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Quality traits and modeling of coagulation, curd firming, and syneresis of sheep milk of Alpine breeds fed diets supplemented with rumen-protected conjugated fatty acid

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ABSTRACT

The aim of this study was to test the modeling of curd-firming (CF) measures and to compare the sheep milk of 3 Alpine breeds supplemented with or without rumen-protected conjugated linoleic acid (rpCLA). Twenty-four ewes of the Brogna, Foza, and Lamon breeds were allotted to 6 pens (2 pens/breed) and fed a diet composed of corn grain, corn silage, dried sugar beet pulp, soybean meal, wheat bran, wheat straw, and a vitamin-mineral mixture. The rpCLA supplement (12 g/d per ewe plus 4 g/d for each lamb older than 30 d) was mixed into the diet of 1 pen per sheep breed (3 pens/treatment) to provide an average of 0.945 and 0.915 g/d per ewe of the *cis*-9,*trans*-11 C18:2 and *trans*-10,*cis*-12 C18:2 conjugated linoleic acid isomers, respectively. The trial started at 38 ± 23 d after parturition, and individual morning milk samples were collected on d 16, 23, 37, 44, and 59 of the trial. Milk samples were analyzed for composition, and duplicate samples were assessed for milk coagulation properties (MCP). A total of 180 CF measures for each sample (1 every 15 s) were recorded. Model parameters were the rennet coagulation time, the asymptotic potential CF, the CF instant rate constant, the syneresis instant rate constant, the maximum CF achieved within 45 min (CF_{max}), and the time at achievement of CF_{max}. The data were analyzed using a hierarchical model that considered the fixed effects of breed, diet, lamb birth, and initial days in milk, which were tested on individual ewe (random) variance; the fixed effect of sampling day, which was tested on the within-ewe sample (random) variance; and the fixed effect of instrument or cuvette position (only for MCP), which was tested on the residual (replicates within samples) variance. The local Alpine sheep breeds displayed similar milk compositions, traditional MCP, and CF modeling parameters. Supplementation with rpCLA triggered changes in milk composition and worsened MCP (e.g., delayed rennet coagulation time, slower CF instant rate constant, and a doubling of syneresis instant rate constant), but did not influence potential CF. Overall, our results indicate that rpCLA supplementation reduced the actual maximum CF (CF_{max}) but did not modify the interval between rennet addition and CF_{max} or time to CF_{max}.
Key words: ovine milk, milk coagulation property, conjugated fatty acid, curd-firming modeling

INTRODUCTION

For decades, bovine milk coagulation properties (**MCP**) have been evaluated using mechanical lactodynamographs (Bittante et al., 2012). Three single-point parameters are defined: the rennet coagulation time (**RCT**, min), which is the interval from the addition of the enzyme to the gelation of the milk; the curd-firming (**CF**) rate ($\mathbf{k_{20}}$, min), or the time from gelation to a CF of 20 mm; and the CF measured 30 min after rennet addition ($\mathbf{a_{30}}$, mm). Combinations of these parameters are used to categorize milk samples for their cheese-making properties. Computerized lactodynamographs can record continuous repeated measurements of CF. Bittante (2011b) and Bittante et al. (2013b) proposed a model that fully represents the temporal evolution of CF on the basis of rennet RCT, the asymptotical potential CF at infinite time ($\mathbf{CF_P}$, mm), the curd-firming instant rate constant ($\mathbf{k_{CF}}$) from RCT to infinite time, and the syneresis instant rate constant ($\mathbf{k_{SR}}$). Although MCP have not been widely studied among small ruminants, partial studies in this field have been made on sheep (Pellegrini et al., 1997; Jaramillo et al., 2008; Pazzola et al., 2013) and goats (Park et al., 2007; Pazzola et al., 2011, 2012). Notably, the traditional MCP procedure is sometimes considered inadequate for evaluating the milk of these animals (Bittante et al., 2012). Compared with bovine

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milk, the milk-coagulation process of small ruminants is typically much faster and of greater magnitude; thus, a_{30} often measures CF after the maximum value has been reached, and k_{20} measures only a limited tract of the steep increase in CF (Bittante et al., 2012). Thus, the use of the model proposed by Bittante (2011b) and Bittante et al. (2013b) could provide new insights into the coagulation properties of ewe milk, and permit us to evaluate whether these properties can be influenced by breed or different diets and feed additives.

Conjugated linoleic acid has gained attention in recent years for its beneficial effects on human health (Dilzer and Park, 2012). Although these effects have largely been studied in animal models and in vitro (McCrorie et al., 2011), further research is needed (Gebauer et al., 2011). The main source of natural CLA for humans is the consumption of food from ruminant species, especially those fed on pasture or diets containing oil seeds (Nuernberg et al., 2005; Scollan et al., 2006; Woods and Fearon, 2009). However, supplementing the animal diets with rumen-protected CLA (**rpCLA**; produced by the feed industry) is an effective way to increase the CLA content of beef (Gillis et al., 2004; Schiavon et al., 2011) and lamb (Terré et al., 2011) meat. The rpCLA has shown favorable effects on the efficiency of energy and nitrogen utilization in growing young bulls, and appears to exert a limited effect on fat deposition (Schiavon et al., 2010, 2012; Schiavon and Bittante, 2012). In dairy ruminants, however, the most notable effect of rpCLA is its ability to decrease the fat content in milk from cows (Baumgard et al., 2000; Bauman et al., 2008; Glasser et al., 2010), goats (Lock et al., 2008; Shingfield et al., 2009; Ghazal et al., 2012), and sheep (Oliveira et al., 2012; Weerasinghe et al., 2012). However, whereas numerous studies regarding the effects of rpCLA on dairy ruminants exist, little is known about its influence on MCP.

The aims of the current study were to (1) examine the effect of rpCLA supplementation of lactating ewes on their milk composition; (2) model the CF process of sheep milk; (3) compare the effect of 3 different sheep breeds on milk quality and MCP; and (4) study the effects of rpCLA supplementation on the composition and MCP of sheep milk.

MATERIALS AND METHODS

Animals

The present study was carried out at the Lucio Toniolo Experimental Farm of the University of Padova (Legnaro, Italy), using a flock of sheep representing endangered Alpine breeds native to the Veneto Region (northeast Italy). Animals were treated following the *Guideline for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1988). The study involved 24 ewes, of them, 10, 9, and 5 ewes belonged to the Brogna, Foza, and Lamon breeds, respectively. The 24 ewes were allotted to 6 pens of 3 × 6 m (2 for each breed) with their 31 suckling lambs (15, 10, and 6 for the Brogna, Foza, and Lamon breeds, respectively). At the start of the trial, the ewes were 38 ± 23 DIM and weighed 61 ± 13 kg. The trial lasted 63 d. Ewes and lambs were individually weighed each week. Animals were monitored daily by a technician and health status was controlled 3 times per week by a veterinarian following the experimental protocol for animal care.

Feeds and Feeding

The basal diet was composed of corn grain, corn silage, dried sugar beet pulp, soybean meal, wheat bran, wheat straw, and a vitamin-mineral mixture (Table 1). Dietary ingredients were mixed and fed as a TMR, offered ad libitum, and prepared daily using a mixer-wagon equipped with a computer-assisted weighing scale that was calibrated monthly. Three pens, 1 for each breed, with 5, 4, and 3 Brogna, Foza, and Lamon ewes, respectively, received the basal diet; the remaining 3 pens (with 5, 5, and 2 Brogna, Foza, and Lamon ewes, respectively, 12 in total) received the same basal diet top dressed and then mixed with TMR, with an rpCLA product (SILA, Noale, Italy) equal to 12 g/d per ewe plus 4 g/d per lamb aged 30 d or more. This rpCLA dose was established to provide averages of 0.945 and 0.915 g/d per ewe for the *cis*-9,*trans*-11 C18:2 and *trans*-10,*cis*-12 C18:2 CLA isomers, respectively. The composition of the commercial rpCLA used was previously reported by Schiavon et al. (2010). The amount of each feed ingredient loaded into the mixer-wagon and the weight of the mixture uploaded in the manger of each pen were recorded daily. The orts remaining in the mangers were weighed weekly by pen. The average DMI was computed on a pen basis.

Samples of each feed ingredient were analyzed for their proximate compositions (AOAC International, 2000) and their NDF and ADF contents (Van Soest et al., 1991). The ME of the basal ration was computed from the actual ration ingredient composition and tabular values of each feed ingredient (NRC, 2007).

Milk Sampling and Analyses

The ewes were separated from their lambs for at least 2 h and then hand milked on d 16, 23, 37, 44, and 59. After collection, milk samples (without preservative) were immediately divided into subsamples A (35 mL)

Table 1. Ingredients, chemical composition, DM, and ME of a TMR and its ingredients

TMR ingredient	TMR, g/kg of DM	DM, g/kg	Chemical composition, g/kg of DM				ME,[1] MJ/kg of DM
			PG	NDF	ADF	Starch	
Corn grain, ground	373	884	89	127	25	686	13.1
Corn silage	260	351	77	402	228	365	10.6
Dried sugar beet pulp	111	897	93	439	252	0	11.4
Soybean meal	110	891	491	139	90	0	13.3
Wheat bran	64	881	169	375	113	0	10.7
Wheat straw	66	917	23	810	491	0	6.0
Vitamin-mineral mixture[2]	16	920	—	—	—	—	—
TMR	1,000	504[3]	130	293	146	347	11.4

[1]Values taken from NRC (2007).

[2]Content per kilogram of DM: 12.4 g of Ca, 1.7 g of P, 2.5 g of Na, 100 mg of Cu, 300 mg of Zn, 1.0 mg of Co, 3 mg of I, 1 mg of Se, 200 mg of Mn, 22,000 IU of vitamin A, 83 IU of vitamin E, and 2,750 IU of vitamin D_3.

[3]Including water added to the mixer wagon to increase moisture of the TMR.

and B (20 mL), stored in portable refrigerators (4°C), and transported to the cheese-making laboratory at the Department of Agronomy, Food, Natural Resources, Animals and Environment of the University of Padova for analyses. All samples were processed within 5 h after collection.

For each ewe, milk subsample A was analyzed for fat, protein, lactose, TS, and nonfat solids contents using a MilkoScan FT2 (Foss, Hillerød, Denmark). In addition, SCC were performed using a Fossomatic FC counter (Foss). Each SCC was converted to the SCS by means of logarithmic transformation as SCS = ($\log_2$ SCC × $100{,}000^{-1}$) − 3. The energy of milk was calculated using the values proposed by the NRC (2007) and converted to kilojoules per gram (fat = 38.89 kJ/g; protein = 23.90 kJ/g; lactose = 16.53 kJ/g).

Analysis of MCP

The B subsamples were assessed for MCP using 2 mechanical lactodynamographs (Formagraph, Foss). All experimental conditions (milk temperature, rennet concentration, and rennet type) were applied as described in detail by Cipolat-Gotet et al. (2012). In brief, a rack containing 10 cuvettes (1 rack per instrument) was prepared. Two milk subsamples (10 mL) for each ewe were randomly allotted to the 2 racks, heated at 35°C, and mixed with 200 μL of rennet solution (Hansen Standard 215 with 80 ± 5% chymosin and 20 ± 5% pepsin; Pacovis Amrein AG, Bern, Switzerland) diluted to 1.2% (wt/vol) in distilled water [to yield 0.051 international milk clotting units (**IMCU**)/mL]. The instruments recorded the width (in millimeters) of the oscillatory graph during the test every 15 s. The observation period lasted for 45 min after rennet addition. Traditional MCP (RCT, k_{20}, and a_{30}) were provided directly by the instrument. Recording was prolonged to 45 min after enzyme addition to achieve an additional measure of CF ($\mathbf{a_{45}}$, mm). Relatively few samples (8 of 206 samples) failed to coagulate within the 45-min duration of the test.

Modeling Curd Firmness and Syneresis

As CF was measured every 15 s for 45 min, a total of 180 CF values were recorded for each sample. The comparison of the much shorter RCT values of ovine milk to those of bovine milk, and the prolongation of recording to 45 min enabled the use of the 4-parameter model described by Bittante et al. (2013b):

$$\mathrm{CF}_t = \mathrm{CF_P} \times \left[1 - e^{-\mathrm{k_{CF}} \times (t - \mathrm{RCT})}\right] \times e^{-\mathrm{k_{SR}} \times (t - \mathrm{RCT})},$$

where CF_t is the curd firmness at time t (mm); $\mathrm{CF_P}$ is the asymptotic potential maximum value of curd firmness (mm); $\mathrm{k_{CF}}$ is the curd-firming instant rate constant (%/min); $\mathrm{k_{SR}}$ is the curd syneresis instant rate constant (%/min); and RCT is the rennet coagulation time (min).

This model uses all available information to estimate the 4 parameters; therefore (unlike the traditional MCP), these are not single-point measurements. The $\mathrm{CF_P}$ parameter is conceptually independent from test duration and (unlike a_{30}) is not intrinsically dependent on RCT. The parameter $\mathrm{k_{CF}}$ is assumed to increase CF toward the $\mathrm{CF_P}$ asymptotic value, whereas $\mathrm{k_{SR}}$ is assumed to decrease CF toward a null asymptotic value. In the initial phase of the test, the first rate constant prevails over the second, so CF_t increases to a point in time ($\boldsymbol{t}_{\mathbf{max}}$) at which the effects of the 2 parameters are equal but opposite in sign and CF_t attains its maximum level ($\mathbf{CF_{max}}$). Thereafter, CF_t begins to decrease, tending toward a null value because of the effect of curd syneresis and the corresponding expulsion of whey. The RCT parameter is still a traditional measure, but it is now estimated using all available data.

Statistical Analyses

The CF_t observations available for each sample were fitted with curvilinear regressions using the nonlinear procedure (PROC NLIN) of SAS (Version 8.2, SAS Institute Inc., Cary, NC). The parameters of each individual equation were estimated employing the Marquardt iterative method (350 iterations and a 10^{-5} level of convergence). In some late-coagulating samples (6 of 206 samples), the data did not converge. Samples in which the CF_P exceeded the replicate (3 cases) or the mean (3 cases) by 3 SD were considered outliers and excluded from our analysis of all equation parameters. No data editing was performed for the other parameters.

The data regarding milk analyses, traditional MCP, and CF modeling were analyzed using a linear mixed model employing the MIXED procedure of SAS. The statistical model used to analyze the traditional MCP and the parameters of the CF_t model included the fixed effects of breed (Brogna, Foza, and Lamon), dietary treatment (control vs. rpCLA addition), number of lambs suckling (single or twin lambing), a linear covariate of DIM at the start of the trial, sampling day (16, 23, 37, 44, and 59 d from the start of the trial), and the cuvette location within the 2 instruments (18 levels). The random effects included in the model were the individual animal (24 ewes, 16 df), the milk samples within each ewe (77 df), and the replicates within each milk sample (residual, 70 df). The significances of breed, diet, number of lambs, and DIM were tested on the error line of animal variance; sampling day was tested on the error line of milk sample variance within animals; and cuvette location within instruments was tested on the residual variance. For milk quality traits, the 2 replicates were averaged and the within-ewe samples were assumed to coincide with the residual variance.

In the case of each ewe's live weight (1 observation per animal, data not shown), the model was simplified because it did not include the effects of sampling day or the cuvette location within the instrument, and the only random effect included (residual) coincided with the animal. In the case of DMI (1 observation per pen, data not shown), the model included only the effect of diet and breed, and the residual coincided with their interaction.

RESULTS

Brogna ewes presented a lower BW compared with the Foza and Lamon ewes (51.8 vs. 71.1 and 71.2 kg, respectively; $P < 0.01$), and Brogna and Foza ewes consumed less DM than Lamon ewes (2.45 and 2.50 vs. 3.00 kg of DM/d, respectively; $P < 0.05$). Supplementation with rpCLA did not influence the DMI of ewes and lambs (expressed per unit of ewe present; 2.63 vs. 2.67 kg of DM/d, respectively, for control and supplemented diets), or influence the BW and average body gains of ewes and lambs, regardless of breed.

However, rpCLA supplementation did affect the milk composition, reducing the protein and nonfat solids contents as it increased the SCS (Table 2). All of the other factors included in the model, with the exception of sampling day (which was significant for almost all traits), had limited effects; the Lamon breed yielded milk with a lower nonfat solids content compared with Foza ewes, and ewes that had lambed twins or had longer intervals of DIM at the beginning of the trial produced milk with greater protein and nonfat solids contents.

The only breed effect observed among the traditional traits used to depict MCP was a lower a_{45} for Lamon versus Foza sheep (Table 3). The MCP were significantly worse when rpCLA was added to the basal diet, as RCT was delayed, CF was slowed, and a_{30} and a_{45} were lower. Twin lambing had positive effects on both measures of CF. Days in milk at the beginning of the trial did not affect any MCP, whereas sampling day and cuvette location influenced all traits except RCT.

For the CF_t model parameters, milk from Lamon ewes was characterized by slower curd firming and faster syneresis compared with the Foza breed (Table 4). Similar to the traditional MCP, the parameters obtained from CF_t modeling showed that rpCLA supplementation had negative effects on the parameters of coagulation and curd firming, except for the asymptotic CF value and the interval between the addition of the enzyme and the moment of maximum CF. In milk sampled from rpCLA-supplemented ewes, the expulsion of whey from the curd (i.e., the syneresis rate) was much faster and the number of samples showing no detectable syneresis within 45 min from rennet addition was halved.

The birth type affected the CF modeling, as samples from ewes with twin lambs showed more rapid decreases of CF after reaching the maximum (i.e., more rapid syneresis). The sampling day and instrument or cuvette location affected all modeling parameters except for RCT (both factors) and CF_P (sampling day).

DISCUSSION

Traditional Coagulation Properties of Sheep Milk

The enzymatic coagulation of milk, the firming of curd, and the subsequent expulsion of whey (syneresis)

Table 2. Effect of breed, rumen-protected conjugated linoleic acid (rpCLA) supplementation, birth type, DIM at first sampling, and date of sampling on quality traits of ewe milk

Item	Fat, %	Protein, %	Lactose, %	TS, %	Nonfat solids, %	Energy, MJ/kg	SCS, U
Breed							
Brogna (Br)	6.60	5.74	5.06	17.9	12.0	4.77	4.89
Foza (Fo)	5.45	5.71	5.28	17.0	12.1	4.35	4.35
Lamon (La)	6.69	5.51	5.08	17.7	11.7	4.75	5.96
Contrasts, *P*-value							
Br vs. (Fo + La)/2	0.52	0.34	0.40	0.45	0.47	0.50	0.74
Fo vs. La	0.21	0.22	0.24	0.41	0.05	0.29	0.09
Diet							
Control	6.56	5.82	5.12	18.0	12.1	4.79	4.37
rpCLA addition	5.94	5.48	5.16	17.1	11.7	4.47	5.76
P-value	0.41	0.02	0.77	0.21	0.007	0.29	0.06
Birth type							
Single	6.25	5.46	5.12	17.3	11.7	4.58	5.22
Twin	6.24	5.85	5.16	17.7	12.1	4.68	4.90
P-value	0.99	0.01	0.79	0.56	0.01	0.77	0.69
DIM							
<50	6.68	5.60	5.16	17.9	11.9	4.79	5.18
50–75	6.39	5.42	5.12	17.4	11.7	4.63	4.57
>75	5.67	5.93	5.14	17.3	12.2	4.47	5.44
P-value	0.75	0.04	0.95	0.82	0.05	0.83	0.59
Ewe RMS[1]	1.88	0.05	0.06	1.53	0.06	0.29	1.89
Sampling day, *P*-value	<0.001	0.08	0.01	<0.001	0.02	<0.001	0.13
RMSE[2]	2.00	0.07	0.04	1.44	0.08	0.28	0.84

[1]Ewe RMS = ewe root mean square.

[2]RMSE = root mean square error.

Table 3. Effect of breed, rumen-protected conjugated linoleic acid (rpCLA) supplementation, birth type, DIM at first sampling, date of sampling, and instrument or cuvette position on traditional milk coagulation properties of ewe's milk[1]

Item	RCT, min	k_{20}, min	a_{30}, mm	a_{45}, mm
Breed				
Brogna (Br)	8.68	1.62	59.3	54.8
Foza (Fo)	7.18	1.57	58.7	55.2
Lamon (La)	8.05	1.67	54.6	47.8
Contrasts, *P*-value				
Br vs. (Fo + La)/2	0.36	0.99	0.17	0.16
Fo vs. La	0.51	0.41	0.07	0.01
Diets				
Control	6.50	1.46	60.6	56.9
rpCLA addition	9.44	1.78	54.5	48.3
P-value	0.01	0.004	0.003	<0.001
Lambs born				
Single	7.66	1.66	55.2	50.0
Twin	8.29	1.58	59.9	55.2
P-value	0.59	0.44	0.02	0.03
DIM				
Regression coefficient	−0.028	−0.004	−0.006	−0.113
P-value	0.38	0.17	0.91	0.08
Ewe RMS[2]	1.78	0.16	2.10	2.14
Sampling day, *P*-value	0.56	0.02	<0.001	0.002
Sample RMS[3]	2.46	0.20	NE[4]	NE
Instrument/position, *P*-value	0.11	<0.001	<0.001	<0.001
RMSE[5]	1.08	0.16	7.86	10.46

[1]RCT = rennet coagulation time; k_{20} = time interval between coagulation and attainment of a curd firmness of 20 mm; a_{30} (a_{45}) = curd firmness after 30 (45) min from rennet addition.

[2]Ewe RMS = ewe root mean square.

[3]Sample RMS = sample root mean square.

[4]NE = not estimable.

[5]RMSE = root mean square error.

Table 4. Effect of breed, rumen-protected conjugated linoleic acid (rpCLA) supplementation, birth type, DIM at first sampling, date of sampling, and instrument or cuvette position on modeling of coagulation, curd firming, and syneresis of ewe milk[1]

Item	RCT, min	CF_p, mm	k_{CF}, %/min	k_{SR}, %/min	No k_{SR}, %	CF_{max}, m	t_{max}, min
Breed							
Brogna (Br)	9.1	68.6	42.8	0.70	25.2	63.2	25.3
Foza (Fo)	7.6	66.8	45.9	0.58	28.2	62.7	24.6
Lamon (La)	8.4	67.5	37.8	0.99	16.4	59.1	22.5
Contrasts, *P*-value							
Br vs. (Fo + La)/2	0.33	0.35	0.77	0.53	0.69	0.19	0.37
Fo vs. La	0.54	0.69	0.04	0.01	0.17	0.08	0.37
Diet							
Control	7.0	67.3	49.5	0.51	30.8	64.0	24.1
rpCLA addition	9.8	68.0	34.8	1.00	15.7	59.3	24.2
P-value	0.01	0.64	<0.001	<0.001	0.03	0.007	0.96
Birth type							
Single	8.1	67.6	41.6	0.90	26.7	60.4	24.6
Twin	8.7	67.7	42.7	0.61	19.9	63.0	23.7
P-value	0.58	0.99	0.73	0.04	0.35	0.15	0.66
DIM							
Regression coefficient	−0.027	0.086	0.162	0.005	−0.409	0.038	−0.173
P-value	0.39	0.05	0.08	0.13	0.05	0.42	0.003
Ewe RMS[2]	1.75	1.14	4.28	NE[3]	4.8	2.61	2.35
Sampling day, *P*-value	0.52	0.38	<0.001	0.003	0.002	0.001	<0.001
Sample RMS[4]	2.43	NE	3.88	NE	9.7	NE	2.77
Instrument/position, *P*-value	0.16	<0.001	0.02	<0.001	<0.001	<0.001	<0.001
RMSE[5]	1.04	7.54	10.92	0.69	33.7	4.99	7.69

[1]RCT = rennet coagulation time; CF_P = asymptotic potential curd firmness; k_{CF} = curd firming instant rate constant; k_{SR} = syneresis instant rate constant; No k_{SR} = incidence of milk samples with not estimable k_{SR}; CF_{max} = maximum curd firmness achieved within 45 min; t_{max} = time at achievement of CF_{max}.

[2]Ewe RMS = ewe root mean square.

[3]NE = not estimable.

[4]Sample RMS = sample root mean square.

[5]RMSE = root means square error.

are the key processes in cheese-making, and thus affect cheese yield and quality (Bittante et al., 2013a; Cípolat-Gotet et al., 2013). However, the mechanical and optical near infrared (NIR) lactodynamographs (Cipolat-Gotet et al., 2012) that are generally used to measure these traits are nonautomated and time-consuming. In the bovine dairy industry, the Fourier-transform infrared spectrum of milk, which is heritable (Bittante and Cecchinato, 2013), was recently introduced as a method for predicting the parameters traditionally measured by lactodynamographs. The Fourier-transform infrared prediction is very rapid and inexpensive, does not need enzymes or mechanical tools, and does not require milk coagulation to occur. Such indirect predictions may also prove useful for breeding programs (Cecchinato et al., 2009). To our knowledge, however, these techniques have not previously been tested on milk from small ruminant species and more research is needed on this topic.

The data obtained in the present study confirmed that large differences exist between ovine and bovine MCP, as reported in Table 3. The average RCT measured from control ewes (6.5 min) was much shorter than that commonly found in bovine species (10 to 20 min), as reviewed by Bittante et al. (2012). The average k_{20} revealed that a much steeper increase in the CF of ovine milk (1.5 min) compared with bovine milk (5 to 10 min). The average CF after 30 min was also greater for sheep milk (61 mm) than cow milk (25 to 42 mm). Notably, the rennet concentration used in the present study (0.051 IMCU/mL) was smaller than those in all but one of the papers on bovine MCP reviewed by Bittante et al. (2012; 0.061 to 0.150 IMCU/mL), and this disparity widens if the amount of rennet is expressed per unit protein instead of per unit of milk. Moreover, the milk of cows and ewes reacts differently to acidification, temperature changes, calcium addition, and variation in rennet concentration (Bencini, 2002).

Modeling the Coagulation, CF, and Syneresis of Sheep Milk

Two other shortcomings of the traditional MCP parameters are the increasing percentage of bovine milk samples that do not coagulate within the commonly used time interval of 30 min from rennet addition (Ikonen et al., 1999; Cecchinato, 2013) and the increasing proportion of milk samples that do not allow computation of the k_{20} trait. These increases reflect the worldwide spread of the Holstein breed, which are known for having inferior MCP compared with breeds of Alpine origins (Cecchinato et al., 2011). A similar problem has been noted in small ruminants (Pazzola et al., 2012, 2013). The traditional MCP analysis uses only 3 data points, whereas computerized rennet meters (lactodynamographs) use continuous repeated measurements. Bittante (2011b) modeled a data set recorded from individual bovine milk samples using computerized rennet meters over 30 min (120 records, 1 every 15 s, in the case of Formagraph lactodynamographs) and proposed to use the obtained CF curve to estimate the RCT, the asymptotical potential CF at infinite time (CF_P), and the CF instant rate constant (k_{CF}) from RCT to infinite time. Later, Bittante et al. (2013b) expanded the CF model to account for the decrease in CF often recorded after (though sometimes before) 30 min from rennet addition, using a forth parameter called the syneresis rate constant (k_{SR}).

In the case of sheep milk, the traditional MCP traits are considered even less reliable in depicting the process of CF, largely because the process is much faster and of greater magnitude compared with bovine milk, such that a_{30} often measures CF after the maximum value has already been reached and k_{20} measures only a limited tract of the steep increase in CF (Bittante et al., 2012). The present study showed that the model proposed by Bittante et al. (2013b) overcomes the concerns linked to traditional MCP measures, yielding results that can depict the evolution of CF over time for sheep milk. In fact, the large majority of individual samples converged, allowing us to estimate the values for all 4 parameters of the model. Compared with the bovine milk studied by Bittante et al. (2013b), the control sheep milk samples analyzed in the present study showed, on average, a much earlier gelation (RCT of 7.0 vs. 19.3 and 20.7 min compared with Brown Swiss and Holstein Friesian cows, respectively), a greater asymptotic potential CF (CF_P of 67 vs. 54 and 36 mm, respectively), a much steeper increase in CF (k_{CF} of 49.5 vs. 12.0 and 13.0%/min, respectively), and a slower decrease in CF due to syneresis (k_{SR} of 0.5 vs. 1.4 and 1.7%/min, respectively). Regarding this last parameter, ~30% of milk samples from control ewes did not exhibit any apparent decrease of CF during the 45 min after rennet addition, meaning that the k_{SR} could not be estimated and was assumed to be null. If we excluded these samples, the average k_{SR} of the remaining samples was 0.6%/min. In practice, the CF equation of the samples characterized by nonestimable k_{SR} values coincided with the 3-parameter model that was initially proposed by Bittante (2011b) to depict the CF trends of lactodynamograms generated over a short observation interval (30 min for cow milk). It is probable that prolonging the observation interval beyond 45 min would have allowed us to estimate k_{SR} for the samples that failed to show any significant decrease within the test period. Bittante et al. (2013b) prolonged their recording interval to 90 min and observed that almost all of their bovine milk samples presented an inflection, allowing them to compute k_{CF} for all samples. In any case, the samples with late CF decreases were characterized by very slow syneresis rate constants.

For each milk sample, knowing the 4 parameters of the CF curve allowed us to calculate the maximum CF value (CF_{max}), which reflects the potential CF attainable and the 2 opposite effects of CF rate and syneresis rate, and the time interval from rennet addition to the attainment of the maximum CF (t_{max}), which also incorporates the RCT. Compared with the milk of Brown Swiss cows tested by Bittante et al. (2013b), the sheep milk examined in the current study showed a greater CF_{max} (64 vs. 35 mm, respectively) and reached t_{max} earlier (24 vs. 41 min, respectively). The average t_{max} of the sheep milk samples presented in Table 4 includes samples that failed to show any decrease within 45 min from rennet addition; for these last samples, t_{max} was assumed to be 45 min. When these samples were excluded from the analysis, we obtained an average t_{max} of 15 min. We cannot compare these parameters to others in the literature because the present study is the first to model the output of computerized rennet meters when examining ovine milk.

Comparison Among the Sheep Breeds of the Veneto Alps

Brogna, Foza, Lamon, and Alpagota are the only autochthonous sheep breeds of the Italian Alps still present in the northern part of the Veneto region (Bittante, 2011a). Brogna sheep, which are reared in the province of Verona, are of medium size (similar to Alpagota) and characterized by red spots on a white coat (Pastore, 2002; Pellattiero et al., 2011). Of the 3 breeds studied herein, Brogna is the only breed that was often used (and in some cases is still used today) as a dairy ewe; its milk can be used to produce a local pecorino (Pegorin) cheese. The other 2 local breeds, which are both in danger of extinction, are reared in very low numbers in the provinces of Vicenza (Foza) and Belluno (Lamon). Both are large breeds with high growth rates among their lambs; they have long ears and small black spots (especially on the head) against a white coat (Pastore, 2002). These breeds are traditionally reared for meat production (mainly from weaned lambs and castrated yearlings). The Lamon breed has been studied in the past decades (Bittante et al., 1996; Ramanzin et al.,

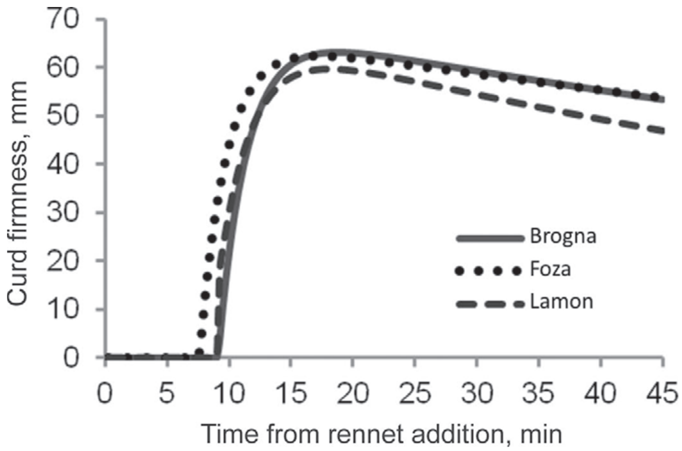

Figure 1. Effect of sheep breed on coagulation, curd firming, and syneresis processes of ewe milk.

1991), but no previous study has examined the quality and coagulation properties of milk from the Veneto sheep breeds.

The contents of fat, protein, and lactose in the Veneto sheep breeds are close to the average values reported by Barłowska et al. (2011) for ovine milk in their literature review on the composition of ruminant milk, and are very good if compared with milk traits recorded for specialized dairy ewes, such as the Sarda sheep (Mura et al., 2012; Vacca et al., 2013). The milk compositions of the 3 local Alpine breeds were very similar, with the sole exception of higher nonfat solids content in Foza milk compared with Lamon milk (Table 2).

The 2 larger breeds also presented some differences in terms of the traditional MCP, as Foza milk samples tended to have greater a_{30} ($P = 0.07$) and a_{45} ($P = 0.01$) values. A picture of the difference between the 2 breeds, given in Figure 1, shows that milk from Foza ewes was characterized by a steeper increase (k_{CF}) and a slower decrease (k_{SR}) in CF compared with the milk of Lamon ewes. The graphical representation of CF evolution over time provides a much clearer picture of the pattern of coagulation, CF, and syneresis than we would obtain using only the 3 points considered by the traditional MCP procedure (RCT, a_{30}, and k_{20}). From the shapes of the curves, we see that traditional k_{20} measures only about one-third of the increase of the CF_t curve, that a_{30} is in the decreasing tract of the curve and not in the increasing one, as for bovine milk, and it is influenced by all the other parameters. Finally, the differences in the milk CF_t curves due to single or twin lambings (Figure 2) were mainly caused by differences in syneresis.

Effect of rpCLA on the Composition of Sheep Milk

We found that rpCLA supplementation strongly modified milk composition in sheep, even though the ewes received less than 1 g of each CLA isomer per day. The rpCLA-induced decrease in milk fat content was only nominal (−0.62 percentage points; not significant), but it was similar in magnitude to that found in dairy cows that received similar CLA dosages expressed per unit of metabolic weight (Baumgard et al., 2000; Selberg et al., 2004; Castañeda-Gutiérrez et al., 2005). The rpCLA-induced decrease in milk fat observed herein was much lower than that previously observed in sheep (∼−2.57 and −2.26 percentage points; $P < 0.001$) (Weerasinghe et al., 2012). However, whereas those authors used sheep (breed unspecified) of similar BW and DIM compared with those used in the present study, their sheep were milked twice a day and received a restricted diet (DMI of 1.8 vs. 2.6 kg/d in the prior and present studies, respectively) that had a lower dietary energy concentration (ME of 10.9 vs. 11.4 MJ/kg of DM, respectively) and a greater dietary CP content (CP of 163 vs. 130 g/kg of DM, respectively). In addition, whereas the CLA source was the same, the daily supply in the prior study was about twice that used in the current study. In dairy cows, the effect of rpCLA supplementation on milk fat content was lower in animals fed high-concentrate diets compared with those fed low-concentrate diets. The available energy supply may influence the response of the mammary gland to CLA isomers, particularly *trans*-10,*cis*-12 C18:2, as suggested by Glasser et al. (2010). Oliveira et al. (2012) also recorded a large reduction (−1.76 percentage points) of milk fat content in CLA-supplemented ewes of the Lacaune breed (a specialized dairy sheep breed), but the authors used 10-fold more (not rumen-protected) CLA isomers compared with the current study.

Supplementation with rpCLA decreased the protein and nonfat solids contents of sheep milk (−0.34,

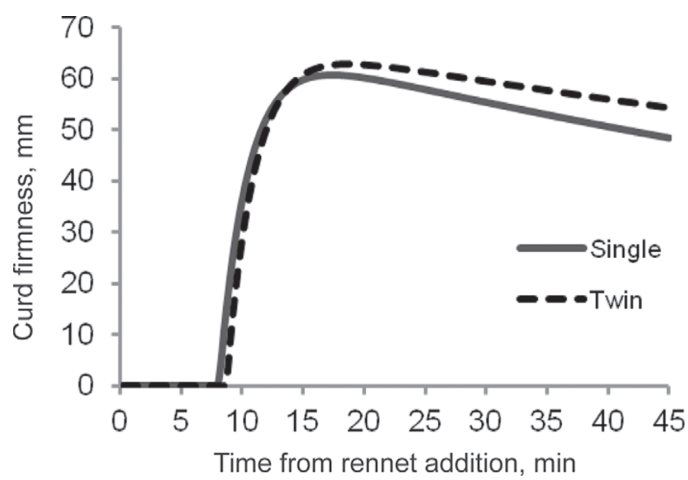

Figure 2. Effect of number of lambs born on coagulation, curd firming, and syneresis processes of ewe milk.

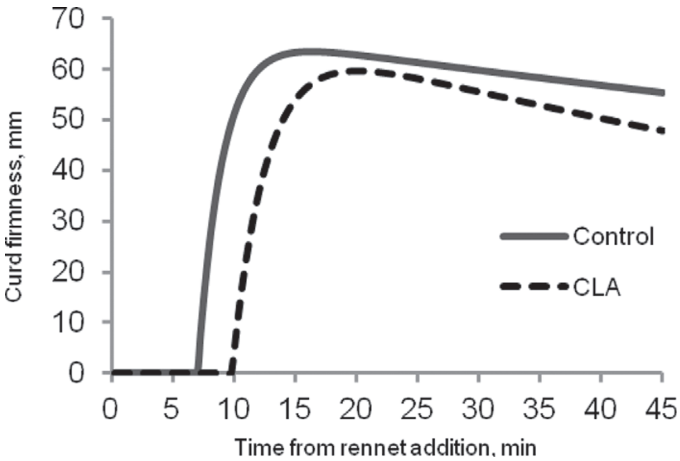

Figure 3. Effect of rumen-protected conjugated linoleic acid supplementation to diet on coagulation, curd firming, and syneresis processes of ewe milk.

−0.40, and −2.7 percentage points, respectively) compared with the control. Previously cited studies found inconsistent effects of CLA on milk protein content. In sheep, Weerasinghe et al. (2012) found a negative effect, whereas Oliveira et al. (2012) found a positive effect. In cows, the effect of CLA on milk protein content generally appears to be low or null (Maxin et al., 2011; Hötger et al., 2013).

In terms of other possible effects on milk quality, we observed that rpCLA supplementation tended to increase the SCS compared with the control. In bovine milk, the observed changes in milk composition (especially those in protein content) are generally considered negative for MCP because of their well-known phenotypic and genetic correlations (Cecchinato et al., 2011; Bittante et al., 2012).

Effect of rpCLA on Curd Firmness and Syneresis

In the literature, no information exists regarding the effects of rpCLA administration on coagulation, CF, and syneresis in both bovine and sheep milk. In the present study, almost all parameters of the CF model were affected by rpCLA supplementation of ewes (Table 4). Supplementation did not affect the potential CF; however, it delayed milk gelation (RCT of 7.0 vs. 9.8 min for control and rpCLA, respectively), slowed CF (k_{CF} of 49.5 vs. 34.8%/min, respectively), and doubled the rate of whey expulsion (k_{SR} of 0.51 to 1.00%/min, respectively). Furthermore, the incidence of samples that did not show any CF decrease during the test period was halved in ewes receiving rpCLA supplementation (No k_{SR} of 30.8 vs. 15.7%, respectively). When we excluded the samples with an apparent lack of syneresis, the average values of k_{SR} increased to 0.6 and 1.2%/min, respectively, for control and rpCLA-treated ewes. The effect of rpCLA on the CF curve is shown in Figure 3. The rpCLA-induced changes in the 2 first parameters would be considered negative for cheese-making, whereas the effect of the change in the third parameter on cheese-making is not yet known.

CONCLUSIONS

The present study provides new insights into the complex processes of coagulation, CF, and syneresis in ovine milk, and shows that rpCLA supplementation can influence these processes. Despite the phenotypic and genetic diversity of the 3 local sheep breeds tested herein, the ewes produced milk with similar compositions and technological properties. Ovine milk is characterized by a faster gelation after rennet addition, a steeper increase of CF, and a slower decrease of CF caused by syneresis. These trends are not effectively captured by the single-point analysis of traditional coagulation traits (RCT, k_{20}, and a_{30}), but the present study showed that they can be fully captured by modeling the entire CF curve over time. This modeling requires the estimation of only 4 parameters, which can be achieved by prolonging the observation time up to 45 min from rennet addition. In sum, we show that rpCLA supplementation of sheep can change the composition and worsen the cheese-making properties of their milk (i.e., by delaying gelation, slowing curd firming, and accelerating syneresis). Future studies are warranted to examine the effects of CLA on cheese yield or quality, assess the relationships with milk coagulation, CF, and syneresis, and identify causal mechanisms.

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