

Effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling in East Friesian ewes

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ABSTRACT: The evaluation of the effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling was carried out over the period of three successive years using milk samples ($n = 162$) obtained from a total of 27 ewes of the East Friesian (EF) breed, reared on a small sheep farm in Juřinka in the region of Wallachia. The stage of lactation had a highly significant effect on the contents of all milk components. However, only the contents of total solids (TS), solids non-fat (SNF), fat (F), protein (P) and casein (CN) gradually increased with the advancement of lactation. The stage of lactation also had a highly significant effect both on all the properties of milk and the rennet curdling quality (RCQ). All phenotypic correlations between the particular contents of TS, SNF, F, P, CN and urea nitrogen (UN) were positive and high ($P \leq 0.001$). On the other hand, all phenotypic correlations between milk yield and particular contents of TS, SNF, F, P, CN and UN were negative and high ($P \leq 0.001$). The majority of phenotypic correlations between rennet clotting time (RCT) and the other particular parameters was insignificant. However, the phenotypic correlations between lactose (L) and RCT and between pH and RCT were positive and high ($P \leq 0.001$) whereas the phenotypic correlation between titratable acidity (TA) and RCT was negative and high ($P \leq 0.001$). The majority of phenotypic correlations between the rennet curdling quality (RCQ) and the other particular parameters was insignificant. Nevertheless, the phenotypic correlations between pH and RCQ and between RCT and RCQ were positive and high ($P \leq 0.001$) whereas the phenotypic correlation between TA and RCQ was negative and high ($P \leq 0.001$).

Keywords: stage of lactation; ewe; milk composition; milk properties; quality of rennet curdling; East Friesian

The composition of sheep milk and its production per lactation are influenced by a large number of factors; however, the most important factors are breed, nutrition, health of the animals, environ-

ment and the number and stage of lactation. The effect of the stage of lactation on milk composition in different sheep breeds was studied by Jelínek et al. (1990), Maria and Gabina (1993), Čapistrák

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et al. (1995), Hassan (1995), Fuertes et al. (1998) and Antunovič et al. (2001). The effect of different nutrition on the production and composition of sheep milk in the Tsigai and Improved Wallachian breed was studied by Kica et al. (2000). The relationships between feeding and milk composition in Italian dairy sheep were investigated by Pulina et al. (2005).

Unlike cow or goat milk, sheep milk is used, due to its specific composition, almost exclusively for the production of cheese so that its quality is based not only on its nutrient content but also on its renneting ability. The rennet clotting properties (RCP) of sheep milk are important in cheese-making and in the sensorial characteristics of the product obtained (Calvo, 2001). The RCPs of sheep milk are influenced by a large number of factors; however, the most important factors are the composition of milk, pH, titratable acidity, somatic cell count, temperature of milk, quality of rennet and the environment. The effect of the somatic cell count and the stage of lactation on the plasmin activity and cheese-making properties of ewe milk was studied by Albenzio et al. (2004). The evolution of the pH and renneting properties of milk in Massese ewes during lactation was examined by Pugliese et al. (2000). The correlations between the components of sheep milk and its rennetability in the crossbreds of Tsigai and East Friesian breeds were investigated by Gajdůšek and Jelínek (1992). The evolution of the physicochemical characteristics and renneting properties in ewe's milk collected in the Roquefort area were studied by Pellegrini et al. (1994).

Generally, it is possible to state that the Czech production of sheep cheese is very low. However, especially due to the relatively good economics of this production and the growth of consumer demand for sheep cheese, the domestic production of sheep cheese is expected to rise slightly in future. The East Friesian breed (EF) is the most important dairy breed of sheep in the Czech Republic. Nevertheless, its proportion in the total number of sheep is only ca. 2.6% (Holá, 2005). A significant part of EF sheep in the Czech Republic is reared as purebred. However, the rams of this breed are very often used in improvement crossing with the ewes of Improved Wallachian breed.

The objective of this study was to evaluate the effect of the stage of lactation on milk composition, its properties and quality of rennet curdling in East Friesian ewes and to determine phenotypic

correlations between tested variables. Attention was also paid to evaluating the effect of the stage of lactation on daily milk yield (DMY).

MATERIAL AND METHODS

The evaluation of the effect of the stage of lactation on milk composition, its properties and quality of rennet curdling was carried out over the period of three successive years using milk samples ($n = 162$) obtained from a total of 27 ewes of the EF breed, reared on a small sheep farm in Juřinka in the Wallachian region. The age of ewes varied from 2 to 7 years. In all years under study, lambing occurred indoors and during March. After lambing, the ewes with lambs were reared indoors until the end of April. In all years under study, the weaning of lambs was carried out during the last ten days of April. In the period from lambing until the end of April, the daily feed ration of ewes consisted of 1 kg fodder beet, 0.5 kg of concentrate mixture (50% barley and 50% wheat), meadow hay (*ad libitum*) and mineral lick (*ad libitum*). From the beginning of May until the end of lactation, the main part of the daily feed ration of ewes was *ad libitum* grazing on the permanent pasture, supplemented with 1 kg of meadow hay, mineral lick (*ad libitum*) and 0.25 kg of the aforementioned concentrate mixture. The natural mating of ewes with rams (the so-called harem system) was used during the whole study period. Different rams were used each year. During the whole period under study, all ewes were reared in one group under identical conditions and without any principal differences in nutrition and management. All ewes were in good body condition and clinically healthy (including their udders) during the whole period under study.

Milk records and samplings were carried out six times each year under study whereas the first milk record and sampling was carried out on the average 33rd day of lactation. The following milk records and samplings were carried out on the average 67th, 95th, 129th, 158th and 191st day of lactation. Ewes were milked by hand twice a day and their milking was finished on the average 216th day of lactation. Milk records and sampling were carried out during the morning (7 a.m.) milking. Milk yield was also recorded during the evening (7 p.m.) milking. In the period up to the weaning of lambs, before each milk record and sampling, the lambs were separated from their mothers 12 hours prior to the morn-

Table 1. Evaluation of RCQ

Category	Appearance and firmness of curd and appearance of whey
1	very good and hard curd, keeping its shape after its removal from the container; whey is clear, of yellow-greenish colour
2	good but a little softer curd, not keeping its shape quite perfectly; excretion of whey not perfect; whey is greenish
3	not good, soft curd, partly not keeping its shape; milky white whey
4	very bad curd, not keeping its shape; milky white whey
5	very weak or invisible flocculation of casein

ing milking. After the evening milk recording, the lambs were allowed back to their mothers.

Milk samples were not conserved, but after milking, all samples were cooled to 5 to 8°C and transported in a thermo-box to a specialised milk laboratory at the Mendel University of Agriculture and Forestry in Brno. Analyses always started within 4 hours from sampling. As part of the laboratory analysis, the following milk components were determined: total solids (TS), solids non-fat (SNF), fat (F) and lactose (L), N-compounds (protein (P), casein (CN) and urea nitrogen (UN)) and calcium (Ca). The following milk properties were determined: pH, titratable acidity (TA), specific gravity (SG) and rennet clotting time (RCT). The evaluation of rennet curdling quality (RCQ) and daily milk yield (DMY) in the course of lactation was also an integral part of this study. TS content (%) was determined gravimetrically, by oven drying at 102°C to constant weight (Czech State Standard ISO No. 6731, 1998). F content (%) was determined by Gerber's acidobutyrometric method (Czech State Standard ISO No. 2446, 2001). SNF content (%) was computed by subtracting milk fat content from TS content. P and CN contents (%) were determined using a PRO-MILK apparatus (manufactured by the Danish Co. Foss Electric; Czech State Standard No. 570530, 1974). Ca content in milk (g/l) was determined by complexometric titration with fluorexone as an indicator (Czech State Standard No. 570530, 1974). L content (%) was determined polarimetrically (Czech State Standard No. 570530, 1974). UN content (mg/100 ml) was measured spectrophotometrically using the method with *p*-dimethylamino-benzaldehyde (Gajdůšek et al., 1996). Active acidity (pH) was measured with the pH-meter WTW 95 with the electrode WTW SenTix 97 (Czech State Standard No. 570530, 1974). Titratable acidity (SH) was determined by titration using the Soxhlet–Henkel method

(Czech State Standard No. 570530, 1974). Specific gravity (g/cm³) was measured lactodensimetrically (Czech State Standard No. 570530, 1974). The rennet clotting time (RCT) was the time period (in seconds) between the application of 2 ml of the rennet solution to 100 ml of the milk which was tempered at 35°C and the aggregation of milk. The rennet solution was obtained by diluting 5 ml of Fromase[®]750 TL (DSM Food Specialties, Netherlands) to 95 ml of distilled water. Fromase[®]750 TL is a liquid microbial coagulant preparation derived from a selected strain of *Rhizomucor miehe*. The renneted milk was placed for 1 hour in a thermostat at 35°C. Next, the curd was tipped out into Petri dish and the RCQ was evaluated by a score description according to the appearance and firmness of the curd and the appearance of the whey (Table 1). The DMY was determined by weighing. Weighing was carried out to the nearest 0.1 kg.

Data were subjected to a four-way analysis of variance (ANOVA) using the SAS for Windows v. 9.1 (SAS, 2005) statistical package. This mathematical model was used:

$$Y_{ijkl} = \mu + R_i + L_j + S_k + T_l + e_{ijkl}$$

where:

- μ = overall mean
- R_i = fixed effect of the year of study ($i = 1, 2$ and 3)
- L_j = fixed effect of the number of lactation ($j = 1, \dots, 6$)
- S_k = fixed effect of litter size ($k = 1$ and 2)
- T_l = fixed effect of sampling time ($l = 1, \dots, 6$)
- e_{ijkl} = random residual error

Statistically significant effects were further analyzed and the means were compared using Sheffe's multiple range test. The null hypothesis was tested: the means for each dependent variable did not differ between the six sampling times. Correlations between variables were calculated using the CORR (Pearson) procedure of SAS.

RESULTS AND DISCUSSION

The effect of the stage of lactation on the contents of all milk components under study and on the daily milk yield is summarised in Table 2. The stage of lactation had a highly significant effect on all milk components, however only the contents of TS, SNF, F, P and CN increased gradually with the advancement of lactation. The above-mentioned tendency concerning the contents of TS, SNF, F and P is in line with the results published by Casoli et al. (1989), Gonzalo et al. (1994), Čapistrák et al. (1995), Fuertes et al. (1998) and Ploumi et al. (1998). However, Jelínek et al. (1990) and Hassan (1995) reported that during the first 2–5 weeks of lactation the contents of the above-mentioned components decreased as a result of the increased DMY in this period. The contents of TS, F and P increased depending on the day of lactation from 15.59% to 20.68%, from 4.96% to 7.80%, and from 4.69% to 6.66% respectively, while this data is comparable to that published by Čapistrák et al. (1995), Jandal (1996) and Sahan et al. (2005). On the other hand, Hassan (1995) reported markedly higher contents of TS and F at the end of lactation. The CN content, which markedly influences the RCT of milk (Gajdůšek and Jelínek, 1992), increased during lactation from 3.35% to 4.94%. A similar trend and comparable CN contents were reported by Jelínek et al. (1990) and Pugliese et al. (2000). DMY gradually decreased depending on the stage of lactation, which is in line with the results published by Hassan (1995) and Ochoa-Cordero et al. (2002). The lactose content in the course of lactation was relatively the most constant of all components of milk under study, confirming its role as an osmotic regulator and a compensator for variations in all other components. The highest contents of lactose were found on the 33rd and the 67th day of lactation and at the end of lactation. On the contrary, its lowest content was found on the 129th day of lactation. This tendency is in line with the results published by Ochoa-Cordero et al. (2002). On the other hand, Pavič et al. (2002) reported the highest L content at the beginning of lactation and the lowest at the end of lactation. The contents of Ca varied from 1.81 g/l to 2.14 g/l depending on the day of lactation. The highest content of Ca was found at the end of lactation. However, relatively high contents of this mineral were also found on the 33rd and 67th day of lactation. On the other hand, the lowest Ca content was found in the middle of lacta-

tion. A similar fluctuation in Ca content was also reported by Sahan et al. (2005). However, Sahan et al. (2005), and Polychroniadov and Vafopoulou (1985) reported markedly higher Ca contents in the course of lactation. The urea nitrogen contents were in the range between 15.22 mg/100 ml and 25.31 mg/100 ml. In the period from 67th to the 129th day of lactation a gradual increase in UN contents was found. However, thereafter it continued to fall gradually until the end of lactation. A similar tendency and approximately the same urea nitrogen contents during the lactation were observed in goat milk by Kuchtík and Sedláčková (2003).

The effect of the stage of lactation on milk properties and RCQ is summarised in Table 3. The stage of lactation had a highly significant ($P \leq 0.01$) effect both on all milk properties under study and on RCQ. The pH of milk is influenced by hygienic and climatic conditions, and slightly higher acidity is typical of sheep milk compared to cow milk (Pavič et al., 2002). Martini and Caroli (2003) reported that the pH of milk was significantly influenced by the breed of sheep. In our study, the pH of milk gradually decreased between the 33rd and 129th day of lactation. However, thereafter the pH rose gradually until the end of lactation. A similar tendency and similar values of pH were reported by Pugliese et al. (2000). On the other hand, Pavič et al. (2002) observed a gradually increasing trend in pH values during lactation. In comparison with pH values, the values of TA had an opposite tendency. This means that its value gradually increased until the 129th day of lactation and thereafter the TA fell gradually until the end of lactation. On the other hand, Boroš et al. (1985) and Jelínek et al. (1990) reported that the TA values of ewe's milk gradually increased during lactation, whereas Sahan et al. (2005) reported that the TA of milk in Awassi ewes decreased during lactation and reached its lowest values in the final week of lactation. The specific gravity (SG) decreased slightly until the 129th day of lactation. However, thereafter the opposite trend was found and its highest value was recorded at the end of lactation. On the other hand, Voutsinas et al. (1988) and Sahan et al. (2005) reported that the SG of sheep milk decreased during the first six or seven weeks of lactation. However, thereafter its values remained relatively stable until the end of lactation. Sheep milk contains more F, SNF, P, CN, whey proteins and total ash compared to goat and cow milk and these differences make the rennet clotting time (RCT) for sheep milk shorter (Jandal,

Table 2. The effect of the stage of lactation on the contents of milk components and daily milk yield

Characteristic	Day of lactation							F-test
	33	67	95	129	158	191		
Total solids (%)	mean	15.59 ^A	16.41 ^B	17.42 ^C	18.10 ^C	19.04 ^D	20.68 ^E	162.13**
	S.E.	0.22	0.13	0.23	0.12	0.11	0.15	
SNF (%)	mean	10.64 ^A	11.22 ^B	11.36 ^B	11.39 ^B	12.07 ^C	12.88 ^D	85.20**
	S.E.	0.12	0.10	0.11	0.07	0.07	0.13	
Fat (%)	mean	4.96 ^A	5.19 ^A	6.07 ^B	6.71 ^C	6.97 ^C	7.80 ^D	125.30**
	S.E.	0.14	0.11	0.15	0.08	0.09	0.09	
Protein (%)	mean	4.69 ^A	5.23 ^{ab}	5.55 ^{bBC}	5.90 ^C	6.28 ^D	6.66 ^E	121.44**
	S.E.	0.08	0.07	0.09	0.06	0.05	0.10	
Casein (%)	mean	3.35 ^{aA}	3.66 ^{bA}	4.01 ^B	4.21 ^B	4.61 ^C	4.94 ^C	97.99**
	S.E.	0.05	0.07	0.06	0.07	0.06	0.08	
Ca (g/l)	mean	2.02 ^{AC}	2.00 ^{aABC}	1.81 ^{bBC}	1.85 ^{BC}	1.81 ^B	2.14 ^A	12.93**
	S.E.	0.35	0.22	0.30	0.29	0.52	0.55	
Lactose (%)	mean	4.87 ^{AC}	4.98 ^{AC}	4.79 ^{AC}	4.43 ^{ab}	4.68 ^{bBC}	5.00 ^A	15.80**
	S.E.	0.06	0.03	0.05	0.05	0.05	0.08	
Urea nitrogen (mg/100 ml)	mean	15.34 ^A	15.22 ^A	21.74 ^{ab}	25.31 ^{bb}	23.25 ^B	21.94 ^B	35.56**
	S.E.	0.53	0.68	0.63	1.32	0.53	0.64	
Daily milk yield (kg)	mean	1.19 ^A	1.16 ^{aA}	1.01 ^{bA}	0.82 ^{cb}	0.64 ^{dB}	0.42 ^C	91.48**
	S.E.	0.04	0.04	0.04	0.03	0.03	0.02	

the values in the same line marked with different letters (a–d) and (A–E) differ significantly ($P \leq 0.05$) and highly significantly ($P \leq 0.01$), respectively

Table 3. The effect of the stage of lactation on milk properties and rennet curdling quality

Characteristic	Day of lactation							F-test
	33	67	95	129	158	191		
pH								
mean	6.65 ^{AB}	6.61 ^{AB}	6.51 ^B	6.26 ^C	6.62 ^{AB}	6.76 ^A	24.63 ^{**}	
S.E.	0.02	0.02	0.02	0.06	0.03	0.04		
Titration acidity (°SH)								
mean	7.24 ^A	8.33 ^{AD}	9.80 ^B	11.88 ^C	9.44 ^{BD}	8.93 ^{BD}	38.82 ^{**}	
S.E.	0.21	0.13	0.25	0.12	0.11	0.15		
Specific gravity (g/cm ³)								
mean	1.036 ^{ab}	1.035 ^{ab}	1.035 ^{ab}	1.033 ^b	1.034 ^{ab}	1.036 ^a	3.79 ^{**}	
S.E.	0.0001	0.0003	0.0004	0.0003	0.0003	0.001		
Rennet clotting time (s)								
mean	113 ^{AB}	123 ^A	78 ^B	78 ^B	112 ^{AB}	107 ^{AB}	6.12 ^{**}	
S.E.	8.55	5.54	6.99	7.31	8.98	9.46		
Rennet curdling quality								
mean	1.93 ^a	1.70 ^{ab}	1.41 ^{ab}	1.26 ^b	1.82 ^{ab}	1.63 ^{ab}	3.89 ^{**}	
S.E.	0.16	0.13	0.11	0.10	0.13	0.12		

the values in the same line marked with different letters (a, b) and (A–D) differ significantly ($P \leq 0.05$) and highly significantly ($P \leq 0.01$), respectively

Table 4. Phenotypic correlations of all characteristics of the study

Characteristic	SNF	Fat	Protein	Casein	Ca	Lactose	UN	pH	TA	SG	RCT	RCQ	Milk yield
Total solids	0.900 ^{***}	0.942 ^{***}	0.902 ^{***}	0.847 ^{***}	0.149	0.152	0.497 ^{***}	0.112	0.271 ^{**}	0.050	0.042	0.002	-0.754 ^{***}
SNF		0.703 ^{***}	0.904 ^{***}	0.834 ^{***}	0.189 [*]	0.360 ^{***}	0.353 ^{***}	0.239 ^{**}	0.120	0.096	0.173 [*]	0.047	-0.645 ^{***}
Fat			0.780 ^{***}	0.741 ^{***}	0.096	-0.029	0.543 ^{***}	0.001	0.349 ^{***}	0.006	-0.059	-0.030	-0.737 ^{***}
Protein				0.915 ^{***}	0.087	-0.033	0.474 ^{***}	0.118	0.293 ^{***}	-0.051	0.089	-0.004	-0.682 ^{***}
Casein					0.101	-0.066	0.398 ^{***}	0.091	0.261 ^{**}	-0.055	-0.025	-0.099	-0.679 ^{***}
Ca						0.157 [*]	-0.080	0.094	-0.103	0.332 ^{***}	0.080	0.089	-0.022
Lactose							-0.167 [*]	0.294 ^{***}	-0.337 ^{***}	0.305 ^{***}	0.270 ^{***}	0.179 [*]	0.032
UN								-0.311 ^{***}	0.565 ^{***}	0.044	-0.090	-0.059	-0.356 ^{***}
pH									-0.685 ^{***}	0.021	0.477 ^{***}	0.388 ^{***}	-0.136
TA										-0.014	-0.451 ^{***}	-0.402 ^{***}	-0.171 [*]
SG											-0.044	0.001	0.145
RCT												0.681 ^{***}	-0.003
RCQ													0.054

*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; UN = urea nitrogen; TA = titratable acidity; SG = specific gravity; RCT = rennet clotting time; RCQ = rennet curdling quality

1996). Martini and Caroli (2003) reported that the RCT of sheep milk was affected by the breed of sheep due to the differences in pH values of milk between breeds. The rennet clotting time (RCT) ranged from 78 to 123 s and the longest RCTs were found on the 33rd and 67th day of lactation. On the other hand, the shortest RCTs were found in the middle of lactation. The RCTs were relatively stable at the end of lactation and ranged from 112 to 107 s. A similar tendency was reported by Pugliese et al. (2000). On the other hand, Jelínek et al. (1990) reported that from the 90th day of lactation the RCT of milk in Tsigai ewes gradually decreased till the end of lactation. The rennet curdling quality (RCQ) was relatively good and uniform in the course of lactation. However, the worst RCQ was found in the first sampling. On the other hand, the best RCQ was found in the middle of lactation (95th and 129th day). To conclude the above, it should be added that the change of feed ration between the first and second sampling did not have a significant effect on the contents of F, Ca, L and UN and on DMY. Neither did this change have a significant effect on all milk properties under study or on RCQ.

The phenotypic correlations of all characteristics of the study are summarized in Table 4. All phenotypic correlations between particular contents of TS, SNE, F, P, CN and UN were positive and high ($P \leq 0.001$). Pavič et al. (2002) also reported that the contents of TS, SNE, F and P were highly and positively correlated with each other. All phenotypic correlations between DMY and particular contents of TS, SNE, F, P, CN and UN were negative and high ($P \leq 0.001$). Maria and Gabina (1993) and Ochoa-Cordero et al. (2002) also reported that the correlations between milk yield and the particular contents of F and P were negative and high. The majority of phenotypic correlations between L content and particular contents of other milk components were not significant, which is in line with the results published by Čapistrák et al. (1995). On the other hand, Ochoa-Cordero et al. (2002) reported that the correlations between L content and the particular contents of TS, F and P were negative and high. The majority of phenotypic correlations between RCT and the other parameters of the study were not significant. However, the phenotypic correlation between L and RCT was positive and high ($P \leq 0.001$), which means that the higher the L content, the longer the RCT. The correlation between pH and RCT was also positive and high ($P \leq 0.001$), which means that the higher

the pH, the longer the RCT. On the contrary, the phenotypic correlation between TA and RCT was negative and high ($P \leq 0.001$), which means that the higher the TA, the shorter the RCT. The majority of phenotypic correlations between RCQ and the other parameters of the study were not significant. However, the phenotypic correlations between pH and RCQ were positive and high ($P \leq 0.001$), which means that the higher the pH, the worse the RCQ. The phenotypic correlation between RCT and RCQ was also positive and high ($P \leq 0.001$), which means that the longer the RCT, the worse the RCQ. On the other hand, the phenotypic correlation between TA and RCQ was negative and high ($P \leq 0.001$), which means that the higher the TA, the better the RCQ. Besides the above-mentioned positive and high correlations concerning pH, there were also positive and high ($P \leq 0.001$) phenotypic correlations between pH and SNF and between pH and L. On the other hand, the phenotypic correlations between pH and U and between pH and TA were both negative and high ($P \leq 0.001$). Pavič et al. (2002) also reported that the correlation between pH and TA was negative and high. Besides the above-mentioned highly significant correlations concerning TA, there were also positive and high ($P \leq 0.01$ and $P \leq 0.001$) phenotypic correlations between TA and the individual contents of TS, F, P, CN and UN. On the other hand, the phenotypic correlation between L and TA was found to be negative and high ($P \leq 0.001$). However, Pavič et al. (2002) reported that the correlation between these parameters was positive and high.

CONCLUSIONS

The stage of lactation had a highly significant effect on the contents of all milk components under study. The stage of lactation also had a highly significant effect on all milk properties under study and on RCQ. The contents of TS, SNE, F, P, CN and UN were highly and positively correlated with each other. On the other hand, all phenotypic correlations between milk yield and the particular contents of TS, SNE, F, P, CN and UN were negative and high ($P \leq 0.001$). The majority of phenotypic correlations between RCT and the other parameters was not significant. The majority of phenotypic correlations between RCQ and the other parameters was not significant either. However, RCQ was above all negatively influenced by an increase in

the pH of milk and by prolongation of the clotting time. On the other hand, the higher the TA, the better the RCQ.

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