POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
www.pan.olsztyn.pl/journal/
e-mail: joan@pan.olsztyn.pl
Pol. J. Food Nutr. Sci.
2007, Vol. 57, No. 1, pp. 39–44

DETERMINING THE QUALITY OF MILK FROM COWS FED ON FEED SUPPLEMENTS ENRICHED WITH ENZYMES AND VITAMINS AND ITS SUITABILITY FOR CHEESEMAKING

Antanas Sederevicius[1], Kazimieras Lukauskas[1], Sigita Urbiene[2], Jolita Balsyte[1]

[1]Lithuanian Veterinary Academy, Kaunas; [2]Lithuanian Agricultural University, Kaunas; Lithuania

Key words: food supplements, somatic cells, milk technological properties, quality

The aim of this research was to determine the effects of enzyme preparations, lysozyme and lysosubtilin in particular, and those supplemented with vitamins A, C and E, added to cows' feed, on the somatic cell count and technological properties of milk which are considered primary important in cheesemaking. Three groups of Lithuanian Black and White cows (10 cows each) were fed on a ration supplemented with Neosomatas 1 preparation containing lysosubtilin, 0.02 g and lysozyme, 0.2 g, and Neosomatas 4 preparation (lysozyme, 0.2 g and vitamins A, C and E). The duration of the trial was 10 days. Measurements were conducted on the 4th, 7th and 10th day of the trial and 7 days following the trial.

The reduction of somatic cell count in milk was observed during and after the feeding period. The highest effect of the feed supplement was observed in the third group which was fed on Neosomatas 4. The enzyme- and vitamin-based feed supplements were also effective with regard to the technological properties of milk. Changes in the acidity of milk during storage were rather slow. Likewise, the process of milk fermentation was slow, the properties of structures that were formed differed compared with control group of cows. However, the technological properties of milk from cows fed on Neosomatas 4 supplement were better as compared with cows fed on Neosomatas 1 feed supplement.

Studies on the enzyme structure formation and the process of syneresis showed that feed supplements enriched with enzymes and vitamins had no effect on the above indices. It was also found that milk under study was characterised by good technological characteristics and therefore was suitable for cheesemaking.

INTRODUCTION

Dairy farming is one of the most important sectors of Lithuania's agriculture both from economical and social viewpoints. In 2000, milk accounted for 17% of the total agricultural produce and dairy exports accounted for more than 30% of agricultural and food products [Sederevicius, 2003]. In 2004, it reached 31%.

Recently, much attention has been paid to the quality and safety of dairy products [Sederevicius, 2004]. The improvement of milk quality is considered to be of primary importance in this respect. The somatic cell count is a commonly used measure of milk quality. In the milk of healthy cows it may vary to 300 x 10^3/mL [Sederevicius, 2004]. Higher somatic cell counts demonstrate deviations from the physiological standards. The most common reason for increased somatic cell count (SCC) is an udder inflammation (mastitis) [Aniulis *et al*., 2000].

It seems important to emphasize that the quality of products made from milk with high SCC is evidently poorer [Aniulis *et al*., 2000]. Consequently, it is very important to reduce SCC in milk. One of the main ways to decrease the somatic cell count in milk is the application of enzyme preparations which have an immunostimulatory effect. Enzyme preparations such as lysozyme and lysosubtilin help to strengthen the immunity [Biziulevicius *et al.,* 2003] of cows. They are also characterised by antimicrobial and immunostimulatory effect [Fuglsang *et al*., 1995; Holzapfel *et al*., 1995; Juozaitiene & Kerziene, 2001]. These enzymes are safe for the animal as they are naturally obtained from animal body as well as from milk [Bachman, 1995; Walstra *et al*., 1999; Scerbakova *et al*., 1986].

There has been increased interest recently in lysozyme participation in functions improving the immunity of cows [Dick, 1981; Stelzner *et al*., 1982]. Lysozyme regulates immune and regenerative processes of the organism, acts against inflammation processes, is characterised by antitoxic and anticancer effect [Dick, 1982]. It has an antimicrobial effect especially against gram-positive bacteria [Juozaitiene & Kerziene, 2001; Pellegrini *et al*., 1992; Kuznecova *et al*., 1985].

Lysosubtilin is applied on a large scale throughout the former Soviet Union for the treatment of gynaecological diseases in cows [Biziulevicius & Lukauskas*,* 1998a]. Lysosubtilin acts through disruption of microbial cell walls and the resulting lysis products (muropeptides, lipopolysaccharides, fungal β-glucans, *etc*.), well-known immunostimulants, are being thought to be responsible for the imunostimulatory effects arising from its oral application [Biziulevicius & Lukauskas*,* 1998b].

Lactic acid and bifido bacteria are known for their resistance to lysozyme [Puzenko, 1983], but if the quantity of lysozyme in milk exceeds 40–80 μg/mL – it can negatively affect the fermentation process. The duration of fermentation

Author's address for correspondence: prof. Antanas Sederevicius, Lithuanian Veterinary Academy, 18 Tilzes Street, LT – 3022 Kaunas; tel.: (8~37) 36 33 62; fax: (8~37) 36 24 17; e-mail: antanas@lva.lt

can be extended up to 1–3 h. Consequently, it seems primarily important to select for fermentation such cultures of bifidobacteria which are resistant to the effect of lysosyme [Swaisgood, 1996; Puzenko, 1983].

However, the effect of lysozyme on milk technological properties and somatic cell count in the case of a higher content of lysozyme and lysosubtilin in feed has not been studied.

An experiment was conducted to evaluate the effect of enzyme preparations (lysosyme and lysosubtilin) and vitamins A, C and E added to cows' feed on the somatic cell count and the technological properties of milk regarding its suitability for cheese production.

MATERIALS AND METHODS

The experiments were carried out on Lithuanian Black and White cows. Clinically healthy 4-year-old cows of second lactation and cows 3–6 months after calving were randomly sampled into 3 groups (n=10). Cows with an increased somatic cell count in milk (from 400 to 1000×10^3/mL) were selected for experiments.

The ration of each cow contained: haylage of permanent grass (12 kg), maize silage (12 kg), hay (2 kg), straw (1 kg), saladin (4 kg), slices of fodder beet (6 kg), concentrates (8 kg on the average), mineral-vitamin supplements and licking salt.

The composition of the ration was as follows: dry matter (solids) – 19.4 kg, digestive protein – 1843 g, sugar – 1940 g, fiber – 3.88 kg, calcium – 124 g, phosphorus – 91 g, carotene – 854 mg and edible salt – 124 g.

Concentrates for cows were supplemented with Neosomatas 1 (Group 2) and Neosomatas 4 (Group 3) preparations, with compositions given in Table 1. Feed supplement contained lysozyme (EC 3.2.1.17) – peptidoglican N – acetilmuramoilhidrolazys and lysosubtilin produced by AB "Biosinteze".

All the cows under trials were of similar weight (500±16 kg), an average productivity reaching 20±0.8 kg. Ration of feed supplements per individual (both compositions) was calculated on the basis of average norms, *i.e.* per 500 kg of life-weight and 20 kg of milk production, which made 500 g/a day. Daily doses were mixed with concentrates and the cows were fed twice. The experimental cows were fed on feed supplements with enzymes (lysozyme and lysosubtilin) and vitamins A, C and E for 10 successive days. The experiments were carried out on the 4th, 7th and 10th day of the experimental feeding and 7 days after it.

Milk samples for somatic cell count were taken individually during morning milking.

Somatic cell count in milk was estimated using the "Somascope" in the state laboratory "Pieno tyrimai" [Savickis, 2003].

The physico-chemical analysis was aimed at determining the sensory properties of raw milk and changes in the acidity of milk during storage [Urbiene, 2001]. All the samples were kept for 24 h at 16°C. The duration of bacteriocidic phase was defined based on changes in the acidity. The suitability of milk for manufacturing rennet cheese was evaluated by means of enzymatic coagulation and enzyme structure formation. During the investigation of enzyme fermentation clotting of raw milk was completed with the enzyme commonly used in cheese production and under the effect of milk microorganisms. Milk quality was defined according to clotting structure and its duration. After a 12-h period milk was divided into three groups according to clotting structure [Urbiene, 1999].

Parameters such as syneresis and protein content were studied. The protein content was studied by a refractometric method based on protein extraction. After the extraction a refraction index was defined in milk sample and the same sample of whey, obtained after protein precipitation. The difference between these parameters is directly proportional to milk protein content [Urbiene, 2001]. Lactic acid was defined using a method from the "Official Methods of Analysis" (Association of Official Analytical Chemists (AOAC)" [Urbiene, 1999]. The process of syneresis was based on a sample filtration when the amount of whey was measured in a formed enzyme structure every 10 min and was later calculated in percent [Urbiene, 1995].

The analysis of each parameter was carried out three times and an average value was calculated.

The data were analysed statistically with the Win Excel program [Juozaitiene & Kerziene, 2001].

TABLE 1. Composition of enzyme mixtures for different groups of cows.

Groups of cows	Feed supplement	Composition of mixture (g)	Number of cows
1 – control	–	–	10
2 – experimental	Neosomatas 1	Lysosubtilin 0.02 Lysozyme 0.2	10
3 – experimental	Neosomatas 4	Lysozyme 0.2 Vitamins A, C, E	10

RESULTS

An average somatic cell count of each group is presented in Table 2.

The results of the trials showed that the somatic cell count in milk of the control group tended to decrease. A considerable decrease in the somatic cell count was observed in milk of cows from Group 2 fed on the ration supplemented with enzymes on day 7 of feeding ($p<0.05$). However, the results reverted to their initial norm ($p<0.05$). In the milk of cows Group 3 the somatic cell count reduced to 4.8 times.

TABLE 2. Somatic cell count in milk ($\times10^3$/mL).

Milk samples from the groups of cows	Before feeding	Feeding period (days)			7 days after feeding
		4	7	10	
1 control	741.7±40	547.6±20	835.8±40	847.9±30	1270.1±40
2 experimental	719.8±30	1387.0±30	476.0±50	641.0±35	730.8±20
3 experimental	729.6±20	206.1±45	305.3±40	267.7±30	205.2±30

TABLE 3. Physicochemical parameters of milk from cows under trial**.**

Feeding period (days)	Milk samples from particular groups	Parameters				
		Protein content (%)	Fat content (%)	Carbohydrate content (%)	Lactic acid content (%)	Milk acidity, (°T)
4	1 control	3.02±0.01	4.15±0.03	4.66±0.04	0.130±0.004	15.0±0.5
	2 experimental	3.10±0.01	3.95±0.03	4.53±0.05	0.144±0.005	16.0±0.5
	3 experimental	3.05±0.02	4.26±0.02	4.57±0.05	0.153±0.003	17.0±0.5
7	1 control	2.98±0.01	4.20±0.04	4.52±0.03	0.126±0.004	14.0±0.5
	2 experimental	3.04±0.01	3.96±0.03	4.50±0.05	0.130±0.003	14.5±0.5
	3 experimental	3.10±0.02	4.12±0.04	4.55±0.04	0.139±0.004	15.5±0.5
10	1 control	2.96±0.01	3.95±0.03	4.52±0.05	0.144±0.005	16.0±0.5
	2 experimental	3.12±0.02	4.01±0.03	4.56±0.03	0.153±0.003	17.0±0.5
	3 experimental	3.16±0.02	4.11±0.02	4.37±0.04	0.162±0.004	18.0±0.5
7 days after feeding	1 control	3.01±0.02	3.97±0.01	4.57±0.05	0.144±0.004	16.0±0.5
	2 experimental	3.21±0.02	4.02±0.02	4.59±0.03	0.144±0.004	16.0±0.5
	3 experimental	3.16±0.02	4.22±0.02	4.65±0.05	0.153±0.004	17.0±0.5

TABLE 4. Quality characteristics of milk samples after enzymatic coagulation.

Feeding period (days)	Milk samples from different groups of cows	Character of cheese structure quality	
		Sample of enzyme fermentation	Modified sample of enzyme fermentation
4	1 control	Grade I. Hard cheese.	Grade I. Hard cheese.
	2 experimental	Grade II. Soft structure.	Grade II . Porous cheese.
	3 experimental	Grade III. Hard cheese. Hard surface.	Grade I. Hard cheese.
7	1 control	Grade II. Hard cheese, rough surface.	Grade II. Bad structure, untaught.
	2 experimental	Grade II. Hard cheese. Tattered, rough surface.	Grade II. Poor structure. Sporadic structure flakes observed.
	3 experimental	Grade II. Smooth but soft surface. Porous cheese	Grade II. Good structure but untaught.
10	1 control	Grade III. Poor structure, sporadic flakes.	Grade III. Poor structure, soft, swollen cheese.
	2 experimental	Grade III. Poor structure, sporadic flakes.	Grade II. Smooth, but porous interior.
	3 experimental	Grade III. Poor structure, sporadic flakes.	Grade II. Smooth, but porous interior.
7 days after feeding experiment	1 control	Grade II. Soft, porous cheese.	Grade III. Poor structure, multiple flakes.
	2 experimental	Grade I. Hard cheese, hard surface.	Grade II. Satisfactory quality but the cheese is soft and porous interior.
	3 experimental	Grade II. Satisfactory quality of structure, porous interior.	Grade II. Satisfactory quality but soft and porous interior.

TABLE 5. Formation and character of curd structure.

Feeding period (days)	Milk samples from different groups of cows	Time of structure formation (min)		Protein content of whey (5)	Character of structure formed
		Beginning	End		
4	1 control	16.0±0.5	26±1.0	0.52±0.03	Standard structure, clear whey.
	2 experimental	28.0±0.5	42.0±1.0	0.65±0.04	Soft structure.
	3 experimental	28.0±0.5	42.0±1.0	0.67±0.05	Soft structure.
7	1 control	15±0.5	25.0±1.0	0.50±0.05	Standard hard structure, clear whey.
	2 experimental	28.0±0.5	42.0±1.0	0.68±0.04	Hard structure, cloudy whey.
	3 experimental	28.0±0.5	40.0±1.0	0.67±0.04	Structure better than with Neosomatas 1, but worse than with control group.
10	1 control	16±0.5	25±1.0	0.53±0.04	Hard structure, clear whey.
	2 experimental	28±0.5	41±1.0	0.68±0.03	Soft structure, cloudy whey.
	3 experimental	25±0.5	31±1.0	0.67±0.04	Structure sufficiently hard, but worse as compared to the control.
7 days after feeeding	1 control	13±0.5	26±1.0	0.53±0.03	Structure worse than in the control group. Clear whey.
	2 experimental	10±0.5	20±1.0	0.51±0.02	Hard structure, clear whey.
	3 experimental	10±0.5	20±1.0	0.50±0.02	Hard structure, clear whey.

Data of the samples with regard to physicochemical characteristics of milk from cows fed on feed supplements with enzymes and vitamins during and after the feeding are given in Table 3.

Analysis of the results leads to the conclusion that the effect of feeding cows with vitamins A, C and E had no considerable effect on milk composition and acidity. However, the effect of the feed supplements enriched with enzymes and vitamins A, C and E had a greater effect on the changes in the acidity during storage.

All the samples were kept at 16°C for 24 h. Changes in the acidity were observed during storage. It was determined that the acidity of milk obtained during feeding cows with enzyme preparations remained unchanged during storing (24 h) at 16–17°C.

The sample of enzymatic coagulation demonstrates the effect of milk quality on the curd structure formation and its characteristics. The modified sample of enzymatic coagulation was studied on heat-treated milk. The characteristics of curd structures are presented in Table 4.

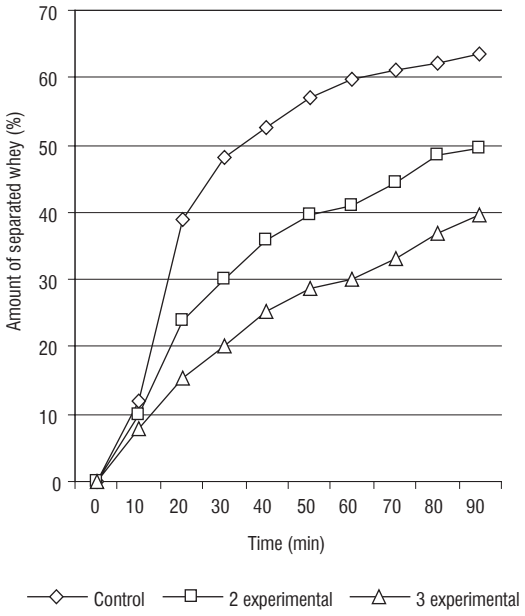

FIGURE 1. Process of the curd structure syneresis during the feeding period.

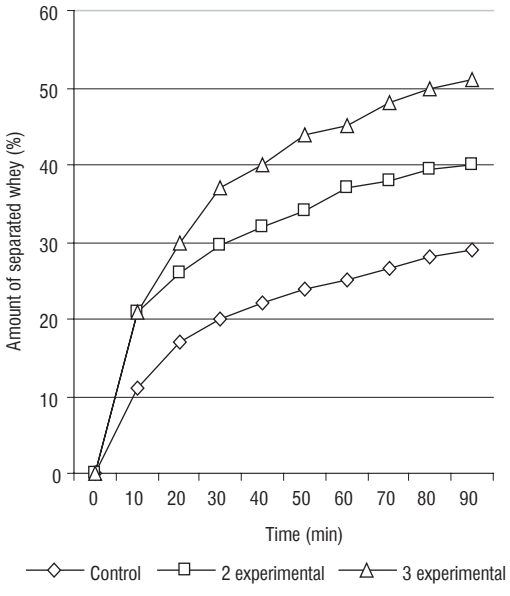

FIGURE 2. Process of the curd structure syneresis after feeding.

Evaluation of the sample of enzyme coagulation (Table 4) revealed that during the entire feeding period milk was characterised as typical of Grade II.

Determination of the impact of feed supplements with enzymes and vitamins on the characteristics of milk was an important stage of the experiment in relation to cheese manufacturing. During the research the duration of curd structure formation, characteristics of the structure formed and the process of curd structure syneresis were evaluated. The process of curd structure formation and its character (Table 5) reveals that during the feeding period the formation of curd structure in the samples of milk from Groups 2 and 3 was 1.3–1.6 times longer compared to the samples from the control group.

The curves of the curd structure syneresis in the samples of milk 10 days after feeding cows on feed with enzymes and vitamins and those 7 days after the experiment are given in Figures 1 and 2.

It is evident that whey separation from milk samples of Groups 2 and 3 (Neosomatas 1 and Neosomatas 4) was worse compared to those in the control group. However, 7 days after the feeding the curve of syneresis changed. It was worse in the samples of the control group than in those of groups fed on Neosomatas 1 and Neosomatas 4.

However, after the feeding period the structure of the curd was formed two times faster than in the samples of milk from control group.

DISCUSSION

It was found during the feeding trials that the somatic cell count in the milk of Group 2 cows fed on feed supplements with lysozyme and lysosubtilin decreased insignificantly, although in the milk of individual cows the decrease was 2.05–2.23 times as low. The enzymes and vitamins based feed supplement fed to cows of Group 3 was found to have a significant effect on the somatic cell count in milk. Due to the effect of the preparation the average somatic cell count in milk was reduced by 2.8 times during the feeding period and after the feeding, the tendency of reduction in SCC was observed. A considerably high effect of this supplement was observed on milk somatic cell count in cows of Group 3. At the end of the feeding period, the somatic cell count in the milk of this group of cows showed a reduction from 3.2 to 4.8 times. It indicates a significant improvement in the quality of milk after feeding [Aniulis *et al*., 2000; Biziulevicius *et al*., 1998b; Biziulevicius *et al*., 2003].

According to the data of the trials, the technological properties of milk for cows fed on feed supplements with enzymes and vitamins (Neosomatas 1 and Neosomatas 4) has changed. Changes in the acidity of milk samples were notably slow. When milk samples of the cows of Group 2 and Group 3 were kept for 24 h at 16–17°C, no changes in the acidity were observed. However, all the other samples of milk were characterised for changes in the acidity. However, in the milk samples from the control Group 1 and in all other samples taken 7 days after the feeding on Neosomatas 1 and Neosomatas 4, changes in the acidity ranged from 6 to 7° T. These results led to the conclusion that the lysozyme supplement extends the bacteriocidic phase in milk [Holzapfel *et al*., 1995; Fuglsang *et al*., 1995]. It has been found by Polish scientists that such an effect was observed when lysozyme was added directly into the milk [Pieczonka & Burek, 1994].

The formation of curd structure in the milk samples from cows fed on enzymes and vitamins based feed showed that during 10 days of feeding the structure of cheese was worse and the structure formation process took 1.3–1.6 times longer than that in milk of the control group. This can be explained by a higher somatic cell count in the milk as well as a lower milk quality [Aniulis *et al*., 2000]. However, after the feeding period, these qualities improved considerably. The curd structure was formed 2 times faster than in the samples of milk from the control group.

The analysis of milk samples from both groups of cows 7 days after the feeding trial showed that the milk from cows fed on enzyme and vitamin based supplements was characterised by improved curd characteristics and a better quality of the structure.

The content of proteins in whey during the entire period of feeding cows on enzyme preparations was higher than that after this period. It is assumed that some changes might have occurred in the milk protein system, which positively affected the quality of structure and protein concentration in whey [Walstra *et al.*, 1999]. It can be related to cows' health and improved quality of milk due to the effect of feed with enzymes [Aniulis *et al*., 2000; Biziulevicius *et al*., 1998b].

The process of syneresis reflects the quality of curd structure and its ability to separate whey. It is widely accepted that a hard curd structure with well separated whey is rapidly (~30 min) formed in a high quality milk designated for cheese manufacturing [Walstra *et al*., 1999].

It has been established that the separation of whey in the samples of milk from cows fed on Neosomatas-1 and Neosomatas-4 preparations was more effective than that in the control group milk samples. However, 7 days after the feeding the curve of syneresis changed. It was worse in the samples of the control group than in those of groups fed on Neosomatas 1 and Neosomatas 4. Thus, after feeding cows on feed supplements with enzymes and vitamins the quality of curd structure was much better.

All the above-mentioned changes can be related to an improved physiological state of animals [Brown & Gordon, 2003] and a better milk quality [Biziulevicius *et al*., 2003], which was evidenced by a reduced somatic cell count in milk.

CONCLUSIONS

1. It was established that feeding cows on feed supplements enriched with lysozyme and lysosubtilis enzymes (Neosomatas 1) reduced the somatic cell count in milk by 2.05–2.23 times.

2. Feed supplements based on lysozyme and vitamins (Neosomatas 4) can reduce the somatic cell count in milk from 3.2 to 4.8 times.

3. Feeding cows on feed supplements Neosomatas 1 and Neosomatas 2 extends the bacteriocidic phase in milk. The acidity of milk during the feeding remained unchanged within the period of 24 h at 16–17°C.

4. The feed supplements Neosomatas 1 and Neosomatas 4 had a positive effect on the qualities of milk intended for cheesemaking. After feeding cows with the above preparations the formation of curd structure was approx. 2 times faster and the separation of whey was faster.

REFERENCES

1. Aniulis E., Japertas S., Leipute K., Karviu pieno kokybes analize, atsizvelgiant i somatiniu lasteliu skaiciu. Veterinarija ir zootechnika, 2000, 8, 30, 5–8.
2. Bachman H.P., Butyric acid fermentation in cheese: a literature review. Agrar Forschung, 1995, 11/12, 523–526.
3. Biziulevicius G.A., Kazlauskaite J., Lukauskas K., Ramanauskiene J., Sederevicius A., An enzymatic cow immunity-targeted to reducing milk somatic cell count. 1. A preliminary study using lysosubtilin. Food Agricult. Immunol., 2003, 15, 3–4, 289–292.
4. Biziulevicius G.A., Lukauskas K,. *In vivo* studies on lysosubtilin. II. Efficacy for treatment of post-partum endometritis in cows. Vet. Res., 1998a, 29, 47–58.
5. Biziulevicius G.A., Lukauskas K., *In vivo* studies on lysosubtilin. III. Efficacy for treatment of mastitis and superficial lesions of the udder and teats in cows. Vet. Res., 1998b, 29, 441–456.
6. Brown G.D., Gordon S., Fungal b-glucans and mammalian immunity. Immunity, 2003, 19, 311–315.
7. Dick W., Lysozym. Grundlagen und Diagnostidche Bedentung – Forschritte der Medizin, 1982, Bd. 100, 26, 1230–1234.
8. Dick W., Klinische Bedeutung des Lysozym in Sänglinge und Kleinkinderalter Therapiewoche, 1981. Bd. 31m. 31, 1740–1745.
9. Fuglsang C.C., Johansen C., Christgau S., Adler-Nissen J., Antimicrobial enzymes: Application and future potential in the food industry. Trends Food Sci. Technol., 1995, 12, 390–396.
10. Holzapfel W.H., Geisen R., Schillinger U., Biological preservation of foods with reference to protective cultures, bacteriocins and food – grade enzymes. Int. J. Food Microbiol., 1995, 24, 343–362.
11. Juozaitiene V., Kerziene S., Biometrija ir kompiuterine duomenu analize. 2001, Kaunas, Lietuvos veterinarijos akademija, p. 115.
12. Kuznecova T. A., Kisluchina O. V., Avizienis V. J., Lyzis mikroorganizmov fermentnymi preparatami. Fermentnoe i spirtovae promislinost, 1985, 6, 38–39.
13. Pellegrini A., Thomas U., von Fellenberg R., Wild P., Bactericidal activities of lysozyme and aprotinin against Gram-negative and Gram–positive bacteria related to their basic character. J. Appl. Bacteriol., 1992, 72, 180–187.
14. Pieczonka W., Burek E., Stability of goats milk with added lysozyme. Przem. Spoż., 1994, 48, 112–114.
15. Puzenko I.V., Custvitelnost acidofilnych bakteri k lizocimu. Molocnoe promislinost., 1983, 12, 18–20.
16. Savickis S., The milk testing system of Lithuania. The activity and future prospects of SL "Pieno tyrimai". International conference. Pieno tyrimu sistemos dabartis ir perspektyvos integruojantis i Europos Sajunga, Kaunas, 2003, pp. 24–25.
17. Scerbakova E.G., Rasgunova G.A., Zuravlova T.P., Satanova G.P., Nedaivozova H.H., Detskie molocnie produkty obogascionie lizocimom. Maskva, Nacional Prom, 1986, p. 40.
18. Sederevicius A., Direktyvos 92/46/EEB, nustatančios zalio pieno, termiskai apdoroto pieno ir pieno produktu gamybos ir patiekimo i rinka taisykles, igyvendinimo pasekmiu ivertinimas. Kaunas, 2004, pp. 31–32.
19. Sederevicius A., Milk testing for science and the public. Tarptautinė konferencija. Pieno tyrimu sistemos dabartis ir perspektyvos integruojantis i Europos Sąjunga. Kaunas, 2003, pp. 81–88.
20. Stelzner A., Klein Y.M., Kittlick M., Klein U., Lysozym. 2. Mitteilung: Biologische Funktion. Deutsche Gesundtreits Wesen, 1982, Bd.87, 48, 2033–2038.
21. Swaisgood H. E., Charakteristics of milk. 1996, *in*: Food Chemistry (ed. O.K. Fennema). Marcel Dekker, Inc., New York, p. 1064.
22. Urbiene S., Superkamo pieno kokybes ivertinimas ir tyrimo metodai. Akademija, Kaunas, 2001, p. 49.
23. Urbiene S., Pieno ir jo produktu chemines analizes metodai. Kauno Technologijos Universitetas, Kaunas, 1999, p. 247.

24. Urbiene S., Isliedovanie biochimyceskich svoistv kislomolocnych produktov vo vreme chranienie. Lietuvos maisto instituto ir Kauno technologijos universiteto mokslo darbai. Maisto chemija ir technologija. Vilnius., 1995, 29, 97–101.
25. Walstra P., Geurts T. J., Noomen A., Jellema A., van Boekel M.A.J.S., Principles of Milk Properties and Processes. 1999, Marcel Dekker, Inc., New York, p. 727.

Received November 2005. Revision received June and accepted July 2006.