Effect of protein shortage and conjugated linoleic acid supplementation on quality traits and modelling of coagulation, curd firming and syneresis of Holstein-Fresian milk

Cesaro, G., Schiavon, S.

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Poljoprivredni fakultet u Osijeku, Poljoprivredni institut Osijek

Faculty of Agriculture in Osijek, Agricultural Institute Osijek

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EFFECT OF PROTEIN SHORTAGE AND CONJUGATED LINOLEIC ACID SUPPLEMENTATION ON QUALITY TRAITS AND MODELLING OF COAGULATION, CURD FIRMING AND SYNERESIS OF HOLSTEIN-FRESIAN MILK

Cesaro, G., Schiavon, S.

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SUMMARY

Aim of the present study was to evaluate the effect of diets with optimal (CP 15% DM) or suboptimal (CP 12.3% DM) protein content, supplemented (CLA+) or not (CLA-) with rumen-protected conjugated linoleic acid (rpCLA) on some cheese-making properties. Twenty Holstein-Fresian mid lactating dairy cows have been reared following a 4×4 Latin square experimental design of 4 periods, 3 weeks each. Individual milk samples, collected during the third week of each period, were analysed for chemical composition, traditional milk coagulation properties (MCP: RCT, k_{20} and a_{30}) and for recording curd firmness (CF) every 15 s over a 90 min period. Data acquired from each sample were used to model CF over time calculating the following parameters: rennet coagulation time (RCT_{eq}), asymptotic potential CF (CF_P), CF rate constant (k_{CF}), syneresis rate constant (k_{CF}), maximum CF achieved within 90 min (CF_{max}) and time to CF_{max} (t_{max}). Data were analysed using period, diet and group (random) as sources of variation. Cows evidenced a strong individual variability within groups and were classified as early (RCT<20 min) or late (RCT>20 min) coagulating cows. Dietary protein shortage reduced milk protein and lactose content, while rpCLA supplementation depressed milk fat synthesis. Results showned that traditional MCP parameters were worsened by reduction of dietary protein in the case of milk produced by early coagulating cows, while rpCLA supplementation affected negatively all three traits on all cows. The study of CF model parameters evidenced that CP12 diets have improved CF (CF_P and CF_{max}) respect to CP15 when fed to late coagulating cows while worsened CF (CF_P and CF_{max}) and reduced k_{CF} when fed to early coagulating cows. The results of the present study underline the complex relationship between dietary fat and protein and their consequences on milk technological properties highlighting the need for further investigations.

Key-words: bovine milk, milk coagulation properties, curd firming modelling, dietary protein, rumen-protected conjugated linoleic acid

INTRODUCTION

The use of low protein diets is gaining interest because of environmental concerns and the increasing cost of protein sources (Schiavon et al. 2010 and 2013; Gallo et al. 2015). Conjugated linoleic acid isomers (CLA) content in animal products has gained attention primarily for the beneficial effect of these molecules on human health (Pariza et al. 2001) and have shown a favourable interaction with dietary protein reduction in young bulls fattening (Schiavon et al., 2012; Schiavon and Bittante, 2012). A dietary supply of these isomers decreased milk fat content in dairy cows (Glasser et al., 2010) and worsened milk coagulation properties (MCP) in ewe milk (Bittante et al., 2014). In cheese production interest in MCP of milk is increasing (Bittante et al., 2012). These

Ph.D. Giacomo Cesaro, Post Doc (giacomo.cesaro@unipd.it), Prof. Dr. Stefano Schiavon - University of Padova, Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), Viale dell'Università 16, Legnaro (PD), Italy

information are relevant for cheese processing, particularly for Protected Designation of Origin (PDO) cheeses (Bertoni et al., 2001; Bittante et al. 2011a and 2011b). The combination of dietary protein shortage and the use of fat supplements in dairy cows could exert some effects on MCP, thus more detailed information about this interaction are required.

Traditional measurements of the coagulation attitude are made with the Formagraph (McMahon and Brown, 1982) which usually determine 3 parameters: rennet coagulation time (RCT), time to curd firmness of 20 mm (k_{20}) and curd firmness 30 min after enzyme addition (a_{30}). Different factors affect MCP and especially experimental conditions (Stocco et al., 2015) and cows genetics (Cassandro et al., 2005; Bittante et al., 2012). The existence of late- and non-coagulating milk samples, characteristic of some breeds as the Holstein-Fresian and Scandinavian ones (Tyrisevä et al., 2004), make difficult to measure traditional MCP within the 30 min duration of classical lactodynamographic test and thus the parameters cannot be measured. This problem was overcome by Bittante (2011) and Bittante et al. (2013) who modelled all the observations of curd firmness (CF) collected every 15 s on each individual milk sample extending the period of observation from 30 to 90 min. This experiment aimed to study the effect of a reduced dietary CP, with or without a CLA supplementation, on milk attitude to cheese-making, evaluating milk composition, traditional MCP traits and coagulation, curd firmness and syneresis model parameters of Holstein-Friesian dairy cows.

MATERIAL AND METHODS

The study was conducted at the University of Padova (Legnaro, Italy) and all experimental procedures were reviewed and approved by the University Ethical Committee (CEASA).

Experimental design and diets. Twenty Holstein-Fresian balanced for milk yield, DIM, parity, BW and BCS were randomly assigned to 4 pens (5 cows each), and treated according to a 4×4 Latin square experimental design with 4 periods of 3 weeks each. Cows were fed *ad libitum* total mixed rations based on corn silage, corn grain, meadow hay, sugar beet pulp, alfalfa hay, wheat bran and soybean meal. The control diet (CP15) has been formulated following NRC (2001) recommendations for 30.0 kg/d milk yield (3.5, 3.4 and 4.7 of protein, fat and lactose, respectively). Low CP diet (CP12) was formulated by replacing soybean meal with barley grain, decreasing CP content from 15 to 12.3% DM and increasing starch from 22.7 to 26.3% DM. Other constituents did not vary among diets. A rumen protected mixture of conjugated linoleic acid isomers (rpCLA; SILA, Noale, Italy) was supplemented by top dressing 80 g/d/cow of rpCLA, details about rpCLA composition is given in Schiavon et al. (2011).

Sampling and analysis. Milk samples have been collected during 5 consecutive d of the third experimental week for each of the 4 periods, one sample per cow for each milking event (2 per d), 800 samples were analysed in total. Milk coagulation properties have been analysed using two mechanical lactodynamographs (Formagraph, Foss) according to Cecchinato et al. (2013). Briefly milk samples (10 ml) have been allotted to racks with 10 cuvettes each, heated at 35°C and mixed with 200 μl of rennet solution (Hansen Standard 215, Oacovis Amrein AG, Bern, Switzerland) diluted to 1.2 % (wt/vol). Curd firmness measures (one every 15 sec for 90 min = 360 values each sample) enabled to apply the 4-parameter equation described by Bittante et al. (2013):

$$CF_t = CF_p \times [1- e^{-k_{CF} \times (t-RCT_{eq})}] \times e^{-k_{SR} \times (t-RCT_{eq})}$$

where CF_t is the curd firmness at time t (mm); CF_P is the asymptotic potential maximum value of curd firmness (mm); k_{CF} is the curd-firming instant rate constant (% min^{-1}); k_{SR} is the curd syneresis instant rate constant (% min-1); RCT_{eq} is the rennet coagulation time (min).

In the initial phase of the test the k_{CF} prevail over k_{SR} and CF_t reaches its maximum value at CF_{max} in time t_{max} at which the two rate constant are equal but opposite in sign.

Statistical analysis. The 20 dairy cows differed largely in terms of MCP within each group, and were classified into two sub-groups: early coagulating (r < 20 min; n = 10) and late coagulating (r > 20 min; n = 9) cows that were analysed separately using the mixed procedure in SAS 9.2 (SAS Inst. Inc., Cary, NC) with the following model:

$$Y_{ijklm} = \mu + P_i + G_j + CP_k + rpCLA_l + CP \times CLA_{kl} + e_{ijklm}$$

where y_{ijklm} is the observed trait; μ is the overall intercept of the model, P_i is the fixed effect of the ith period (i = 1, …, 4), G_j is the random effect of the jth group of cows (j = 1, …, 4), CP_k is the fixed effect of the dietary CP level (k = 1, 2); CLA_l is the fixed effect due to the presence or absence of CLA (l = 1, 2), $CP \times CLA_{kl}$ is the interaction between CP and CLA and e_{ijkl} is the random residual. Group was assumed to be independently and normally distributed with a mean of zero and variance σ_j^2.

RESULTS AND DISCUSSION

The traditional MCP traits confirmed a negative effect of rpCLA on milk fat content (Glasser et al., 2010). Early and late coagulating cows differ by about 7 min. for rennet coagulation time, the difference, constant across diets (Table 1), confirms a large variability of MCP among cows of the same breed (Bittante et al., 2015).

Table 1. Effect of diets with a crude protein content of 15 (CP15) or 12.3 (CP12) % DM supplemented or not with 80 g/d of rumen-protected conjugated linoleic acid (CLA) on milk quality and on traditional milk coagulation properties of dairy milk[1]

	Diet				SE	*P*-values		
	CP15	$CP15_{CLA}$	CP12	$CP12_{CLA}$		CP	CLA	CP × CLA
Milk quality (all cows):								
Fat, %	3.66	3.12	3.69	3.12	0.12	0.90	0.002	0.88
CP, %	3.52	3.54	3.42	3.31	0.07	0.026	0.41	0.29
Lactose, %	4.76	4.73	4.72	4.67	0.02	0.020	0.05	0.59
RCT (min):								
early coagulating cows[2]	14.95	15.44	14.86	16.20	0.97	0.47	0.05	0.38
late coagulating cows[3]	23.67	25.34	22.26	26.00	1.89	0.61	**< 0.001**	0.15
k20 (min):								
early coagulating cows[2]	3.90	4.21	4.53	6.16	0.96	**< 0.001**	**0.005**	0.06
late coagulating cows[3]	9.92	11.31	8.93	10.51	0.87	0.15	**0.016**	0.88
a30 (mm):								
early coagulating cows[2]	45.67	45.54	43.13	37.42	4.25	**< 0.001**	**0.037**	**0.049**
late coagulating cows[3]	16.39	13.92	20.90	14.20	2.69	0.08	**< 0.001**	0.12

[1]RCT = rennet coagulation time; k_{20} = time interval to achieve a curd firmness of 20 mm; a_{30} = curd firmness after 30 min from rennet addition; [2]Cows (n=10) with a RCT before the beginning of the trial <20 min. [3]Cows (n=9) with a RCT before the beginning of the trial > 20 min

The supply of rpCLA negatively affected the traditional MCP traits, as observed on bovine (Bittante et al., 2014; Vacca et al., 2015). In fact, RCT was delayed, the k_{20} was increased and a_{30} reduced respect to the diet without rpCLA. The effects were more evident in late- than in early-coagulating cows. The reduction of dietary CP slightly reduced milk protein, lactose contents and worsened traditional k_{20} and a_{30} traits only in the early coagulating cows. Results obtained from CF_t modelling confirmed those observed for MCP. The rpCLA supply delayed milk gelation and decreased the asymptotical potential maximum curd firmness in late coagulating cows. Even though the two instant rate constants depicting increasing and decreasing phases of lactodynamographic pattern (kCF and kSR) were not modified, the final result indicated a CF_{max} decrease (Table 2). The rpCLA supply had no effect on MCP of early coagulating cows. A different effect of dietary CP reduction on technological properties of milk from early- and late-coagulating cows was observed (Table 2). In the first group of the cows the dietary CP reduction worsened the CF_P and k_{CF} model parameters of milk, leading to lower CF_{max}, thus confirming the traditional MCP results. The trend observed for late-coagulating cows was different as the dietary CP shortage had a favourable effect on CFt modelling: CF_P, k_{CF} and CF_{max}, were increased.

Table 2. Effect of diets with a crude protein content of 15 (CP15) or 12.3 (CP12) % DM supplemented or not with 80 g/d of rumen-protected conjugated linoleic acid (CLA) on modelling of coagulation, curd firming and syneresis of dairy milk[1]

	Diet					*P*-values		
	CP15	$CP15_{CLA}$	CP12	$CP12_{CLA}$	SE	CP	CLA	CP × CLA
RCT_{eq} (min):								
early coagulating[2]	15.69	15.90	15.12	16.19	0.94	0.77	0.17	0.36
late coagulating[3]	23.30	24.68	21.95	24.58	1.55	0.28	**0.003**	0.36
CF_P (mm):								
early coagulating[2]	57.78	57.96	55.33	52.76	2.50	**< 0.001**	0.19	0.14
late coagulating[3]	48.47	45.94	50.48	47.74	1.52	0.05	**0.006**	0.91
k_{CF} (% min^{-1}):								
early coagulating[2]	12.62	13.66	11.97	11.04	0.99	**0.0066**	0.93	0.11
late coagulating[3]	6.45	6.41	7.23	7.17	0.51	**0.031**	0.88	0.98
k_{SR} (% min^{-1}):								
early coagulating[2]	0.14	0.15	0.13	0.11	0.03	0.34	0.72	0.67
late coagulating[3]	0.18	0.16	0.14	0.10	0.04	0.14	0.37	0.62
CF_{max} (mm):								
early coagulating[2]	54.88	55.40	52.85	50.04	2.95	**< 0.001**	0.22	0.08
late coagulating[3]	42.72	39.91	45.39	41.71	1.14	**0.027**	**0.0013**	0.66
t_{max} (min):								
early coagulating[2]	72.79	70.37	74.44	76.87	2.547	0.06	0.99	0.27
late coagulating[3]	79.62	83.10	83.06	82.60	2.524	0.37	0.35	0.23

[1]RCT = rennet coagulation time; CF_p = asymptotic potential curd firmness; k_{CF} = curd firming instant rate constant; k_{SR} = syneresis instant rate constant; CF_{max} = maximum curd firmness; t_{max} = time at achievement of CF_{max}. [2]Cows (n = 10) with a RCT before the beginning of the trial <20 min; [3]Cows (n = 9) with a RCT before the beginning of the trial >20 min

CONCLUSION

This study shows that a reduction of dietary CP decreases protein and lactose milk contents, where rpCLA supply decreases milk fat content. For the first time in bovine the effects of both dietary treatments on milk coagulation traits were studied. The parameters of the curd firming model made clear an opposite effect of the reduction of dietary CP on milk produced by early-coagulating cows (unfavourable) and by the late-coagulating cows (favourable). The rpCLA supply did not exert any modification of MCP of early-coagulating cows, but worsened MCP in late-coagulating cows. Further researches are needed to define the upper and lower limit of dietary protein shortage also related to the variability of cow's individual milk yield and characteristics. More studies are required on rpCLA dosage in order to avoid not desired side effects on milk technological characteristics.

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