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# Effect of Dietary Protein Supplementation on Sheep Milk Coagulation Properties

Research Article

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## ABSTRACT

The objectives of this experiment were to evaluate the effects of dietary protein supplementation in lactating dairy ewe's diets on milk coagulation properties. Milk samples (n=126) were analyzed to assess the source of variation for rennet coagulation ability at the 6th ($RCA_6$) and 12th hour ($RCA_{12}$), milk clotting time (MCT, s) and an index of milk clotting time (IMCT, %). In addition, data were used to determine the influence of dietary protein source (sunflower meal (SFM) *vs*. rapeseed meal (RSM)) and lactating day (27, 35, 42, 49, 56, 63 and 70) on $RCA_6$, $RCA_{12}$ and MCT. Eighteen early lactation (4-10 weeks) dairy ewes from the synthetic Bulgarian dairy population breed (SBDP) were allocated into two isonitrogenous (crude protein (CP)=18%, dry matter (DM) basis) dietary treatments (n=9 per diet) formulated to contain equal amounts of fibre, energy, protein digestible in small intestines (PDI) and Ca: P ratio. The rates of MCT (s) were measured as time from rennet addition to onset of rennet-induced gel. RCA values were measured subjectively based on a scale of coagulum characteristics (score 1-3). Results showed a significant effect ($P<0.05$) of supplement (RSM *vs*. SFM) on $RCA_6$ and a tendency at $RCA_{12}$ ($p=0.10$). The influence on MCT and IMCT also showed such a tendency ($p=0.11$). Studied correlations between $RCA_6$, $RCA_{12}$ and MCT values to diet supplements at different lactation stages (days) showed no strong relationships, but the directions and strengths were permanent over the evaluated periods. We can conclude, that the investigated parameters ($RCA_6$, $RCA_{12}$, MCT and IMCT) were affected by dietary protein source (SFM *vs*. RSM) in regards to firmer coagulum ($RCA_6$ and $RCA_{12}$) and shorter MCT for milk collected from ewes fed SFM *vs*. RSM based diets.

KEY WORDS clotting time, coagulation rate, dietary protein source, rapeseed meal, relationship, rennet coagulation ability, sheep milk.

## INTRODUCTION

Milk coagulation during cheese making is a multi-stage process vulnerable to many factors. Milk composition and properties are affected by inherent and external factors. The latter may be associated with nutrition (Storry *et al*. 1983; Politis and Ng-Kwai-Hang, 1988; Aleandri *et al*. 1990; Clark, 1993; Malossini *et al*. 1996; Joudu *et al*. 2009; Harzia *et al*. 2013). Thus, the dietary protein supply, as a critical and limiting component in high-quality ruminant diets, could influence milk quality characteristics such as ewe milk coagulation traits. Sunflower meal (SFM) is a traditional source of protein, that is high in protein and inexpensive and that is used to feed rumimamts in Bulgaria. Meanwhile, rapeseed meal (RSM) is a main by-product of the biodiesel industry, obtained after oil extraction. It is balanced with respect to essential amino acids and nutritional characteristics of this feedstuff mean it is the most widely-used high-protein animal feed after soybean meal (AOF Crop Report, 2012). Its content of bioactive peptides with health and nutritional functionalities also supply various benefits (Marczak *et al*. 2003; Cumby *et al*. 2008;

Yossifov, 2013). The importance of coagulation properties of raw milk is based on their influence on yield and quality of dairy products, as well as on economic efficiency (Bittante *et al*. 2012).

So, milk coagulation properties (MCP) of high interest and investigations in this area are required. Our hypothesis was that the effect of feeding different protein sources may lead to variable coagulation properties as a result of transfer efficiencies from diet to milk.

Thus, the aim of the current investigation was to investigate the influence of dietary protein source in lactating dairy ewe diets (SFM *vs*. RSM) on milk clotting time and rennet coagulation and their relationship with stage of lactation.

## MATERIALS AND METHODS

### Animals, diets and experimental design

The experiment was carried out at the experimental farm of the Institute of Animal Science, Kostinbrod, Bulgaria during the months of January and February. Eighteen ewes (synthetic Bulgarian dairy population) were randomly assigned according to age, lactation, milk production, % milk fats, % milk protein into two iso- nitrogenous, iso- caloric, iso- fibrogenous and equal in PDI, Ca and P dietary treatments (n=9): 1) control-sunflower meal (SFM) and 2) experimental-with rapeseed meal (RSM) in order to evaluate the effects of protein supplementation during the early lactation (27-70 days of lactation). The detailed experimental design followed that described by Yossifov, (2012) and shown in Figure 1.

The total ration (on DM basis) consisted of 74.5% forage (corn sillage-61.5% and meadow hay-13%), 24.5% concentrates (cereal-15.5% and protein source-9%) and 1% vitamin-mineral premix. The diets were fed twice daily-7.00 and 18.00 h. Animals were provided *ad libitum* access to fresh water and salt blocks.

### Sampling and analysis

Individual milk samples were collected on days 27, 35, 42, 49, 56, 63 and 70 of lactation without any preservative, and were analyzed within 1 h of collection (Yossifov, 2014). The investigated cheesemaking measurements followed the scheme described by Dimov *et al.* (1974).

### Rennet coagulation ability (RCA)

In a sterile glass test-tube 10.0 mL of raw milk was mixed with 0.5 mL of a 5% solution of commercial rennet Carlina 850$^{TM}$ (coagulating power 1:12 632). The tubes were plugged up, shaken carefully, and incubated for 6 ($RCA_6$) and 12 h ($RCA_{12}$) at 38-40 ˚C. Results were evaluated subjectively based on three-step scale of coagulum firmness (score 1-3) at 6 and 12 h. The scheme is presented in Table 1.

### Milk clotting time (MCT)

Sterile glass test-tubes containing 20 ml of raw milk were placed in a water bath (milk was tempered up to 35 ˚C) and mixed with 0.4 mL 5% tempered solution (35 ˚C) of commercial rennet Carlina 850$^{TM}$ (coagulating power 1:12 632). Milk clotting time (measured as s) was the time from rennet addition to the formation of the first visible floccules, as measured visually.

The correction factor (0.79164) was used. Clotting time of samples with the longest exposition was designed by index of milk clottingtime (IMCT-100%).

### Statistical methods

Biostatistical analysis was performed and all results were shown as mean values and analysis of variance (ANOVA) standard deviation (SD) and coefficient of variation (Var), of triplicate measurements. The data were analysed using the statistical package (MS Office 2007). Significant differences ($P<0.05$) among treatments were detected using Student's t-test. The influence of lactating day (27, 35, 42, 49, 56, 63 and 70 d) on $RCA_6$, $RCA_{12}$ and MCT was evaluated as a measure of the strength and direction of the linear relationship between two variables.

## RESULTS AND DISCUSSION

Animal performance is reported elsewhere (Yossifov, 2012). Briefly, average daily milk yield (actual and 6.5% fat corrected) was significantly ($P<0.01$) higher for the RSM group than the control group (SFM) without any tangible effects on physico-chemical, technological and nutritional milk parameters. Established coagulation traits for the whole experimental period are presented in Table 2. Values for milk clotting time (MCT) between groups did differ, but differences were not statistically significant ($p=0.11$) 58.35 *vs*. 78.86 s, for samples from SFM and RSM fed ewes, respectively. It can be concluded that substitution of dietary SFM with RSM had no influence on milk clotting time and the period was prolonged (35.1%). Rennet coagulation ability at 6$^{-th}$ ($RCA_6$) and 12$^{-th}$ hour ($RCA_{12}$) of the control group (1.19 and 1.03) exceeded that of the experimental group (1.43 and 1.13). The 12 hour values were statistically significant ($P<0.05$). A possible explanation of these results is concerned with casein hydrolysis and accessibility of casein by the enzyme due to interactions between casein and milk fat, size of the fat globules, casein aggregation ability, milk ionic concentration (Ca-P), etc. (Lucey, 2002; Mellema *et al.* 2002).

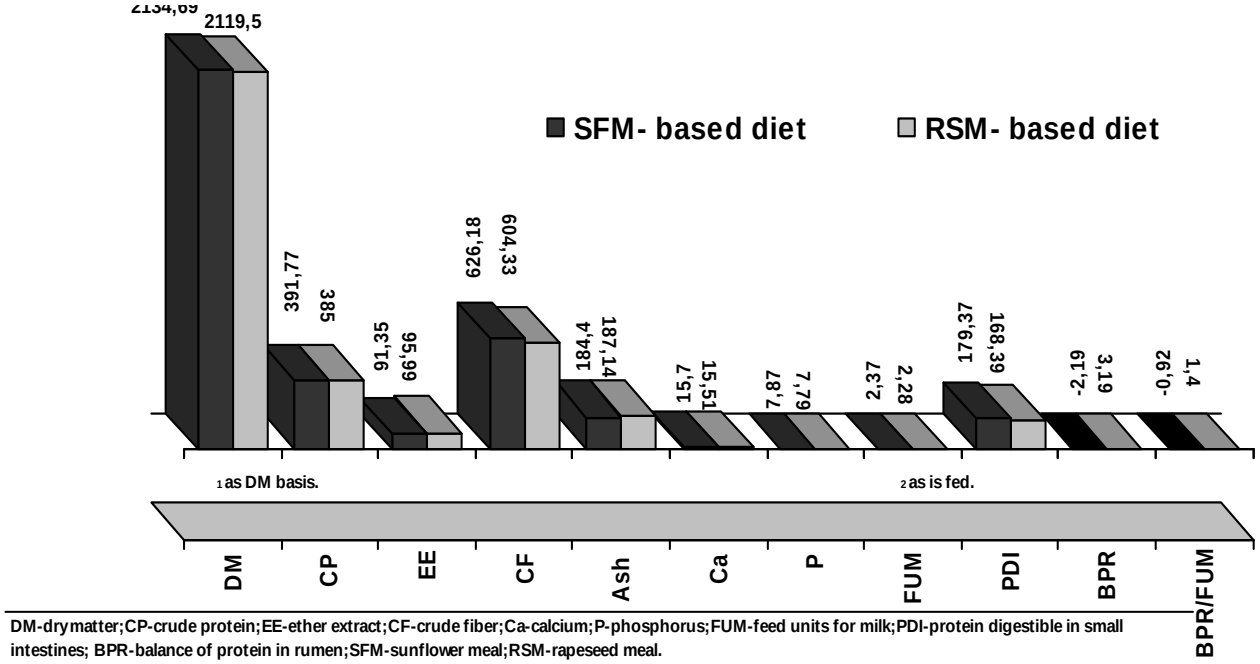


**Figure 1** Chemical composition and nutritional value of SFM and RSM based ewe diets. Adopted by Yossifov, 2012

**Table 1** Scale for determining rennet coagulation ability (RCA)

| Description | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Milk coagulum | | | | | Milk serum | | Rennet coagulation ability |
| | Texture | | | | | | | |
| Score | Shape | Homogeneity | Firmness | Porosity | Heterogeneity | Limpidity | Turbidity | |
| 1 | Compact | Yes | Yes | No | No | Yes | No | Excellent |
| 2 | Tidy | Yes | Yes | Single | Yes | Yes | Yes | Adequate |
| 3 | Loose | No | No | Multitude | Yes | No | Yes | Unfit |

**Table 2** Milk clotting time (MCT) and rennet coagulation ability (RCA) of milk samples, obtained from ewes fed SFM or RSM based diets

| Items | Treatment | | | | | | P-value |
|---|---|---|---|---|---|---|---|
| | SFM | | | RSM | | | |
| | Mean | SD | CV (%) | Mean | SD | CV (%) | |
| $RCA_6$ | 1.19* | 0.17 | 2.76 | 1.43* | 0.26 | 6.86 | <0.05 |
| $RCA_{12}$ | 1.03* | 0.05 | 0.29 | 1.13 | 0.15 | 2.24 | 0.10 |
| MCT | 58.35 | 6.73 | 4.53 | 78.86 | 8.87 | 7.86 | 0.11 |

SFM: sunflower meal based diet; RSM: rapeseed meal based diet; RCA: rennet coagulation ability and MCT: milk clotting time.
SD: standard deviations and CV: coefficient of variation.
* ($P<0.05$).

It can also be assumed that addition of different vegetable protein sources into the diet of lactating dairy ewes can affect coagulation properties through decreased coagulum shape, firmness, porosity and homogeneity, with these effects being reflected in whey limpidity and turbidity (see Table 1 and Figure 2). Clotting time of samples obtained by ewes fed SFM based diets with the longest clotting time was designated an index of milk clotting time (IMCT-100%). As can be seen below (Table 3), the influence of SFM on IMCT was related to lactating day, with its values ranging from 75 to 100%. Compared with control diet, IMCT values of the RSM group after an initial increase (up to 133%), dropped down to 95-130%.

This confirmed the thesis for a positive effect of dietary protein concentration on milk coagulation ability (Joudu *et al*. 2008).

The influence of lactating day (27, 35, 42, 49, 56, 63 and 70 d) on RCA was evaluated (Figures 3 and 4). Greater variation in $RCA_6$ values was observed for the group on an RSM based diet, but trajectories describing RCA were consistent (Figure 3).

Thus, lactation dynamics in coagulum characteristics occurred and estimated values for RCA decreased to the end of the period of analysis ($RCA_{12}$).

Although, the SFM based diet showed better results than the RSM based diet (Figure 3 and 4).

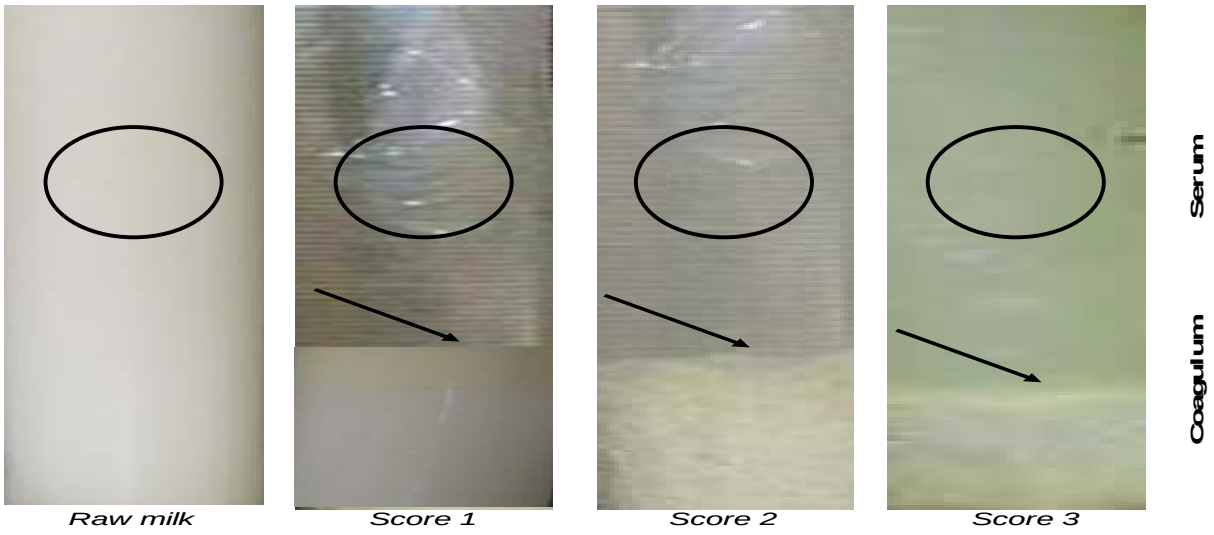


**Figure 2** Comparative analysis on raw sheep milk and its rennet coagulation ability (three-stage scale). Compact coagulum texture with a strong shape, homogeneity and firmness, resulting in limpid serum. In contrast, a heterogenous coagulum with high porosity resulted in turbid serum and a loose texture

**Table 3** Index of milk clotting time as influenced by protein supplement*

| Treatment | Lactating day | | | | | | |
|---|---|---|---|---|---|---|---|
| | 27 | 35 | 42 | 49 | 56 | 63 | 70 |
| | Index of milk clotting time (%) | | | | | | |
| SFM | 86.20 | 79.52 | 100.00 | 74.94 | 81.57 | 99.63 | 95.40 |
| RSM | 112.73 | 115.00 | 133.28 | 94.75 | 129.96 | 119.23 | 129.36 |

* Samples with the longest clotting time at SFM based diets are designed as 100%.
SFM: sunflower meal and RSM: rapeseed meal.

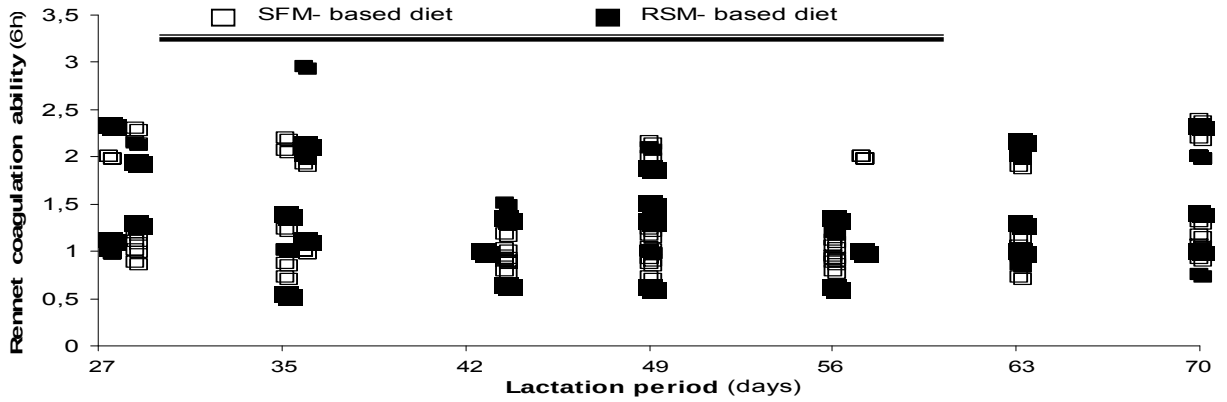


**Figure 3** Changes in RCA scores at 6 hours for the SFM and RSM groups between day 27 and day 70 of lactation

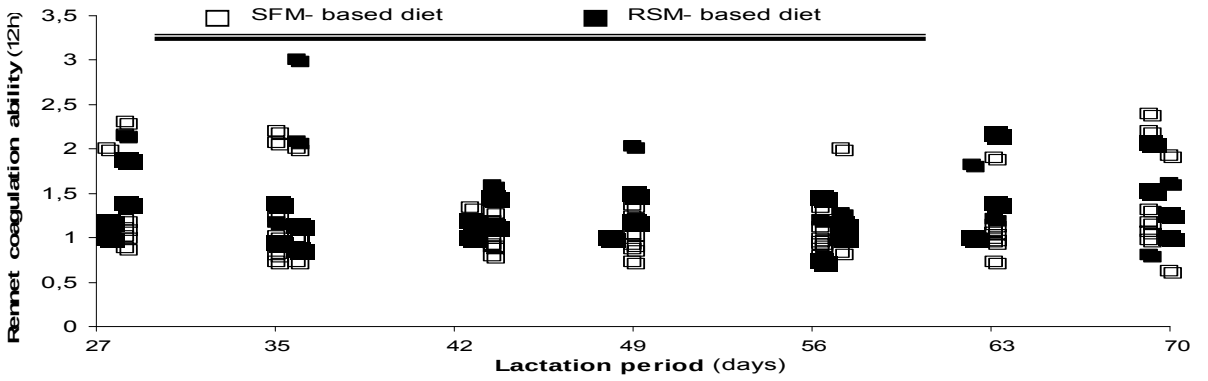


**Figure 4** Changes in RCA scores at 12 hours for the SFM and RSM groups between day 27 and day 70 of lactation

There were few samples with a score of 3, indicating an unsatisfactory RCA value, were found for the RSM based diet group at either 6 or 12 hours (Figure 3 and 4). The findings of the study (Figure 4) depicted priority of SFM based diet over RSM based diet at $RCA_{12}$ values higher percentage of samples formed firmness coagulum (score 1) with serum limpidity (Figure 2 and 4). Changes in MCT values for the two diet groups at different stages of lactation stage showedd no strong relationships, but the direction and strength were similar (Figure 5).

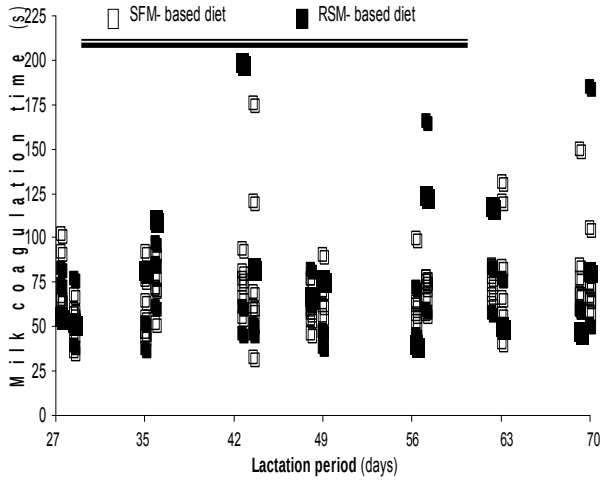


**Figure 5** Changes in MCT scores for the SFM and RSM groups between day 27 and day 70 of lactation

Our results are in agreement with others (Guinee *et al.* 2001; Sundekilde *et al.* 2011; Harzia *et al.* 2012).

## CONCLUSION

Based on the results obtained, it can be concluded that:

A) The type of dietary protein source (SFM *vs*. RSM) does affect rennet coagulation ability, milk clotting time and index of milk clotting time. Lactational changes and dynamics (lactating day, milk composition) and individual animal characteristics also affect the parameters investigated.

B) The results suggest a significant effect ($P<0.05$) of feed (RSM *vs*. SFM) on $RCA_6$ and a tendency at $RCA_{12}$ ($p=0.10$). There tended to be an influence on MCT and IMCT also ($p=0.11$).

C) Changes in $RCA_6$, $RCA_{12}$ and MCT values with lactation stage showed no strong relationships, but the direction and strength were similar for the two groups.

The results revealed that the investigated parameters ($RCA_6$, $RCA_{12}$, MCT and IMCT) were influenced by dietary protein source (SFM *vs*. RSM). Firmer coagulum ($RCA_6$ and $RCA_{12}$) and shorter MCT were observed with milk collected from ewes fed the SFM *vs*. the RSM based diet.

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