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REGULAR ARTICLE

Seasonal effect on milk composition, somatic cell content and milk coagulation properties of Italian Holstein-Friesian cows

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ABSTRACT

This study investigated the seasonal effect on composition, somatic cell content and coagulation properties of bovine milk during two different periods of the year (summer and autumn). 592 samples of raw milk from Italian Holstein-Friesian cows from different locations in the Veneto region, Italy, were collected. The samples were submitted to the following analyses: fat, protein, casein and lactose percentages and pH by infrared spectroscopy; somatic cell counting by optical fluorescence and milk coagulation properties expressed in rennet coagulation time (RCT, min) and curd firmness or consistency 30 minutes after the addition of rennet (a_{30}, mm) by lactodinamography. The index of the aptitude of milk to coagulate (IAC) was also determined from the lactodinamographic parameters that were obtained. To verify the environmental conditions, the temperature humidity index (THI) was calculated for each collection period. No significant difference ($p < 0.05$) was observed between protein, casein, lactose and pH in the samples collected in the summer and the autumn. However, the results for somatic cells, RCT, a_{30} and IAC were significantly different, with lower results in the summer. Over all the total samples analysed, 41.2% showed a milk that did not coagulate in the 30 minutes, with a higher percentage for samples collected in the summer and during this period presented lower results to of RCT, a_{30} and IAC; the THI values, as expected, were higher in the summer than in the autumn. The THI presented statistically different means ($p < 0.05$), which were 73.24 in the summer and 57.43 in the autumn. Milk with this characteristic is not suitable for cheese production; however, it is suitable to produce fluid milk, or for other derivatives where enzymatic coagulation is not part of the process.

Keywords: Clotting; Dairy cows; Lactodinamographic parameters; Temperature-humidity index

INTRODUCTION

From a legal and practical point of view, two main factors are important regarding the quality of milk: chemical composition and hygienic-sanitary characteristics (Brito and Brito, 2004). Knowledge about these characteristics is essential, not only in terms of quality control, but also because they define the sensorial attributes of products like yogurts, cheeses and others, as well as being fundamental in some stages of processing and meeting the standards required by legislation. Cheese manufacturing requires milk coagulation, which is a process involving a series of physicochemical changes, particularly in the casein micelle. For dairy industry, milk characterized with high values of milk coagulation properties (MCP) resulted in higher cheese yield than milk with low values, indicating that MCP could be used as indicators of cheese-making efficiency (Sumner et al., 2002; Cassandro et al., 2008; Frederiksen et al., 2011; Pretto et al., 2013).

The main characteristics that are commonly studied with respect to milk coagulation are: rennet coagulation time (RCT, min); curd-firming time (k_{20}, min) and curd firmness (a_{30}, mm) (Beux et al., 2017). The latter is related to clot firmness or consistency 30 minutes after the addition of rennet. Samples that do not coagulate within 30 minutes are referred to as non-coagulating (NC) samples (Ikonen et al., 1999). In the production of cheese, NC samples would not

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achieve the firmness necessary to be able to cut the curd correctly; consequently, milk with this characteristic, or that has slow coagulation, represents a problem for the dairy industry (Frederiksen et al., 2011).

Knowledge about the profile of milk coagulation makes it possible to determine the appropriate usage of milk, i.e., the production of cheeses or other derivatives in which coagulation is not fundamental. The RCT (min), k_{20} (min) and a_{30} (mm) values are obtained separately, but when combined they express the quality of the milk in relation to coagulation. To facilitate this information, Penasa et al. (2015) combined the most important parameters RCT and A_{30} to provide a new form of measurement, the index of the aptitude of milk to coagulate (IAC). According to the aforementioned authors, the IAC could be adopted by the dairy industry as an overall measure of MCP in order to reward or penalise producers working in milk quality payment systems. According to Cassandro et al. (2016), some dairy producers in Italy have already adopted MCP and provided an economic value.

Several factors can influence coagulation, such as the chemical composition of milk (mainly with respect to casein and its fractions), the type and concentration of the coagulant enzyme, the processing temperature, and the somatic cell contents (Politis and Ng-Kwai-Hang, 1988; O'Connell et al., 2001). The chemical composition of milk ranges according to management, genetics, nutrition and environmental conditions, such as temperature and humidity. The latter is associated with thermal stress, which also has a negative impact on milk production (Zimbelman et al., 2013). The temperature associated with humidity, known as the temperature humidity index (THI), is lower during these periods. This index represents an excellent indicator of thermal comfort (Armstrong, 1994; Bohmanova et al., 2007) and it is used to evaluate the impact of thermal stress on dairy cows (Bouraoui et al., 2002; Brown-Brandl et al., 2003).

The aim of this research was to evaluate the effect of two seasons (summer and autumn) on milk composition, somatic cell content and milk coagulation properties (MCP) of milk from Italian Holstein-Friesian cattle from farms located in the province of Venice, Italy.

MATERIALS AND METHODS

Samples and data collection

A total of 592 milk samples from Italian Holstein-Friesian cows in the province of Venice, Italy were analysed. The milk was collected between the months of July and November. The analyses were carried out in the laboratory of the Breeders Association of the Veneto Region (Padova, Italy). All the samples arrived at the laboratory in refrigerated coolers (4 - 6 °C) with the addition of a preservative (Bronopol, Knoll Pharmaceuticals, Nottingham, UK) at a concentration of 5 uL mL^{-1} milk.

Laboratory analyses

The milk samples were analysed for their fat, lactose, protein (P), casein (CN) contents, and pH using a MilkoScan FT6000 (Foss Electric A/S), (IDF, 2000). The somatic cell count (SCC) was evaluated using Fossomatic 5000 (Foss Electric A/S) equipment (IDF, 2006). For the analyzes described above the samples were preheated at 40 °C for 15 minutes (in thermostated bath) and sequentially taken to the respective equipment for analysis. The SCC values were converted by logarithm transformation to somatic cell scores (SCS) by Cassandro et al. (2008) according to equation:

$$[SCS = 3 + \log_2(SCC/100)] \quad (1)$$

The milk coagulation properties (RCT: time for the beginning of enzymatic coagulation in minutes, concerning the time interval between rennet addition and the onset of clotting; k20: time required in minutes for the gel to reach a consistency with an amplitude of 20mm and a30: curd firmness or consistency which is determined in millimeters obtained 30 minutes after coagulant addition) of the milk samples (10 mL) were measured for 30 min using a formagraph (Foss Electric A/S, Hillerød, Denmark) at 35°C. A quantity of 200 µL of rennet (Naturen Plus 215 IMCU/mL with 80% of chymosin and 20% of pepsin) was added and diluted with 1.2% distilled water (Penasa et al., 2015). The index of the aptitude of milk to coagulate (IAC) was calculated using the following formula (equation 2) (Penasa et al., 2015):

$$IAC = 100 + [(a_{30} - mean_{a30})/SD_{a30} * 2.5] - [(RCT - mean_{RCT})/SD_{RCT} * 2.5] \quad (2)$$

Where: a_{30} = curd firmness; RCT= rennet coagulation time; SD= standard deviation.

The mean and SD of the experimental data were used to calculate the IAC. The milk samples that did not coagulate within 30 min were classified as non-coagulating (NC). The six properties were identified as: PA, PB, PC, PD, PE and PF, and in order to identify the samples from the summer and autumn the letter S (summer) and A (autumn) were added to the acronym. The herds at each properties were composed of 34, 80, 70, 66, 31 and 53 animals, respectively. The test was performed in duplicate.

Temperature-humidity index (THI)

The THI index was calculated according to the following National Research Council formula (NRC, 1971):

$$THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T - 26) \quad (3)$$

Where: T - temperature (°C) and RH - relative humidity (%). The THI was calculated using the average values of environmental temperature and relative humidity. The average was obtained using the values of these parameters in relation to the week of sampling. The data for the temperature and relative humidity were downloaded from the web site Il meteo (Il meteo, 2015).

Statistical analysis

The results were presented in mean values of duplicates, followed by their standard deviation (± SD), and in some cases the minimum and maximum values, which were allocated below the average. The t-test was performed to detect significant differences between the milk that was collected and analysed in the summer and the autumn. Pearson's correlation analysis (r) between the different response variables was applied to evaluate the strength of the correlation between responses. The statistical significance for all the samples was obtained by calculating the p-value (p <0.05 was considered significant). The analysis was performed using STATISTICA v. 13.2 (STATSOFT Inc., Tulsa, Okla, U.S.A.) software. The differences were observed as a function of the period of analysis, the generalised linear model (GLM) was applied to verify the influence of the collection period and properties for each dependent variable. The dependent variables that presented normal distribution were analysed using the identity link function. The GLM analyses were performed using SPSS (version 22) software.

RESULTS AND DISCUSSION

Evaluation of coagulated samples (CS)

58.9% of the samples were considered coagulated samples (CS) and 41.1% non-coagulating (NC), i.e., they did not coagulate within 30 min of analysis. The percentage of NC samples was high when compared to the figures of 9.7% reported by Cassandro et al. (2008) wiht samples collected during winter, spring and summer; 11.5% reported by Pretto et al. (2011) samples collected in the autumn, and 12.9% cited by Toffanin et al. (2015) with samples collected in the winter, all for the same breed of cattle (Italian Holstein) in the North Italy. Of the total samples analysed in the summer (334), 56.6% were NC and of the samples analysed in the autumn, 20.9% (258) were NC. Holstein cows are more sensitive to thermal stress, which may have contributed to the high numbers of NC samples in the summer. The Fig. 1 shows the distribution graph of these samples, as well as the coagulated samples (CS) during the two periods of analysis. In all the properties the highest percentages of NC samples were related to the milk collected during the summer; however, it was expected that some of these samples would coagulate after 30 min. In this study, the analysis was interrupted after this time because in the cheesemaking process. In Italy the curd is usually cut up to 30 minutes after the addition of the rennet (Cassandro et al., 2008).

The k_{20} index was absent in 22% of the samples. This index is only present in milk that reaches 20 mm of curd firmness during coagulation, which does not happen in NC milk or milk with late coagulation. The increasing frequency of samples with these characteristics has meant that k_{20} now has diminshed usefulness as a parameter in this context. Penasa et al. (2016) reported that in 26% of the samples analysed in their study, k_{20} was absent and, in addition, k_{20} values are characterised by lower repeatability and reproducibility in relation to RCT. Based on the above considerations, for the MCP statistical analysis the NC samples were excluded and the k_{20} values were withdrawn. Fig. 1 shows that the percentage of coagulated samples was higher during the autumn (with the lowest THI values), and in the summer only the PF property had a percentage of coagulated samples, which was 70.50%, compared to 75.47% during the autumn. This difference may be partially linked with the variation in THI that was observed during the analysed periods, with summer averages of 73.24 ± 6.74

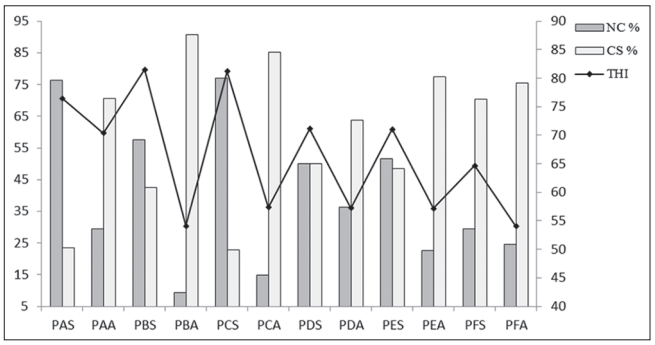

Fig 1. Percentage of CS and NC samples in relation to different properties in the two periods of analysis. Note: * The six properties: PA, PB, PC, PD, PE and PF, and in order to identify the samples from the summer and autumn theletter S (summer) and A (autumn).; THI (temperature humidity index, represented on the secondary axis)

Table 1: Comparison between chemical composition, pH, SCS, RCT, A30, IAC and THI of milk samples collected in summer and autumn using the test t

Season	Trait*									
	Fat g.100mL^{-1}	P g.100mL^{-1}	CN g.100mL^{-1}	Lactose g.100mL^{-1}	pH	SCS	RCT min	A_{30} mm	IAC	THI
Summer	4.08 ± 0.84^a	3.38 ± 0.44	2.61 ± 0.36	4.79 ± 0.24	6.56 ± 0.09	3.91 ± 2.50^a	20.76 ± 4.84^a	17.35 ± 11.21^b	98.46 ± 4.12^b	73.24 ± 6.74^a
Min. - Máx.	2.54 - 6.72	2.42 - 4.62	1.85 - 3.56	3.89 - 5.23	6.30 - 6.67	-2.06 - 10.60	8.88 - 28.38	0.90 - 50.53	92.30 - 108.72	64.66 - 81.53
Autumn	3.72 ± 0.80^b	3.48 ± 0.46	2.71 ± 0.37	4.80 ± 0.18	6.57± 0.07	3.27 ± 2.18^b	17.86 ± 5.32^b	26.75 ± 14.12^a	101.16 ± 4.97^a	57.43 ± 4.99^b
Min. - Máx.	1.85 - 6.42	2.60 - 5.12	2.01 - 3.99	4.24 - 5.23	6.32 - 6.79	-0.64 - 9.60	8.30 - 28.30	2.30 - 56.59	91.99 - 110.87	50.04 - 70.40

a,b Means with different letters in the same column differ statistically (p <0.05). * P = protein; CN = casein; SCS = somatic cell score; RCT = rennet coagulation time; A30 = curd firmness; IAC = index of the aptitude of milk to coagulate; THI = temperature humidity index.

and autumn averages of 57.43 ± 4.99 (Table 1). Factors such as the concentration of κ-CN, the proportion of κ-CN in relation to total CN, the pH of the milk, calcium content among others may influence in the milk coagulation, but these were not addressed in this study.

The THI can be used to determine the influence of ambient temperature on dairy cow productivity (Gantner et al., 2011). Armstrong (1994) classified the degree of thermal stress that was felt by animals as a function of the variation in THI as follows: light between 72-78; moderate between 79-89; and severe between 90-99. THI values below 72 are characterised by a environment that is free of thermal stress and its consequences. However, according to Bernabucci et al. (2010) in a survey of Italian Holstein cows, losses in milk production were associated with THI values close to 68.

Heat stress can also alter milk composition and the frequency of mastitis, which can compromise coagulation properties (Rodriguez et al., 1985, Du Preez et al., 1990) and, consequently, IAC. In Table 2, Pearson's correlation analysis shows that fat, protein, casein and lactose in relation to THI presented a significant correlation (p <0.05); however, low and favourable lactodynographic parameters were associated with lower THI values (0.3119, -0.4048 and -0.3845 for RCT, A_{30} and IAC, respectively) and there was no correlation between THI and pH and SCS. Regarding SCC, the result was different than expected, according to Bouraoui et al., (2002) there is a trend indicating a negative effect of thermal stress on this parameter through impaired mammary defense mechanisms. The same authors reported significantly (p <0.05) increased from 4.1 × 10^5 in the spring (average daily THI 68) to 8.6 × 10^5 in the summer (average daily THI 78).

The period of analysis seems to have influenced the chemical composition of the milk, and consequently the MCP. Table 1 shows the results of the test t applied to verify if there was a difference between the mean values of the variables in the two distinct moments of analysis (summer and autumn).

Table 2: Pearson's correlation (r) and correlation coefficient (R2) between chemical composition, pH, SCS and lactodinamographic parameters

Trait**	Fat	P	CN	Lactose	pH	SCS	THI	RCT	A_{30}	IAC
Fat, g.100mL^{-1}	**1,0000**	0.2633	0.2414	0,0000	0.0043	0.0612	0.0367	0.0056	0.026	0.0119
P, g.100mL^{-1}	0.5131*	**1,0000**	0.9835	0.0743	0.0041	0.0392	0.016	0.0061	0.1145	0.0439
CN,g.100mL^{-1}	0.4914*	0.9917*	**1,0000**	0.152	0.0028	0.0191	0.0247	0.0076	0.1253	0.0482
Lactose, g.100mL^{-1}	-0.0064	0.2726*	0.3899*	**1,0000**	0.0041	0.1402	0.0745	0.0079	0.0462	0.018
pH	-0.0658	-0.0637	-0.0526	0.0641	**1,0000**	0.0013	0.0093	0.0031	0.0045	0.0043
SCS	0.2474*	0.1981*	0.1381*	-0.3743*	-0.0362	**1,0000**	0.0092	0.0172	0.0142	0.0148
THI	0.1916*	-0.1264*	-0.1571*	-0.2730*	-0.0964	0.0957	**1,0000**	0.0973	0.1638	0.1479
RCT, min	-0.0746	-0.078	-0.0874	-0.0888	-0.0556	0.1312*	0.3119*	**1,0000**	0.8067	0.9283
A_{30}, mm	0.1613*	0.3383*	0.3540*	0.2150*	0.0671	-0.1193*	-0.4048*	-0.8982*	**1,0000**	0.9277
IAC	0.1092*	0.2094*	0.2195*	0.1341*	0.0654	-0.1218*	-0.3845*	-0.9635*	0.9631*	**1,0000**

**Significant correlation (p <0.05) and Pearson's correlation (r) below the diagonal, and correlation coefficient (R2) above the diagonal. **P = protein; CN = casein; SCS = somatic cell score; THI = temperature humidity index; RCT = rennet coagulation time; A30 = curd firmness; IAC = index of the aptitude of milk to coagulate.*

The protein, casein, lactose and pH did not present significant difference (p <0.05) between the means of the samples analysed during the summer and autumn. However, there was significant difference (p <0.05) between other variables. The fat content was higher in the samples analysed during the summer, and in that period the THI presented an average of 73.24 (but reached a maximum value of 81.53 at a given moment). Some breeds suffer less influence from environmental conditions in relation to the quantity and quality of milk produced. However, according to Miranda and Freitas (2009), the critical temperature, i.e ., the temperature above which milk production begins to decrease, it is between 24-26 ºC for the Holstein breed and above 29.5 ºC for the Brown Swiss. The higher fat content found in the samples analysed in the summer may have been related to the lower volume of milk produced during this period due to thermal stress (Stelzer et al., 2009). The samples of milk collected in the summer showed a lower percentage of protein and casein than the samples analysed during the autumn. A factor that may have been associated with physiological issues (greater sensitivity of the animals during the summer) and diet (Stelzer et al., 2009); however, these were minor, non-significant differences (Table 1). The effect of casein content is one of the factors that positively contributes to the start of enzymatic coagulation and, even more so, to the final texture of the curd (Wedholm et al., 2006; Hallén et al., 2007). However, it was not observed significant correlation between casein and RCT (-0.0874, Table 2). In terms of A_{30}, the correlation was significant and favourable, but weak (0.3540, p <0.05; Table 2). The values were close to those found by Cassandro et al. (2008) and Tiezzi et al. (2015), who reported that the correlation between casein and RCT was zero (-0.19 and -0.02), and between A_{30} and casein was 0.32 and 0.346, respectively. Zannoni and Annibaldi (1981) argue that milk is considered to be ideal with respect to MCP when it presents an RCT value of approximately 11-18 min and a_{30} of 20-40 mm. The samples collected in the autumn presented a better response regarding the coagulation time (RCT), 17.86 ± 5.32 min, and gel firmness (a_{30}) 26.75 ± 14.12 mm, with mean values close to the ideal (Table 1). The mean for RCT for the samples collected in the summer was 20.76 ± 4.84 min, and 17.35 ± 11.21 mm for a_{30} (Table 1). There was a strong and negative correlation between RCT and a_{30} (-0.8982), which was the same as reported by Ikonen et al. (2004) and Tiezzi et al. (2015), confirming that when RCT decreases the a_{30} increases. This correlation was expected because these traits are measured in consecutive steps during the coagulation process. Thus, if milk starts to coagulate in a short period of time it has more time to firm the curds

and will therefore have better coagulation capacity, usually with higher average a_{30} values. On the other hand, if milk takes longer to coagulate, the curd will have less time to harden and consequently the gel will be weaker (Pretto et al., 2013; Tiezzi et al., 2015). De Marchi et al. (2008) reported mean RCT values (samples collected during the summer) of 18.4 min and a_{30} of 18.9 mm. Cassandro et al. (2008) reported an average of 16.9 min for RCT and a_{30} of 32.0 mm (analysed in the winter and spring). In a study by Penasa et al. (2014) (samples collected in the autumn and winter), the mean value for RCT was 21.0 min, with a_{30} of 20.08 mm. Tiezzi et al. (2015) reported values of 18.9 min for RCT and 23.0 mm for a_{30} in relation to analyses performed in the autumn. All the aforementioned studies were conducted with Holstein cows, and the results were close but with considerable variation. This breed is known for a high milk yield, low protein content, and poor performance with respect to coagulation when compared, for example, with the Jersey and Brown-Swiss breeds (Auldist et al., 2004; De Marchi et al., 2007). They are also more sensitive to the effects of climate, but are the main breed of dairy cattle in Italy, predominantly in the northern region of the country (Pretto et al., 2012). In the present study, the IAC, which is a standard index (Penasa et al., 2015) that associates RCT and a_{30}, varied according to the values of these parameters, and thus the response was better during autumn, with mean values of 101.16 ± 4.97 for autumn and 98.46 ± 4.12 in summer (Table 1). According to Penasa et al. (2016), IAC values above 100 are desirable because they indicate favourable overall MCP values. The previously cited authors also reported that the best IAC values were obtained from samples analysed during the autumn and winter, with the highest value for the month of March (101.2). A slight weakening in coagulation capacity was observed during the summer with a lower value (98.6), which was similar to the results of the present study. In relation to somatic cells, there was a significant difference (p <0.05, Table 1) between the samples analysed in the summer, with SCS of 3.91 (188 x 10^3 cell/mL), and the autumn, with SCS of 3.27 (121 x 10^3 cell/mL). The values found were similar to those reported by De Marchi et al. (2007), Cassandro et al. (2008), and Toffanin et al. (2015), which were 4.01 SCS (211 x 10^3 cell/mL); 3.08 SCS (97 x 10^3 cell/mL) and 4.40 SCS (266 x 10^3 cell/mL), respectively. According to Ikonen et al. (2004), increased numbers of somatic cells resulted in increased RCT and decreased a_{30} in relation to milk from Finnish Ayrshire cows, with a mean SCS of 4.02 (200 x 10^3 cell/mL). The same behaviour was observed by Comin et al. (2005) for Holstein cow milk, with an average SCS value of 4.53 (288.49 x 10^3 cell/mL). Tiezzi et al. (2015) also reported that causal effects of SCS and casein on MCP were observed, which suggests that the relationship between milk coagulation ability and traditional milk quality traits depends more on phenotypic causal pathways than directly on common genetic influence. According to the Pearson's correlation (Table 2), there was a significant but low correlation between RCT, a_{30} and IAC with SCS. The normal somatic cell composition of milk from healthy mammary glands obtained a cell count below 1 x 10^5 cell/mL (Bytyqi et al., 2010). However, all the samples in the present study provided values within the standard required by the European Community (CE no. 853/2004), which is a maximum of 4.0 x 10^5 cell/mL (CE, 2004), and which contributed to low interference in relation to coagulation properties. Because the differences in the means were observed as a function of the period of analysis, the generalised linear model (GLM) was applied in order to verify the influence of this effect, as well as the milk origin (properties) in relation to each dependent variable. Effects model for each dependent variable are summarized in Table 3.

As it was also demonstrated by the test t (Table 1), there was a significant statistical difference (p <0.05) between fat and SCS, and between RCT, a_{30} and IAC in the analysed periods of summer and autumn (Table 3). Among the properties, there was a statistical difference (p <0.05) between the fat contents, protein, casein, SCS, pH, as well as between the lactodinamographic parameters parameters, RCT, a_{30} and, consequently, IAC (Table 3). The only variable that did not show statistical difference between the times periods and properties was lactose, which is in fact one of the most stable components in milk. However, fat, protein, casein, SCS and pH are parameters that are more easily altered in relation to nutrition and the genetic characteristics of animals (Del Prato, 2001; Guetouache et al., 2014). In the present study, although the cows were all of the same breed, the milk came from different farms, which were managed differently. This also contributed to the results, which were in agreement with the scientific literature (De Marchi et al., 2007; Cassandro et al., 2008; Frederiksen et al., 2011, Penasa et al., 2014). The same can be considered for RCT, a_{30} and, consequently, for IAC.

Comparative analysis between the coagulating (CS) and non-coagulating (NC) milk samples

The total of NC samples was 41.1% (31.9% summer and 9.2% autumn), which was high. In order to understand the high number of NC samples, the averages (corresponding to

Table 3 : Test of the effects model for each dependent variable that was studied

Effects	Trait*																			
	Fat g.100mL^{-1}		P g.100mL^{-1}		CN g.100mL^{-1}		Lactose g.100mL^{-1}		SCS		pH		RCT min		A_{30} mm		IAC	
	Wald ChiSq	Sig.	Wald ChiSq	Sig.	Wald ChiSq	Sig.	Wald ChiSq	Sig.	Wald ChiSq	Sig.	Wald ChiSq	Sig.	Wald ChiSq	Sig.	Wald ChiSq	Sig.	Wald ChiSq	Sig.
Seasons	6.52	<0.05	1.84	0.17	2.91	0.08	0.02	0.90	4.13	<0.05	0.09	0.76	18.54	<0.05	27.55	<0.05	19.12	<0.05
Properties	26.62	<0.05	56.33	<0.05	69.94	<0.05	2.06	0.84	20.89	<0.05	125.02	<0.05	15.86	<0.05	23.38	<0.05	29.41	<0.05

**Significant correlation (p <0.05) and Pearson's correlation (r) below the diagonal, and correlation coefficient (R2) above the diagonal. **P = protein; CN = casein; SCS = somatic cell score; THI = temperature humidity index; RCT = rennet coagulation time; A30 = curd firmness; IAC = index of the aptitude of milk to coagulate.*

Table 4: Comparison between chemical composition, pH and SCS of coagulating (CS) and non-coagulating (NC) samples analysed in summer and autumn.

Trait	Summer		Autumn	
	CS*	NC*	CS*	NC*
Fat, g 100^{-1}	4.08 ± 0.84^{a}	3.62 ± 0.73^{b}	3.72 ± 0.80^{a}	3.50 ± 0.67^{a}
P**, g 100^{-1}	3.38 ± 0.44^{a}	3.18 ± 0.37^{b}	3.48 ± 0.46^{a}	3.48 ± 0.41^{a}
CN**, g 100^{-1}	2.61 ± 0.36^{a}	2.44 ± 0.31^{b}	2.71 ± 0.37^{a}	2.70 ± 0.33^{a}
Lactose, g 100^{-1}	4.79 ± 0.24^{a}	4.81 ± 0.24^{a}	4.80 ± 0.18^{a}	4.73 ± 0.21^{b}
pH	6.56 ± 0.09^{b}	6.58 ± 0.08^{a}	6.57 ± 0.07^{a}	6.55 ± 0.08^{b}
SCS**	3.91 ± 2.50^{a}	3.62 ± 2.40^{a}	3.27 ± 2.18^{a}	3.60 ± 2.44^{a}
THI**	73.24 ± 6.74^{b}	76.81 ± 5.53^{a}	57.43 ± 4.99^{a}	58.64 ± 5.85^{a}

**CS (coagulating samples) and NC (non-coagulating samples). a, b; c; dAverages with different letters in the same row (summer and autumn separately) differ statistically ($p < 0.05$, Test t). **P = protein; CN = casein; SCS = somatic cell score; THI = temperature and humidity index.*

summer and autumn) of the chemical composition, pH and SCS of the CS and NC samples were compared by means of the t-test (Table 4). There were significant differences ($p < 0.05$) between fat, protein, casein and pH between the samples that were analysed during the summer; during the autumn, significant differences were only found between lactose and pH. Lactose does not interfere directly with milk coagulation, and even though there was significant difference between the mean pH values this difference was small and was not sufficient to interfere with coagulation. However, as it can be observed in Table 4, in the summer, there was a significant difference ($p < 0.05$) between the casein content for the CS (2.61 ± 0.36 g.mL^{-1}) and the NC samples (2.44 ± 0.31 g.mL^{-1}). In the autumn there were no differences between the samples CS (2.71 ± 0.37 g.mL^{-1}) and the milk NC (2.70 ± 0.33 g.mL^{-1}) in relation to the casein. The mean casein reported by several authors is between 2.52 and 2.70 g.mL^{-1} (Cassandro et al., 2008; De Marchi et al., 2008; Tiezzi et al., 2013; Bonfatti et al., 2014; Toffanin et al., 2015; Penasa et al., 2016). Of these, the lowest casein content (2.55 g.mL^{-1}) presented 12.9% of NC samples (Toffanin et al., 2015) and, of those with the highest casein values (2.65 g.mL^{-1} and 2.70 g.mL^{-1}), the percentage of NC samples was 9.7% for Cassandro et al. (2008) and 3.0% for Penasa et al. (2016).

According to the literature, the casein is an important factor in terms of MCP. However, the association of casein with NC samples is related to the concentration of K-CN relative to the total casein present (Hallén et al., 2010; Fredriksen et al., 2011) and not only in relation to the total casein concentration. The concentration of K-CN is negatively correlated with the size of the casein micelles, and the connection between the size of the micelles and the MCP is that micelles of smaller caseins aggregate faster during coagulation and form a firmer rennet when compared to micelles of larger caseins (Glantz et al., 2010, Frederiksen et al., 2011). Thus, the results obtained in this study corroborate with those in the literature; the percentage of casein was not sufficient to explain the high number of NC samples. Comparing the protein and casein (Table 4) of the CS and NC samples, in the summer there was a significant difference between them, with lower values of these indices for the NC samples. However, in terms of the samples collected in the autumn, comparing the protein and casein contents of the CS and NC samples, the values for both were almost the same.

CONCLUSION

In the present study, 41.1% of the samples of Italian Holstein-Friesian cow milk collected during the summer and autumn were considered non-coagulating (NC). The highest number of such samples was obtained during the summer, representing 56.6% of the total samples analysed in this period; the corresponding figure in the autumn was 20.9%. Between the two studied periods (summer and autumn), there was no significant statistical difference between protein, casein and pH. All samples in the present study provided values within standard required for somatic cell and your correlation whith RCT, A_{30} and IAC was a significant but low. However, the temperature and humidity index (THI) varied greatly between the periods. In the summer, the mean was 73.24 ± 6.74 (values between 64.66-81.53) and during the autumn was 57.43 ± 4.99 (values between 50.04-70.40). Holstein cows are more sensitive to thermal stress, which may have contributed to the high numbers of NC samples. The results for the lactodinamographic parameters (RCT, min; A_{30}, mm and IAC) of the coagulated samples (CS) were best for the samples collected during the autumn. However, the comparative analysis between the chemical composition of the CS and NC samples does not seem to have influenced the results. In the summer, the NC samples presented lower values for protein and casein compared to the CS samples from that same period, with significant differences. However, the values for these parameters were practically the same between the CS and NC samples in the autumn. Non-coagulating milk represents a problem for the cheese industry, and what was observed in this study was that periods with high THI contributed to a high number of samples with this characteristic. The data obtained in this study were not sufficiently conclusive to provide the reason for the high number of NC samples studies should be performed from the genetic point of view regarding the protein composition of milk in order to verify which changes occur in milk during periods of high THI, when the highest percentage of NC samples were found.

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REFERENCES

Auldist, M. J., K. A. Johnston, N. J. White, W. P. Fitzsimons and M. J. Boland. 2004. A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. J. Dairy Sci. 71: 51-57.

Armstrong, D. V. 1994. Heat stress interaction with shade and cooling. J. Dairy Sci. 77: 2044-2050.

Bernabucci, U., N. Lacetera, L. H. Baumgard, R. P. Rhoads, B. Ronchi and A. Nardone. 2010. Metabolic and hormonal adaptations to heat stress in domesticated ruminants. Animal. 4: 1167-1183.

Beux, S., E. A. Pereira, M. Cassandro, A. Nogueira and N. Waszczynskyj. 2017. Milk coagulation properties and methods of detection. Ciênc. Rural. 47(10): 1-8.

Brito, J. R. F and M. A. V. P. Brito. 2004. Qualidade do leite brasileiro e os desafios para atendimento das exigências internacionais. In: Zoccal, R., L. J. M. Aroeira, P. D. C. Martins, M. S. P. Moreira, P. B. Arcuri. Leite: Uma Cadeia Produtiva em Transformação. Embrapa Gado de Leite, Juiz de Fora, p. 235-243.

Bohmanova, J., I. Misztal and J. B. Cole. 2007. Temperature-humidity indices as indicators of milk production losses due to heat stress. J. Dairy Sci. 90: 1947-1956.

Bouraoui, R., M. Lahmar, A. Majdoub, M. Djemali and R. Belyea. 2002. The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. Anim. Res. 51: 479-491.

Brown-Brandl T. M., J. A. Nienaber, R. A. Eigenberg, H. C. Freetly and G. L. Hahn. 2003. Thermoregulatory responses of feeder cattle. J. Thermal Biol. 28: 149-157.

Bytyqi, H., U. Zaugg, K. Sherifi, A. Hamidi, M. Gjonbalaj, S. Muji and H. Mehmeti. 2010. Influence of management and physiological factors on somatic cell count in raw milk in Kosova. Vet. Arch., 80(2): 173-183.

Cassandro, M., A. Comin, M. Ojala, R. Dal Zotto, M. De Marchi, L. Gallo, P. Carnier, and G. Bittante. 2008. Genetic parameter of milk coagulation properties and their relationships with milk yield and quality traits in Italian Holstein cows. J. Dairy Sci. 91: 371-376.

Cassandro, M., D. Pretto, N. Lopez-Villalobos, M. De Marchi and M. Penasa 2016. Estimation of economic values for milk coagulation properties in Italian Holstein-Friesian cattle. J. Dairy Sci. 99: 6619-6626.

De Marchi, M., R. Dal Zotto, M. Cassandro and G. Bittante. 2007. Milk coagulation ability of five dairy cattle breeds. J. Dairy Sci. 90: 3986-3992.

De Marchi, M., G. Bittante, R. Dal Zotto, C. Dalvit and M. Cassandro. 2008. Effect of Holstein Friesian and brown swiss breeds on quality of milk and cheese. J. Dairy Sci. 91: 4092-4102.

Del Prato, O. S. 2001. Trattato di Tecnologia Casearia. Calderini Edagricole, Bologna.

Du Preez, J. H., P. J. Hatting, W. H. Giesecke and B. E. Eisenberg. 1990. Heat stress in dairy cattle and other livestock under Southern African conditions. III. Monthly temperature-humidity index mean values and their significance in the performance of dairy cattle. Onderstepoort J. Vet. Res. 57: 243-248.

Frederiksen, P. D., K. K. Andersen, M. Hammershøj, H. D. Poulsen, J. Sørensen, M. Bakman, K. B. Qvist and L. B. Larsen. 2011. Composition and effect of blending of noncoagulating, poorly coagulating, and well-coagulating bovine milk from individual Danish Holstein cows. J. Dairy Sci. 94: 4787-4799.

Gantner, V., P. Mijić, K. bKuterovac, D. Solić and R. Gantner. 2011. Temperature-humidity index values and their significance on the daily production of dairy cattle. Mljekarstvo, 61(1): 56-63.

Glantz, M., T. G. Devold, G. E. Vegarud, H. L. Månsson, H. Stålhammar. and D. M. Paulsson. 2010. Importance of casein micelle size and milk composition for milk gelation. J. Dairy Sci. 93: 1444-1451.

Guetouache, M., B. Guessas and S. Medjekal. 2014. Review – Composition and nutritional value of raw milk. Biol. Sci. Pharm. Res. 2(10): 115-122. Hallén, E., T. Allmere, J. Näslund, A. Andrén and A. Lundén. 2007. Effect of genetic polymorphism of milk proteins on rheology of chymosin-induced milk gels. Int. Dairy J. 17: 791-799.

IDF: International IDF Standard 141C:2000. 2000. Determination of Milk Fat, Protein and Lactose Content. Guidance on the Operation of Mid-infrared Instruments. International Dairy Federation, Brussels, Belgium.

IDF: International Dairy Federation 148-2E. 2006. Milk-Enumeration of Somatic Cells. Part 2: Guidance on the Operation of Fluoro-opto-electronic Counters. International Dairy Federation, Brussels, Belgium.

Ikonen, T., K. Ahlfors, R. Kempe, M. Ojala and O. Ruottinen. 1999. Genetic parameters for the milk coagulation properties and prevalence of noncoagulating milk in Finnish dairy cows. J. Dairy Sci. 82: 205-214.

Ikonen, T., S. Morri, A. M. Tyriseva, O. Ruottinen and M. Ojala. 2004. Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content and ph of milk. J. Dairy Sci. 87: 458-467.

Il Meteo. 2015. Available from: http://www.ilmeteo.it. [Last retrieved on 2015 Dec 10].

Miranda, J. E. C and A. F. Freitas. 2009. Raças e Tipos de Cruzamentos para Produção de Leite. Embrapa Gado de Leite (Circular Técnica).

NRC. National Research Council. 1971. Commite on Animal Nutrition Agricultural Board. Atlas of Nutritional Data on United and Canadian Feed. National. Academy. Science, Washington. D.C.

O'Connell, D. J., E. P. Mulholland, A. P. Duffy, C. P. O'donnell and F. A. Payne. 2001. Evaluation of hot wire and optical sensors for online monitoring of curd firmness during milk coagulation. Irish J. Agric. Food Res. 40: 227-238.

Okigbo, L. M., G. H. Richardson, R. J. Brown and C. A. Ernstrom. 1985. Variation in coagulation properties of milk from individual cows. J. Dairy Sci. 68: 822-828.

Penasa, M., F. Tiezzi, A. Sturaro, M. Cassandro and M. De Marchi. 2014. A comparison of the predicted coagulation characteristics and composition of milk from multi-breed herds of Holstein-Friesian, Brown Swiss and Simmental cows. Int. Dairy J. 35: 6-10.

Penasa, M., M. De Marchi, S. Ton, L. Ancilotto and M. Cassandro. 2015. Reproducibility and repeatability of milk coagulation properties predicted by mid-infrared spectroscopy. Int. Dairy J. 47: 1-5.

Penasa, M., V. Toffanin, N. Cologna, M. Cassandro and M. De Marchi. 2016. Effects of dairy factory, milk casein content and titratable acidity on coagulation properties in Trentingrana dairy industry. J. Dairy Sci. 83: 242-248.

Politis, I. and K. F. Ng-Kwai-Hang. 1998. Effects of somatic cell counts and milk composition on the coagulating properties of milk. J. Dairy Sci. 71: 1740-1746.

Pretto, D., T. Kaart, M. Vallas, I. I. Jõudu, M. Henno, L. Ancilotto, M. Cassandro and E. Pärna. 2011. Relationships between milk coagulation property traits analyzed with different methodologies. J. Dairy Sci. 94: 4336-4346.

Pretto, D., N. Lopez-Villalobos, M. Penasa and M. Cassandro. 2012. Genetic response for milk production traits, somatic cell score, acidity and coagulation properties in Italian Holstein-Friesian population under current and alternative selection indices and breeding objectives. Livest. Sci. 150: 59-66.

Pretto, D., M. De Marchi, M. Penasa and M. Cassandro. 2013. Effect of milk composition and coagulation traits on Grana Padano cheese yield under field conditions. J. Dairy Sci. 80: 1-5.

Rodriguez, L.W., G. Mekonnen, C. J. Wilcox, F. G. Martin and W. A. Krienk. 1985. Effects of relative humidity, maximum and minimum temperature, pregnancy and stage of lactation on milk composition and yield. J. Dairy Sci. 68: 973-978.

Stelzer, F. S., R. P. Lana, J. M. S. Campos, A. B. Mancio, J. C. Pereira and J. G. Lima. 2009. Performance of milking cows fed concentrate at different levels associated or not with propolis. Rev. Brasil. Zootecn. 38: 1381-1389.

Tiezzi, F., D. B. Valente, M. Cassandro and C. Maltecca. 2015. Causal relationships between milk quality and coagulation properties in Italian Holstein-Friesian dairy cattle. Genet. Select. Evol. 47(45): 1-9.

Toffanin, V., M. De Marchi, N. Lopez-Villalobos and M. Cassandro. 2015. Effectiveness of mid-infrared spectroscopy for prediction of the contents of calcium and phosphorus, and titratable acidity of milk and their relationship with milk quality and coagulation properties. Int. Dairy J. 41: 68-73.

Zannoni, M. and S. Annibaldi. 1981. Standardization of the renneting ability of milk by Formagraph. Sci. Tecn. 32: 79-94.

Zimbelman, R. B, R. J. Collier and T. R. Bilby. 2013. Effects of utilizing rumen protected niacin on core body temperature as well as milk production and composition in lactating dairy cows during heat stress. Anim. Feed Sci. Technol. 180: 26-33.

Wedholm, A., L. B. Larsen, H. Lindmark-Mansson, A. H. Karlsson and A. Andren. 2006. Effect of protein composition on the cheesemaking properties of milk from individual dairy cows. J. Dairy Sci. 89: 3296-3305.