to each breed. A study carried out in the same area on multibreed herds, comparing breeds within the same farm (Stocco et al.,

2017), found lower differences in breed than we did (about 3 instead of 5–6 kg/d for every orthogonal contrast), which means that about half the differences are due to differences in the environment or management, and half to genetic breed specificities.

The fat content was, on average, higher in milk from herds of dairy breeds than in milk from herds of dual-purpose breeds, and, within the former group, higher from BS than from HO herds, and in the latter group higher from SI than AG herds (Table 4). The protein content of milk from herds of dairy breeds and herds of dual-purpose breeds differed little, and within groups it was higher in BS versus HO milk, and in SI versus AG, the difference being much greater in the former group. Lactose content, on the other hand, was higher in milk from AG than from SI herds, and from BS compared with HO herds, although in the latter case the difference was modest.

Regarding the FTIR-predicted cheese yield traits, Table 4 shows that, unlike milk composition traits, the yield from dual-purpose herds surpasses that of dairy herds, regardless of whether cheese productivity is expressed in terms of fresh cheese or cheese solids. Within groups, cheese yield traits were higher in milk from BS herds compared with HO herds, and in AG herds than in SI herds.

The superiority of BS over HO milk reflects only partially the composition of the milk from the herds of the 2 breeds, and confirms the results obtained from multibreed herds in the same area. The superiority of AG milk over SI milk (and also over dairy breeds) does not parallel the differences in milk composition. In the study on mixed-breed farms, where cheese yield traits were obtained experimentally through the model cheese-making procedure and not predicted from milk FTIR spectra (Stocco et al., 2018), these traits in AG milk were similar to (slightly lower than) those in SI milk obtained from the same farms. However, more

Table 4. Effect of breed of cows (LSM) on milk yield and composition and on cheese yield (HO = Holstein, BS = Brown Swiss, SI = Simmental, AG = Alpine Grey)

Item	Dairy breed		Dual-purpose breed		Orthogonal contrast		
	HO	BS	SI	AG	HO+BS vs. SI+AG	HO vs. BS	SI vs. AG
Milk yield, kg/d	26.8	21.9	22.0	15.6	***	***	***
Milk composition, %							
Fat content	3.90	4.06	3.94	3.66	***	***	***
Protein content	3.14	3.40	3.29	3.25	***	***	***
Lactose content	4.73	4.75	4.74	4.80	***	***	***
Predicted cheese yield, %							
Fresh cheese ¹	14.72	14.83	14.99	15.13	***	***	***
Cheese solids ²	7.12	7.16	7.24	7.30	***	***	***

¹Predicted cheese yield of fresh cheese is the weight of the fresh cheese after salting expressed as a percentage of the weight of the milk processed.

²Predicted cheese yield of solids is the weight of the DM of the fresh cheese expressed as a percentage of the weight of the milk processed.

*** $P < 0.001$.

fresh cheese and cheese solids were produced from the AG milk than expected based on milk composition, giving it a better estimated cheese-making efficiency. The cheese-making efficiency index was defined as the percentage ratio between the cheese yield measured experimentally and the cheese yield predicted on the basis of the milk fat and protein content. Other authors have defined efficiency as the ratio between the measured value of a trait and the maximum predicted value of the same trait in the given conditions (Caballero-Villalobos et al., 2018). The cheese-making efficiency index of AG milk (Stocco et al., 2018) was 102.2 (vs. 101.0 for SI, 102.4 for BS, and 96.1 for HO). It should be considered that the large majority of AG cows are reared on traditional farms, often in tiestalls, and fed a diet based on hay and some compound feed. In the aforementioned study on multibreed farms, the milk from less-intensive farms was found to be better for cheese-making than milk from intensive farms (cheese-making efficiency index 102.6 vs. 99.4, respectively), independently of the breed. Thus, FTIR predictions seem able to capture differences in cheese yield properties of milk that go beyond its fat and protein contents and that reflect differences in both genetics and dairy systems.

The reasons for these differences in the cheese-making efficiencies of different breeds, or even of different individuals within a given breed, may lie mainly in the proportions of the different protein fractions of the milk protein. Whereas the proportion of α_{S1} -CN, α_{S2} -CN, and β -CN, despite being significant, differed little among the 6 different breeds (Amalfitano et al., 2020), the proportion of κ -CN was much lower in the milk from HO cows than in the milk from all the other breeds, and the proportion of β -LG was much higher, SI excluded. The comparisons are further complicated by the fact that the milk protein fractions have different genetic variants, which not only affect the expression level of the gene in the udder, but also often differently affect the cheese-making properties of milk. Because the frequency of the different alleles of the genes codifying for milk proteins differs greatly in different breeds, we can expect that genetic variants—and not just the proportions and concentrations of the protein fractions—also contribute to explaining the differences in cheese-making efficiency observed in different breeds.

The FTIR spectra can predict the concentrations of different protein fractions in milk (De Marchi et al., 2009; Rutten et al., 2011; Sanchez et al., 2017), albeit with a moderate level of accuracy, and milk infrared spectra can capture information that goes beyond simply the quantity of protein in milk. Furthermore, it is well known that FTIR spectra can also be used for predicting the coagulation properties of milk (Ferragina et al., 2015; El Jabri et al., 2020), and that some cor-

relation exists between milk coagulation properties and cheese yield (Cecchinato and Bittante, 2016). In any case, coagulation traits represent a valuable technological information per se, but they do not seem to be very useful as predictors of cheese yield, although their accuracy of prediction based on FTIR spectra is often lower than that found for directly predicting cheese yield (Ferragina et al., 2013), and coagulation traits are less affected by dairy system, herds, and parity of cows than cheese yield and nutrient recovery (Bittante et al., 2015).

Effects of Herd Intensiveness and Cow Productivity Levels on Milk Yield and Composition

Before analyzing the effects of the level of intensiveness of the herds and the level of productivity of individual cows, it is worth pointing out that their stratification was based on dMEO—that is, the quantity of net energy secreted daily by each cow (CL) or as an average of all the cows in the herd (HL), after correcting for all the factors included in the statistical model (year, calendar month, parity, lactation stage). The main reason for choosing dMEO is that it is probably the best indicator of the cow's metabolic load during lactation, and also of the herd's nutrient production level, because dMEO represents the major net energy requirement (net energy for lactation, NE_L , MJ/d) of the individual cows and of the herd (NRC, 2001).

Stratifying the cows and herds on the basis of uncorrected daily milk yield (kg/d) would have given an advantage to those cows and herds producing proportionally more water and less fat and protein (and cheese). This would have resulted in the highest HL and CL classes being characterized by milk of a worse quality than the lowest classes. In contrast, dMEO is the quantity of fat, protein, and lactose secreted daily and expressed in terms of energy, and is calculated by multiplying the quantity of milk produced by the percentage contents of fat, protein, and lactose. In this case, we expect the highest HL and CL classes to be characterized by more milk of a better quality than the lowest classes. From the genetic point of view, it is well known that selecting only for increased milk yield is highly negatively correlated with milk quality, whereas selecting for increased daily production of fat and protein is moderately positively correlated with milk quality (Miglior et al., 2005).

The 6 plots in Figure 3 depict the factorial combinations of 2 types of data stratification (HL and CL) by 3 milk quality traits (fat, protein, and lactose). In each plot, the LSM of the 5 HL (or CL) classes of each breed for a given quality trait are plotted against their corresponding corrected daily milk yields. This makes it

possible to illustrate the effect of the HL or CL of the different breeds, taking into account their respective daily milk yields.

Taking the first plot (the effect of HL on fat content by herd breed), we have confirmation of the effect of herd breed (whose median value is approximately

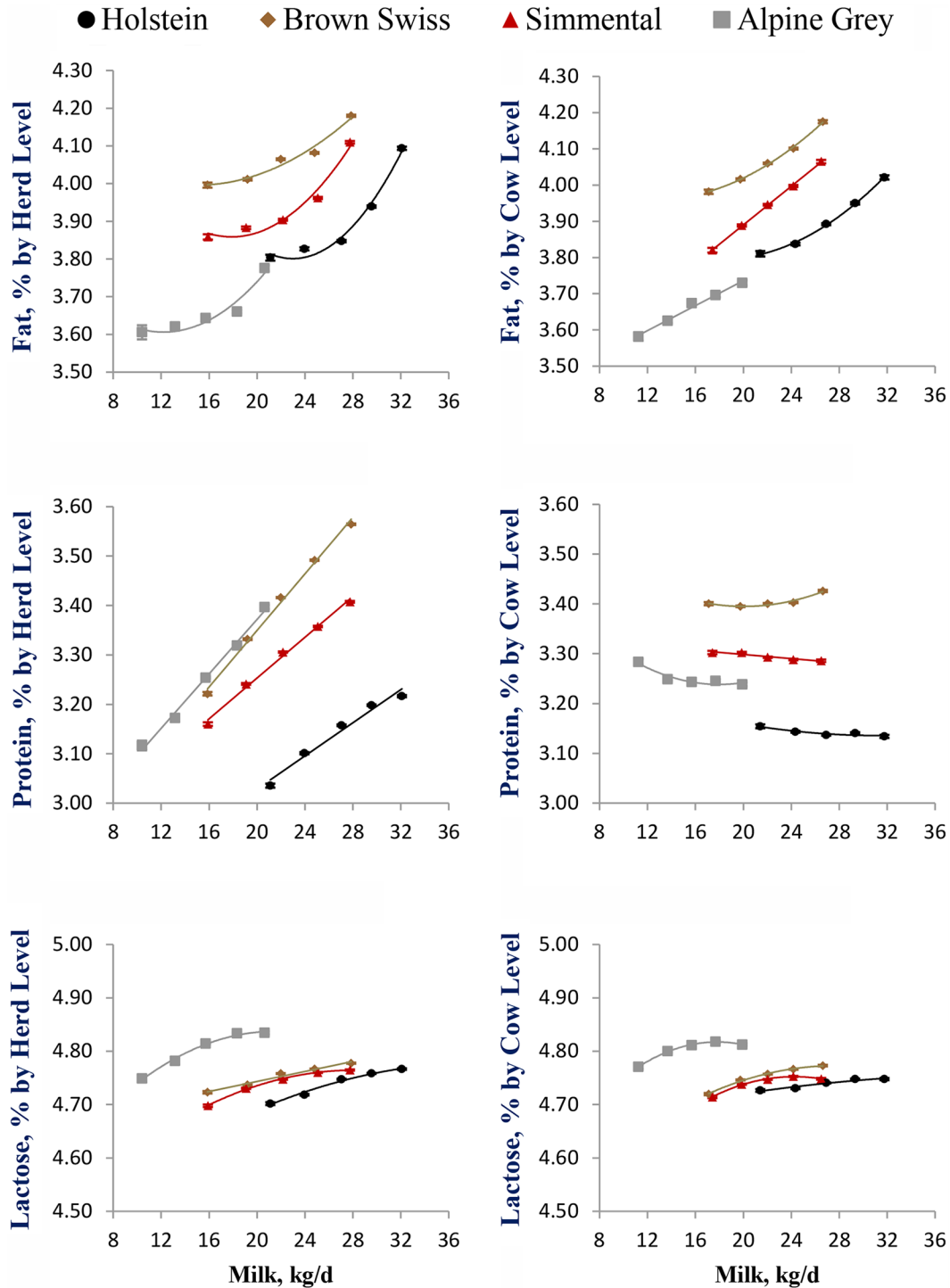


Figure 3. Milk composition (LSM ± SE) by breed, herd intensiveness class, or cow productivity class, plotted against actual cow yield (kg/d).

represented by the central class, HL-3, of each breed), previously reported in Table 4. Regarding the corrected milk yield, we can see along the x-axis that the AG herds are the lowest-producing herds, the SI and BS herds are intermediate and very similar to each other, and HO are the highest-producing herds. We can also see that the class of the highest-producing AG herds has an average daily milk yield similar to the class of the lowest-producing HO herds. Along the y-axis we can see a different, but not opposite, ranking for milk fat content: AG herds are still the lowest, followed by HO herds, then SI herds, then BS herds, the highest. Comparison of the averages of the 4 breeds (Table 4) shows that milk quantity and fat content are not correlated, either positively or negatively. The original characteristics and the selection history of the 4 breeds, and their uses in different dairy systems, are specific to each of them and result in them having different, uncorrelated characteristics.

Looking at the trend in fat content in the intensiveness HL (Figure 3), we can see first of all that it is positive in all 4 breeds: that is, within each breed, the dairy herds producing more milk energy per day per cow are producing more milk with more fat than the lower-producing herds. This improvement is not linear, because moving from HL-1 to HL-2 to HL-3 and to HL-4 a slight increase in fat content occurs in each case, but this increase is much larger when moving from HL-4 to HL-5 for all the breeds (Figure 3) and seems to be particularly large in herds with HO and SI cows, which could explain the relatively high F-value of the interaction “HL by breed” reported in Table 3.

Moving on to the effect of CL class on milk fat, we find that here, too, within breed and herd the improvement in cow productivity is accompanied by an improvement in fat content. In addition, the differences between the 2 extreme classes (CL-E vs. CL-A) of each breed differ little from the differences in HL (HL-5 vs. HL-1), and the increase in milk fat content for each breed is, unlike HL, almost linear from the first to the fifth CL class.

In our previous study on multibreed herds (41 herds with 1,508 cows of 6 breeds), we stratified the herds into 2 classes (high and low intensiveness) using the same criterion as here—corrected dMEO—and there, too, we found a greater fat content in the milk from high-intensive herds (4.44%) than from low-intensive herds (4.20%; Stocco et al., 2017). We could, of course, attribute this result to the criteria used to stratify the herds and the cows. However, in another study (85 herds with 1,264 BS cows) the herds were not stratified according to dMEO but were clustered into 4 different dairy systems, defined according to their facilities, feeding regimen, milking technique, and use or nonuse of summer pasture, without including any information on

the cows’ production levels. Compared with the traditional dairy system (prevalence of tiestalls, diet of hay with little compound feed, widespread transhumance to summer highland pasture), the modern dairy system (loose housing, total mixed rations with or without silages, milking parlor, no pasture) had a higher milk yield (28 vs. 21 kg/d, respectively), which was also accompanied here by an increased fat content (4.6 vs. 4.2%).

Regarding the effect of HL class on milk protein, we again found a clear improvement in milk quality with increasing herd intensiveness of herd, and that this improvement was greater and much more linear than the effect of HL on fat content, especially in herds with BS and AG cows. The plot of the effect of CL class on milk protein content by breed (Figure 3) offers a completely different picture: here the trend is almost flat (slightly decreasing, except BS). This pattern is more consistent with expectations based on genetic classification: the cows with the highest breeding value for daily yield of fat and protein are not expected to differ largely from those with the lowest breeding values in terms of milk protein content. These results for milk protein content also confirm, on a much wider scale, previous results showing a favorable relationship between farm intensiveness and milk protein content in multibreed herds (3.59–3.80%; Stocco et al., 2017) and in BS herds (3.65–3.83%; Bittante et al., 2015).

Lactose content also had a favorable association with dMEO at both the farm and the individual cow levels. But the variation in lactose content was much smaller than the variation in fat and protein content (Figure 3). In both HL and CL, this improvement seems to be slightly curvilinear, being greater from the lower to intermediate classes than from the intermediate to the higher classes in all breeds. In the previous studies, lactose was either not reported (BS herds) or exhibited no variation according to the intensity level of the farms (multibreed herds).

Effects of Herd and Cow Productivity Levels on Cheese Yield Traits and Cheese-Making Efficiency

An increase in the intensiveness of dairy farms is associated with an almost linear increase in the yield of fresh cheese from milk of all breeds (Figure 4). The improvement was large, from about 1.05 percentage points for HO herds (+7% of the average) to 1.20 to 1.25 for BS and SI herds (+8%), and 1.45 for AG herds (almost +10%). The improvement in cheese solids was obviously lower (less than half), and maintained the same ranking as the average breed values. It is worth noting that the improvement in cheese solids yield is

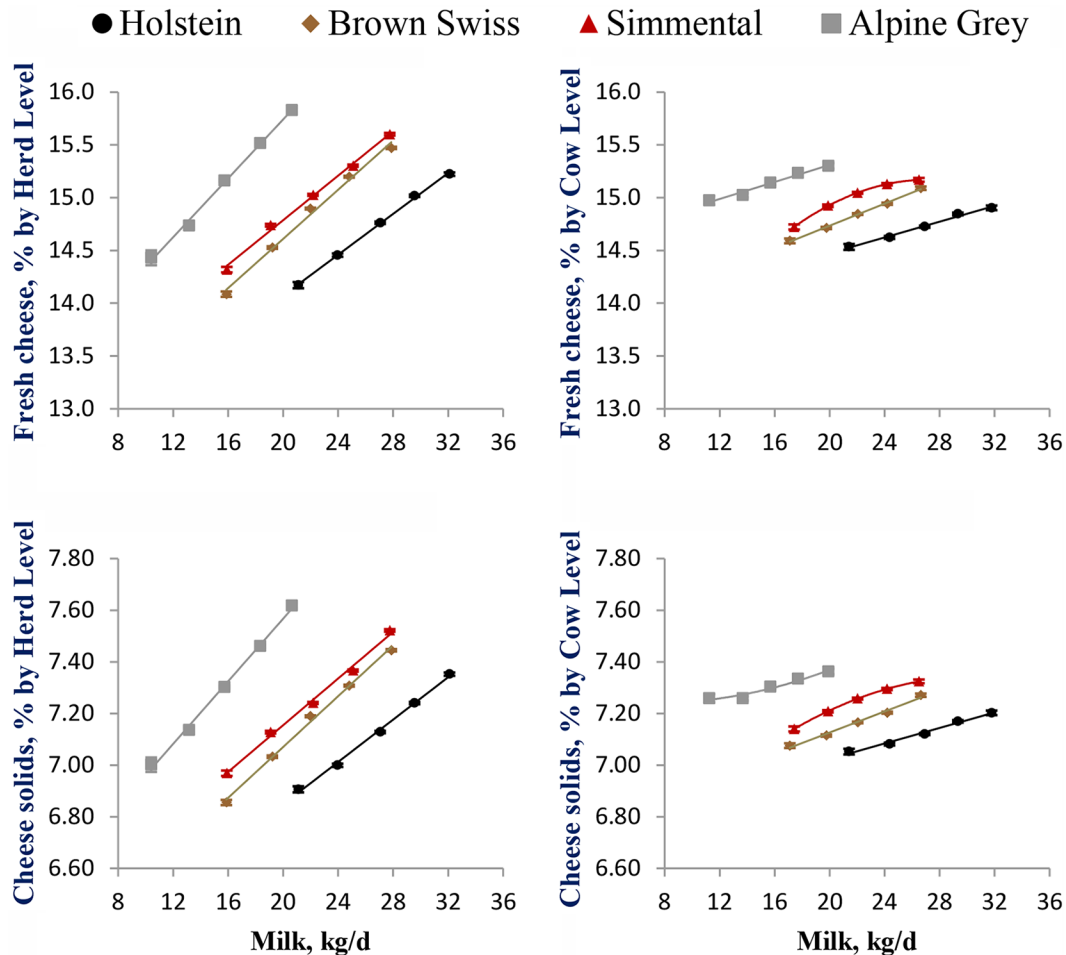


Figure 4. Cheese yield (LSM \pm SE) by breed, herd intensiveness class, or cow production class, plotted against actual cow yield (kg/d). Cheese yield of fresh cheese is the weight of the fresh cheese after salting, expressed as a percentage of the weight of the milk processed; cheese yield of DM is the weight of the DM of the fresh cheese, expressed as a percentage of the weight of the milk processed.

greater for AG, intermediate for BS and SI, and slightly smaller for HO herds. From this we can speculate that increasing intensiveness improves cheese-making efficiency in the case of AG herds and slightly reduces it in the case of HO herds. It is also worth noting that, in the study on multibreed farms using the individual model cheese-making procedure, both the laboratory-measured cheese yields and the theoretical cheese yield calculated from a fat-protein formula were higher in the more-intensive than the less-intensive farms (Stocco et al., 2018). However, the improvement in the measured trait was less than expected based on milk composition, so that cheese-making efficiency was lower in the more-intensive farms, reflecting the results found here for HO but not those for AG. Comparing the BS herds in the 4 dairy farming systems, Cipolat-Gotet et al. (2020) found that the higher yields of fresh cheese from intensive farms using total mixed rations were maintained during ripening. However, after correcting the cheese

yields for milk composition, this higher yield from the more-intensive dairy systems was no longer significant, leaving only a modestly lower weight loss during ripening.

Regarding cow productivity levels, it is immediately clear that they have a positive, almost linear effect on the yields of both fresh cheese and cheese solids, but that CL is much less important than HL in all 4 breeds (Figure 4). The improvement at the individual cow level is, in fact, about one-third of that at the herd level in the case of AG and about half in the case of BS, the other breeds being intermediate. The effect of CL on cheese solids yield is even lower, especially for AG and HO cows, and very similar to the sum of the effects on the fat (positive) and protein (slightly negative) contents of milk. There does not seem to be any variation in the cheese-making efficiency of milk from cows with different productivity potentials in all breeds.

CONCLUSIONS

Fourier-transform infrared prediction of cheese yield seems able to capture information that goes beyond fat and protein content, explaining differences in milk nutrient recovery in different cows and herds, and selection on predictions could be more efficient than those based on milk fat and protein. The different cheese yields from different breeds can be explained only partly by milk fat and protein composition, and less productive breeds can partially compensate with a higher milk nutrient content and recovery in cheese. High-intensive herds produce much more milk, with a higher nutrient content and a higher cheese yield. Within individual herds, more productive cows have a much greater milk yield, a greater content of fat but not of protein, and a cheese yield differing little from expectations. Finally, the effects of herd intensiveness and cow productivity are similar but not identical in different breeds, the less productive ones having some advantage.

ACKNOWLEDGMENTS

The authors thank the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Italy, projects DOR1815378/18 and DOR1970230/19, for financial support. The authors confirm that there is no conflict of interest regarding the publication of this article.

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