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## The influence of pasturable and stall period diets of dairy cows on the electrical conductivity of milk

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## Abstract

The aim of this study was to analyse the influence of dairy cows' diet on electrical conductivity (EC) of milk during pasturable and stall periods and to assess EC relationship with milk yield and somatic cell count (SCC). The experiments were carried out during 2009–2013 with 421 dairy cows which were milked using an automated voluntary milking system "DeLaval VMS" ("DeLaval", Sweden). All year round the cows were kept untied indoors and fed on farm-made grass silage forage, during pasturable period the diet was supplemented with a fresh legume/ grass mixture. The data consisted of 462582 milking records. The average EC of milk  $(4.92 \pm 0.001 \text{ mS cm}^{-1})$  met zootechnical standards. The average milk yield was  $25.12 \pm 0.020 \text{ kg}$ , milk SCC  $- 188.95 \pm 1.032$  thousand ml<sup>-1</sup>. Milk yield during pasturable period was in average 1.4% lower and SCC was 7.9% higher than during the stall period. The average EC during pasturable period of the experiment was 3.6% higher than during the stall period in all lactations (p < 0.001). The higher (23.03 g) cows' ruminal N-balance (RNB) during pasturable period had no positive effect on milk yield as SCC increased during that period. The correlation between milk yield and EC of milk during pasturable and stall periods was negative (r = -0.124--0.226, p < 0.001). The observed SCC was lower than 200 thousand ml<sup>-1</sup> in the milk samples where EC was in the range of 4.6 to 5.8 mS cm<sup>-1</sup>. As EC increased, SCC had a statistically significant (p < 0.001) rise both in pasturable and stall periods (r = 0.356-0.403). EC of milk could provide an alternative way for early diagnosis of mastitis.

Key words: correlation, forage, milk yield, somatic cell count.

## Introduction

Milk production is one of the most complicated agricultural industries. The main factors governing the cost-effectiveness of milk production are the genetic potential of livestock, feed and nutrition, and animal keeping technologies (Juozaitienė, Žakas, 2002; Moussaoui et al., 2004). Milk production and quality of milk are greatly influenced by the diet and feeding techniques. These factors account for approximately 50–60% of the dairy farm costs (Mockevičienė et al., 2008).

Bovine mastitis remains as the disease causing the biggest economic losses to the dairy industry (Seegers et al., 2003; Moussaoui et al., 2004). De Mol and Ouweltjes (2001) indicated that early detection of mastitis is very important, not only because of the economic impact due to yield losses, but also because of the negative effects on the animals' welfare. Some authors state that somatic cell count (SCC), which is widely regarded as mastitis indicator, depends on time of the year (Riekerink et al., 2007; Kondrotaitė et al., 2013). Riekerink et al. (2007) indicate that the highest SCC is observed in late summer and early autumn. Green et al. (2006) state that the increase of SCC in the early summer months is influenced by the cows with subclinical

mastitis. The immune system of cows after the winter season is very low and with the start of pasturable season and increased productivity the incidence of mastitis starts to grow (Klimienė et al., 2005). The seasonal fluctuation of SCC is caused by seasonal mastitis, overheat, cold and contusion of the udder tissue (Juozaitienė, Žakas, 2002), thus cows require properly balanced diets during this time of the year. Nutrition can influence the cow's resistance to mastitis. The major impact of nutrition on udder health is via suppression of the immune system.

Electrical conductivity (EC) of milk was introduced as an indicator of mastitis several decades ago. Until now EC has solely been used for detection of bovine mastitis on the phenotypic level. However, EC may be used as an indicator of mastitis in a breeding program where selection against mastitis is included. SCC has been widely considered to be the most useful indirect measure of mastitis. However, using SCC for genetic evaluation has some disadvantages such as low recording frequency. Most automatic milking systems are equipped with sensors for measuring EC, and EC can be easily recorded at every milking. Genetic correlations between EC and mastitis have been estimated to be in the

range from 0.65 to 0.8, hence, obtaining genetic response for mastitis should be possible by using information of EC in genetic evaluation. However, collecting and implementing EC information in a breeding program may be a challenge (Norberg, 2005).

Modern automatic milking systems can register EC data relatively cheaply and rapidly. Musser et al. (1998) state that absolute and differential EC scores for cows with subclinical mastitis are significantly higher than those for healthy cows, and absolute EC score is significantly associated with detection of the disease. Various studies show positive correlation between EC and SCC, which can be used as mastitis indicators (Musser et al., 1998; Norberg, 2005). Modern dairy farms use these indicators for electronic udder health status control. Milk SCC and EC were associated with daily production loss; the effects were additive. Therefore, EC and SCC can be utilized as indirect tests of subclinical mastitis (Nielen et al., 1993).

The aim of this experiment was to investigate the potential influence of cows' diets during pasturable and stall periods on EC of milk, milk yield and SCC in dairy herds and to analyse the relationship among these traits.

## Materials and methods

The study was carried out during 2009–2013 at Veterinary Academy of Lithuanian University of Health Sciences, state owned company Agriculture Information and Rural Business Centre and three high production milk farms in Lithuania. Data from a total of 421 dairy cows which were milked using automated voluntary milking system "DeLaval VMS" ("DeLaval", Sweden) were collected for the period from June 2008 to November 2011. The data consisted of milk yield, electrical conductivity (EC) of milk and somatic cell count (SCC), a total of 462582 milking records were evaluated and analysed.

All year round the test cows were kept untied indoors and fed on farm-made grass silage forage. Periods

taken into consideration in the study are pasturable period (from 15<sup>th</sup> May till 15<sup>th</sup> October) and stall period (from 15<sup>th</sup> October till 15<sup>th</sup> May). The combined feed had identical nutritional and energy values during pasturable and stall periods. During pasturable period the diet was supplemented with a fresh legume/grass mixture. The diets were optimised using computer feed consultancy programme *HYBRIMIN Futter 2008*. The feed intake was controlled by weighing the feed given to the cows in the automated milking system. RNB was calculated using the formula (Weiss et al., 2005):

RNB = (CP - uCP) : 6.25, where RNB is ruminal N-balance, CP – crude protein, uCP – usable crude protein.

Statistical analysis. The data of milk yield, EC of milk and SCC were processed using MS SQL Server 2005 database management system and R statistical package (http://www.r-project.org) was used for statistical analysis. Arithmetical averages ( $\overline{X}$ ), standard errors ( $\pm m\overline{X}$ ), average square deviation (SD) and coefficients of variation (CV) were calculated. The data of SCC were log transformed to base 10. This logarithmic transformation achieved normality of distribution.

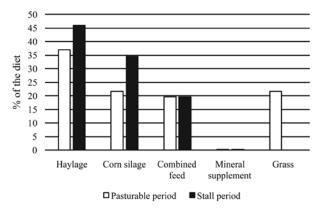
## **Results**

The average composition of cows' diet is displayed in Table 1. Both during pasturable and stall periods the test cows were fed diets that contained 18.6 kg dry matter, net energy of lactation was almost identical (stall period diet had 1.44 MJ more energy). During pasturable period the diet was enriched by adding a fresh legume/grass mixture. According to results of our research, the diet during pasturable period had 159 g more crude protein, as well as usable crude protein – 0.56% more than during stall period. The comparison of diet composition also shows that RNB was higher by 23.03 g in the diet of pasturable period in comparison with the stall period diet.

*Table 1.* Dairy cows' diets in pasturable (15<sup>th</sup> May – 15<sup>th</sup> October) and stall (15<sup>th</sup> October – 15<sup>th</sup> May) periods and their differences

Diet	Unit	Pasturable period	Stall period	Difference between diets in pasturable and stall periods	
				unit	%
Haylage	kg	17	20	-3	-15
Corn silage	kg	10	15	-5	-33.33
Combined feed	kg	9	8.56	0.44	5.14
Mineral supplement	kg	0.007	0.01	-0.003	-30
Grass	kg	10	0	10	-
Composition of the diet:					
dry matter	kg	18.6	18.6	0	0
net energy of lactation	MJ	116.16	117.6	-1.44	-1.22
crude protein	g	3025	2866	159	5.55
crude fiber	kg	3.81	3.78	0.03	0.79
usable crude protein	g	2713	2698	15	0.56
Ruminal N-balance	g	50.02	26.99	23.03	85.33
calcium	g	137.94	131.37	6.57	5
phosphorus	g	72.5	69.92	2.58	3.69
calcium:phosphorus	ratio	1.90:1	1.88:1	0.02:1	1.06

The composition of diets during pasturable and stall periods is displayed in Figure. During stall period the haylage accounted for 45.9% of the diet, which was 8.94% more than during pasturable period. Stall period diet also had more corn silage – 34.43% of the whole diet compared to 21.73% of the diet during pasturable period (12.7% less). The amount of combined feed was similar both during pasturable and stall periods, and accounted for 19.56% and 19.65% of the diets, respectively. The cows were fed fresh grass only during pasturable period and it accounted for 21.73% of the diet. The diet of stall period contained 3.78 kg of crude fiber, 2.488 kg of structural fiber, neutral detergent fiber (NDF) made up 31.92% of dry matter (DM), acid detergent fiber (ADF) - 18.31% of DM. Pasturable period diet had a slightly higher content of crude fiber – 3.81 kg, structural fiber was almost identical - 2.39 kg. NDF and ADF content in DM was by 4.92 and 3.31% lower, respectively than during pasturable period.



*Figure.* Composition of dairy cow diets during pasturable (15<sup>th</sup> May – 15<sup>th</sup> October) and stall (15<sup>th</sup> October – 15<sup>th</sup> May) periods

Analysis of milking system data showed that the average EC of milk  $(4.92 \pm 0.001 \text{ mS cm}^{-1})$  and variation (13.02%) for the total period of our study met zootechnical standards. The data of milk EC for each month of year are displayed in Table 2. As the data in the table suggest the highest EC ranging from 5.03 to 5.11 mS cm<sup>-1</sup> was recorded in the summer months, while the lowest EC from 4.73 to 4.81 mS cm<sup>-1</sup> was recorded in the winter months. All the results when compared to preceding months were statistically significant (p < 0.001) with the variation coefficients ranging from 11.98 to 13.81.

According to Table 3, during the pasturable period EC of milk was by 0.18 mS cm<sup>-1</sup> higher than during the stall period (p < 0.001). The average EC of milk during both periods met zootechnical standards and variation was similar.

When analysed by lactation number during pasturable and stall periods (Table 4), the average EC of milk showed similar trend. During pasturable period all the lactations recorded higher EC values than during stall period, ranging from 0.01 mS cm<sup>-1</sup> in the third lactation cows' milk to 0.29 mS cm<sup>-1</sup> in first lactation (p < 0.001). With increasing number of lactations EC of milk showed an upward trend rising from 4.91 to 5.40 mS cm<sup>-1</sup> (9.98%) in pasturable period and from 4.91 to 5.40 mS cm<sup>-1</sup> (15.58%) in stall period.

*Table 2.* Electrical conductivity (EC) data for each month of year (average data for June 2008 – November 2013)

Month	EC of milk, mS cm <sup>-1</sup>					
	n	$\overline{X}$	$\pm m\overline{X}$	SD	CV	
January	36150	4.73***	0.003	0.63	13.25	
February	34104	4.81***	0.003	0.62	12.86	
March	40055	4.84***	0.003	0.62	12.89	
April	38862	4.90***	0.003	0.63	12.88	
May	41016	5.02***	0.003	0.63	12.53	
June	37092	5.05***	0.003	0.63	12.52	
July	38614	5.11***	0.003	0.61	11.98	
August	40582	5.03***	0.003	0.69	13.81	
September	39079	4.98***	0.003	0.64	12.83	
October	41787	4.85***	0.003	0.65	13.40	
November	39208	4.91***	0.003	0.59	12.06	
December	36025	4.79***	0.003	0.61	12.67	
Total / mean	462574	4.92	0.001	0.64	13.02	

*Note.* n – number of records evaluated,  $\overline{X}$  – arithmetical average,  $\pm m\overline{X}$  – standard error, SD – average square deviation, CV – coefficient of variation; \*\*\* – differences are statistically significant at p < 0.001 as compared to the preceding month.

*Table 3.* Electrical conductivity (EC) of milk during pasturable (15<sup>th</sup> May – 15<sup>th</sup> October) and stall (15<sup>th</sup> October – 15<sup>th</sup> May) periods (average data for June 2008 – November 2013)

Period	EC of milk, mS cm <sup>-1</sup>					
	n	$\overline{X}$	$\pm m\overline{X}$	SD	CV	
Pasturable period	238170	5.01	0.001	0.65	12.97	
Stall period	224404	4.83***	0.001	0.62	12.83	

Explanations under Table 2; \*\*\* – differences are statistically significant at p < 0.001 as compared to the pasturable period

**Table 4.** Electrical conductivity (EC) data during pasturable (15<sup>th</sup> May – 15<sup>th</sup> October) and stall (15<sup>th</sup> October – 15<sup>th</sup> May) periods by lactation number (average data for June 2008 – November 2013)

		EC of milk, mS cm <sup>-1</sup>						
Period	EC 01 IIIIK, m5 cm							
renod	n	$\overline{X}$	$\pmm\overline{X}$	SD	CV			
1st lactation								
Pasturable period	94349	4.91	0.002	0.59	12.09			
Stall period	105802	4.62***	0.002	0.54	11.60			
2 <sup>nd</sup> lactation								
Pasturable period	72713	5.00	0.002	0.62	12.49			
Stall period	62529	4.92***	0.002	0.60	12.11			
3 <sup>rd</sup> lactation								
Pasturable period	27962	5.20	0.004	0.73	13.97			
Stall period	20269	5.19***	0.005	0.67	12.92			
4 <sup>th</sup> and subsequent lactations								
Pasturable period	23098	5.40	0.004	0.65	11.94			
Stall period	22062	5.34***	0.004	0.53	9.86			
Explanations under Table 2								

Analysis of the dairy cows' diets shows that both during pasturable and stall periods their composition was balanced according to nutrient levels. Diets also met the dairy cow nutrition physiological rates by crude and structural fiber content. Average milk yield in all three dairy farms during the experiment was 25.12  $\pm$  $0.020 \text{ kg day}^{-1}$ , SCC averaged at  $188.95 \pm 1.032$  thousand ml<sup>-1</sup>. As the data in Table 5 show, averge milk yield during pasturable period was 1.4% lower than during stall period (p < 0.001). Average milk SCC during pasturable period was 7.9% higher than that of stall period (p < 0.001). The correlation between milk yield and EC of milk was slightly negative, r = -0.124 and r = -0.226 during pasturable and stall periods, respectively; however, the correlation was highly significant (p < 0.001). During stall period the correlation between those parameters was 1.8 times stronger in comparison with pasturable period. When evaluated over the total period of this study, the correlation between these parameters was also statistically significant negative (r = -0.168, p < 0.001).

*Table 5.* Milk yield, somatic cell count (SCC) and their correlation with electrical conductivity (EC) of milk during pasturable (15<sup>th</sup> May – 15<sup>th</sup> October) and stall (15<sup>th</sup> October – 15<sup>th</sup> May) periods (average data for June 2008 – November 2013)

	Milk yield	l, kg day-1	SCC, thousand ml <sup>-1</sup>				
Period	$\overline{X}$	$\pm m\overline{X}$	$\overline{X}$	$\pm m\overline{X}$			
Pasturable period	24.92	0.028	196.56	1.531			
Stall period	25.28***	0.029	183.12***	1.266			
Average	25.12	0.020	188.95	1.032			
Correlation coefficients with EC							
Pasturable period	-0.124***		0.356***				
Stall period	-0.226***		0.403***				
Total study period	-0.168***		0.379***				

Explanations under Table 2

Our study results show that SCC did not exceed 200 thousand ml<sup>-1</sup> in milk samples where EC was in the range from 4.6 to 5.8 mS cm<sup>-1</sup>. The correlation coefficient between EC and SCC had similar values both during pasturable and stall periods, 0.356 and 0.403 (p < 0.001) respectively, the relationship being slightly higher during the stall period. When evaluated over the whole study period, with the increase of EC of milk, SCC also had a statistically significant rise (r = 0.379, p < 0.001).

## Discussion

Our experiment, carried out in three milk farms around Lithuania, shows that both during pasturable and stall periods cows were fed balanced diets, but the pasturable period diet contained 5.55% more crude protein and 0.56% more usable crude protein. In addition, RNB was 85.33% higher during pasturable period. Diets also met the dairy cow nutrition physiological rates by crude and structural fiber content.

Microbes in the cow's rumen break down rumen degradable protein and non-protein nitrogen into amino acids and ammonia to build more microbial protein. However in high production milk farms, due to deficient or excess nitrogen, rumen microbes are unable to produce enough amino acids to meet the cow's productivity. Therefore, maintaining a healthy RNB is essential for keeping the rumen functioning efficiently (Weiss et al., 2005). Proportionate RNB setting ensures that no shortage of nitrogen is experienced in the rumen (von Lengerken, 2004). When composing diets, RNB setting should be positive (above zero). The best setting for better milk yields is when RNB is in the rage of 20-50 g, otherwise crude proteins tend to lose their "efficiency". Positive RNB setting has a positive impact on the synthesis of rumen microbial proteins and overall fermentation processes in the rumen. However, nitrogen overload should also be avoided, because it undermines the quality of milk, harms animals and pollutes the environment (Mikulionienė, Stankevičius, 2002). In our study during the pasturable and stall periods, corn silage accounted for 21.7% and 34.4% of the diet, respectively. It was shown that microbial inoculants can have a positive effect on silage characteristics in terms of lower ammonia-N concentrations and reduced dry matter losses (Jatkauskas, Vrotniakienė, 2011). Negative RNB balance is an indicator that the ratio of digestible to non digestible proteins should be adjusted in the diet. When cows are fed grass silage and cereal flour in winter and fresh grass and the same concentrates in summer, the common problem is the excess of digestible protein. In Lithuania, the main protein components in combined forage are soybean, canola or sunflower wholegrain and squeeze. Their crude protein digestibility is 70-80%, and the usage of low digestibility protein is not economically efficient, their thermal or chemical processing lowers the assimilation of amino acids in the rumen (Juozaitienė et al., 2004).

In our study we found that the average EC of milk  $(4.92 \pm 0.001 \text{ mS cm}^{-1})$  was consistent with that reported by other authors. Hamann and Zecconi (1998) presented values between 4.0 to 5.5 mS cm<sup>-1</sup> for healthy and 5.0 and 9.0 mS cm<sup>-1</sup> for clinically infected cows from several experiments. Juozaitienė et al. (2010) found that the average EC of milk from Lithuanian Black cow population was  $6.30 \pm 0.09$  mS cm<sup>-1</sup>. Norberg et al. (2004) state that the average EC of Danish cattle milk ranged from 5.5 to 6.5 mS cm<sup>-1</sup>. Average EC from healthy udder quarters was 4.87 mS cm<sup>-1</sup>, cows diagnosed with subclinical and clinical forms of mastitis showed EC of 5.37 and 6.44 mS cm<sup>-1</sup>, respectively. Other authors (Hillerton, Walton, 1991; Hamann, Gyodi, 2000) indicate that milk EC from healthy cows ranges from 4.0 to 5.5 mS cm<sup>-1</sup>. If EC rises above 6.0 mS cm<sup>-1</sup>, pathological processes in the udder tissue can be suspected. pakauskas et al. (2006) suggest that EC of milk in the samples taken from healthy cows was 4.3–5.7 mS cm<sup>-1</sup>, while that of milk taken from cows with subclinical mastitis was 6.1-8.5 mS cm<sup>-1</sup> and SCC increased from 500 to 1500 thousand ml<sup>-1</sup>. When evaluated over the total period of our study, EC of milk

had a statistically significant correlation with milk SCC (r = 0.379, p < 0.001). The high correlation between milk SCC and EC has also been estimated by Povinelli with co-authors (2005) and Norberg et al. (2006). The correlation between milk yield and milk EC in our study was slightly negative (r = -0.168, p < 0.001). Nielen et al. (1993) state that a rise of 1 mS cm<sup>-1</sup> of the mean EC causes a decline of 0.88 kg a day in milk production, the correlation between mean EC and SCC being 0.32.

The study results show that the higher RNB during pasturable period had no positive effect on milk yield as SCC increased during that period and it had a negative correlation with milk yield. Analysis of literature data (Windig et al., 2005; Kul, Erdem, 2008) reveals that the level of SCC in milk has a direct correlation with udder health and a marked effect on milk production. The average EC in our study met zootechnical standards. During the pasturable period of the experiment, milk EC was 3.6% higher than during the stall period in all lactations (p < 0.001). The correlation between milk SCC and EC was positive (r = 0.379, p < 0.001), as also reported in other studies (Bansal et al., 2005).

## **Conclusions**

- 1. Both during pasturable and stall periods the cows were given balanced diets. During pasturable period the diet contained 5.55% more crude protein, 0.56% more usable crude protein, ruminal N-balance (RNB) was 1.85 times higher than that in stall period diet.
- 2. Electrical conductivity (EC) of milk in our experiment met zootechnical standards. The highest EC of milk was observed during May-August and reached 5.02 – 5.11 mS cm<sup>-1</sup>. The average EC during pasturable period was 3.6% higher than that during stall period in all lactations (p < 0.001).
- 3. Daily milk yield during pasturable period was in average 1.4% lower compared to that during stall period (p < 0.001), somatic cell count (SCC) – 7.9% higher (p < 0.001)0.001). The higher cows' RNB during pasturable period had no positive effect on milk yield as SCC increased during that period.
- 4. The experiment showed that the correlation between milk yield and EC was slightly negative both during pasturable (r = -0.124) and stall (r = -0.226)periods; however, the correlation coefficients were highly significant (p < 0.001).
- 5. The EC of milk had a statistically significant correlation with SCC both during pasturable (r = 0.356, p < 0.001) and stall (r = 0.403, p < 0.001) periods. SCC was lower than 200 thousand ml-1 in the milk samples with EC in the range of 4.6 to 5.8 mS cm<sup>-1</sup>.
- 6. The EC of milk could provide an alternative way for early diagnosis of mastitis in dairy cows.

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# Karvių ganyklinio ir tvartinio laikotarpių racionų įtaka pieno elektriniam laidumui

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#### Santrauka

Tyrimo tikslas – ištirti karvių raciono įtaką pieno elektriniam laidumui (EL) ganykliniu bei tvartiniu laikotarpiais ir įvertinti EL ryšį su karvių produktyvumu bei pieno somatinių ląstelių skaičiumi (SLS). Automatizuota savanoriško melžimo sistema "DeLaval VMS" ("DeLaval", Švedija) 2009–2013 m. buvo ištirta 421 karvė, kurios visus metus buvo laikomos tvartuose nepririštos. Tvartiniu ir ganykliniu laikotarpiais karvės šertos ūkyje pagamintais žoliniais silosuotais pašarais, ganykliniu laikotarpiu papildomai buvo duodama miglinių ir pupinių žolių mišinio. Duomenų masyvą sudarė 462 582 įrašai. Vidutinis tirtų karvių pieno EL (4,92 ± 0,001 mS cm<sup>-1</sup>) atitiko zootechninę normą. Tyrimo laikotarpiu karvių vidutinis primilžis buvo 25,12 ± 0,020 kg pieno per parą, o pieno SLS – 188,95 ± 1,032 tūkst. ml<sup>-1</sup>. Ganykliniu laikotarpiu karvių pieno primilžis per parą buvo vidutiniškai 1,4 % mažesnis, o SLS – 7,9 % (p < 0,001) didesnis nei tvartiniu laikotarpiu. Tirtų karvių visų laktacijų pieno EL vidurkis buvo vidutiniškai 3,6 % didesnis (p < 0,001) ganykliniu laikotarpiu nei tvartiniu. Geresnis karvių raciono ruminalinis azoto balansas (23,03 g) ganykliniu laikotarpiu neturėjo teigiamos įtakos karvių produktyvumui, nes šiuo laikotarpiu pieno SLS buvo didesnis. Koreliacija tarp pieno kiekio ir jo EL ganykliniu bei tvartiniu laikotarpiais buvo neigiama (r = -0,124--0,226, p < 0,001). Pieno SLS neviršijo 200 tūkst. ml<sup>-1</sup> mėginiuose, kuriuose EL svyravo nuo 4,6 iki 5,8 mS cm<sup>-1</sup>. Didėjant pieno EL, pieno SLS esmingai (p < 0,001) didėjo ir ganykliniu, ir tvartiniu laikotarpiais (r = 0,356-0,403), todėl galima teigti, kad šis rodiklis gali būti naudojamas mastitui prognozuoti.

Reikšminiai žodžiai: koreliacija, pašarai, produktyvumas, somatinių ląstelių skaičius.