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ORIGINAL ARTICLE

The influence of cows stresses resistance on cheese yield and quality

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The study of the influence of types of stress resistance of Ukrainian red dairy cows on milk safety indicators, technological properties of milk associated with the production of rennet cheese, and the organoleptic qualities of cheese and its yield are presented. The experimental animals were divided into three groups according to the standard deviation value of 0.67 from the average concentration of cortisol in the studied group of animals of the same age during the calving. Blood for the study of cortisol concentration in cows was taken in the morning hours before feeding after a stress load. The stress load was a complex of technological factors associated with blood sampling, fixation of animals for one hour, and the presence of veterinary specialists and support personnel. Among 117 experimental cows, groups of 12 animals were formed to study the cheese suitability of milk and cheese quality. The method of balanced analog groups was applied. The animals were of the same breed, peers in age, average body condition, gentle-dense constitution type, and the intergroup difference in body weight were not reliable. The need for these studies arose due to the constant technological stresses that animals experience in the conditions of their operation, and the effect of the type of stress resistance on the cheese suitability of milk and the quality of rennet cheese produced from it remains an insufficiently studied issue, which determined their scientific novelty and relevance. It was found that milk, which was obtained from cows with high and medium resistance to stress, meets the requirements for raw materials for cheese preparation and has high technological properties, in contrast to peers with low-stress resistance, in which milk according to the fermentation test was attributed to the third class and found unsuitable for the production of rennet cheese. This is evidenced by the twice lengthened phases of milk coagulation under milk-clotting enzyme and double quality of rennet fermenter for its coagulation, which led to a decrease in the quality of the finished product - rennet cheese "Lyubitelskiy", which, according to the results of the examination, had a bitter taste and a rubbery consistency. However, the yield of finished products did not depend on the group of cows differing in stress resistance. It is concluded that milk obtained from cows of the first two groups can be used for the production of cheese according to a reduced technological process, as a result of which the energy costs for the production of finished products will be reduced and the cost of cheese will decrease.
Keywords: cortisol, milk safety, rennet fermentation test, coagulation and gelation phase, organoleptic properties of cheese, cheese yield.

Introduction

In the context of industrial technology, animals are affected by various negative environmental factors or stressors. They often worsen livestock's well-being, reduce productivity, and lead to a violation of milk production technology. Temperament and behavioral types of animals are hereditary traits (Salak-Johnson et al., 2006). The individual characteristics of animals are manifested because homeostasis is restored much faster in some individuals. Simultaneously, without adverse health consequences, a sharp decrease in productivity and reproductive capacity (Beilharz et al., 1982; Borell et al., 2007; Chernenko, 2015). In others, the same operational loads cause destructive consequences in the body, and homeostasis is not recovered for a long time (Wolfenson et al., 2000; Carroll et al., 2013).

To restore the constancy of the internal environment of the body, significant additional energy resources are required. First of all, it needs to get them due to its reserves, which are achieved, among other things, resulting from gluconeogenesis. However, this process is not possible without the presence of "building material". They are free amino acids and fatty acids. Therefore, under the influence of stress hormones, cortisol, protein synthesis, and fats are blocked (Giesecke et al., 1985), which is part of the so-called General Adaptation Syndrome (GAS). The effectiveness of the body's recovery depends on the duration of the action of the stress factor (Frank et al., 1989), on the intensity of its effect (Lacetera et al., 2001), as well as on the individual adaptive properties of the organism (Salak-Johnson et al., 2006) and type of constitution (Chernenko, 2015). Stress can cause significant lactation fluctuations (Gorewit et al., 1992; Wenzel et al., 2003), threaten the antimicrobial efficacy of natural udder

defense mechanisms, increase the risk of physiological regression of breast tissue, leading to subclinical and clinical mastitogenic udder infections, change the composition, technological properties of milk and its safety (Chernenko et al., 2019). Abeykoon et al. (2016) studied the differences in milk coagulation properties obtained from three different types of dairy cattle raised in Sri Lanka. The milk coagulant's various properties, product yield, technological properties of milk were evaluated, and the biochemical composition (lactose, protein, fat, non-fat milk solids) of milk samples was determined. It was concluded that there is a significant correlation between milk coagulability and genetically determined variants of milk protein. However, there is no data on the influence of the type of animals' nervous system on the cheese suitability of milk and rennet cheese's organoleptic properties.

Our research aimed to establish the influence of cows' stress resistance on milk suitability for cheese production and rennet cheese quality.

Material and methods

The research was carried out on 117 first-calf cows of the Ukrainian red dairy breed, which belong to the agricultural company Olimpeks-Agro (Dnipropetrovsk region). The stress level in cows' bodies was determined by cortisol concentration in the blood after stress load in the morning hours before feeding. The stressor was the fixation of animals, veterinarians' presence, and the very process of taking blood for its general planned analysis. Besides, the animals remained in a fixed position for one hour, unable to approach the trough or drinker. The method for determining cortisol concentration in the blood serum of experimental animals is based on the competition between an unlabeled antigen and an enzyme-labeled antigen for a certain number of bonds with the antibody. The amount of enzyme-labeled antigens bound to antibodies is inversely proportional to the unlabeled test sample concentration. We used the reagents of the Diagnostic system laboratory (USA), as well as the STAT FAX 2100 Microplate Reader at the wavelengths of 450 and 600 or 620 nm, Stat Fax 2200 Incubator Shaker at 500-700 movements per minute, Stat Fax 2600 Automated microplate washer, and semi-automatic dispensers for 5-50, 50-200 and 200-1000 µl. The solution's optical density was measured at a wavelength of 450 and 620 nm (within 30 minutes after adding the Stop- Solution). Before taking the data, the absorption was set to "0", respectively, with a zero standard (Blank). The obtained values of cortisol standards were applied to construct a standard curve, which was used to determine cortisol concentration in the studied blood samples. Samples that exceeded the high standard were further diluted with the 0 µg/dL standard and retested. Since all standards are given in µg/dL, the value in µg/dL was multiplied by 27.6 to convert them to nmol/L.

The cows were clustered into three groups using a standard deviation value of 0.67D from the mean cortisol value for the experimental group of animals of the same age in the calving. The first group included cows whose blood cortisol concentration was in the range of 19.9-112.8 nmol/L. They received the conventional designation "highly stress-resistant". The second group includes cows with a cortisol concentration in their blood in the range of 112.8-205.7 nmol/L (modal class - "average resistance to stress"). The third group included animals in which the concentration of cortisol was high and amounted to more than 205.7 nmol/L (the symbol is "low-stress-resistant") (Fig.1).

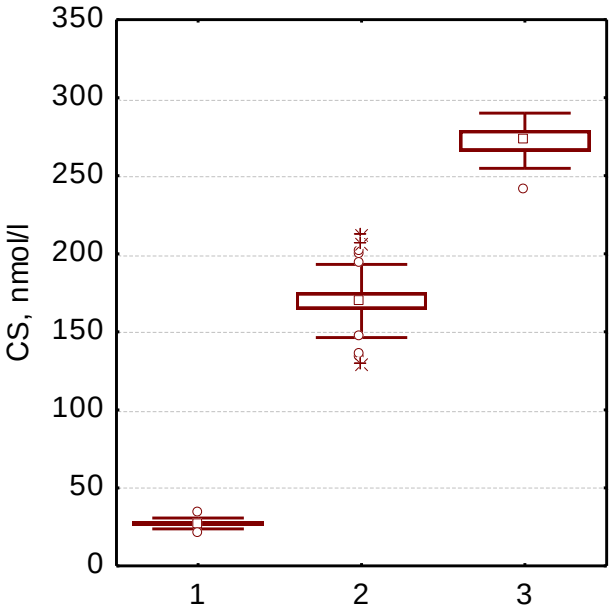

Fig. 1. The concentration of cortisol in the blood of experimental animals after stress load: 1 – highly stress-resistant; 2 – average resistance to stress; 3 – low-stress resistant; Friedman ANOVA

According to GOST 3662: 2015 "Raw milk cow. Technical conditions", we investigated the bulk cow's milk of the highest grade. Rennet coagulation was carried out under the action of the milk-clotting enzyme CHY-MAX Extra 600 IMCU from Chr. Hansen (Denmark) and calcium chloride according to GOST 450-77 "Technical calcium chloride. Technical conditions" by Browin (Poland). From the milk of experimental cows in a private cheese factory, rennet cheese "Lyubitelsky" was made according to GOST 4395: 2005 "Soft cheeses".

Results

The most numerous were the cows from the first group with high-stress resistance, which comprised 61.5% of the studied animals. The least of all (third group) were animals with low-stress resistance – 14.5%. The number of cows with average stress resistance was 24.0%. To test milk for its cheese suitability, we formed three groups with 12 cows each according to balanced analog groups. The animals were clustered by breed, origin, calving age, and lactation period, while the intergroup difference in body weight was insignificant. We investigated the milk of cows in the laboratory of a cheese factory (table 1).

Table 1. Composition and technological properties of cow's milk

Index	Stress resistance of cows		
	high (first group)	middle (second group)	low (third group)
Content of nitrates in milk, mg/kg	3.95	3.95	4.20
Content of pesticides in milk, HCH (gamma isomer, mg/kg	0.012217	0.01239	0.01161
Fat content in milk, %	4.10	3.81	4.11
Casein content in milk, %	3.34	3.25	3.02
Density of milk, °A	27	27	27
Acidity of milk, °T	17	17	18
Calcium content, mg%	120.8	128.2	138.8
Phosphorus content, mg%	92.1	99.3	104.2
Somatic cells, thousand/ml	<500	<500	<500
Heat resistance of milk, group	I	I	I
Cheese suitability of milk, class	I	II	III

The given data show that the milk of cows of the first and second groups in its technological properties meets the requirements for rennet cheeses' production following GOST 3662: 2015 "Raw milk cow. Technical conditions". However, according to the rennet fermentation test, the milk of cows with low-stress resistance belongs to the third class and is not suitable for cheese production.

The content of pesticides and nitrates in the milk of animals of all groups met the safety requirements established in SSR 4.4.4.011 and SLPR 1.8.20-1.05; however, the milk concentration from low-stress resistance cows was 6.3% higher than in other groups. The significant difference between animals of different stress resistance in terms of milk cheese suitability is explained by the peculiarity of metabolism in the body of animals of different biological types. We revealed a noticeable difference in the hormonal composition of cows' blood, depending on their sensitivity to stress.

The ability of milk to curdle under the influence of a milk-clotting enzyme characterizes a certain amount of microflora capable of producing gas, is detected by the rennet fermentation test, and is an essential indicator for cheese making. We conducted a study to investigate the cheese milk suitability of cows of different types of stress resistance. For this, three milk samples were taken, collected from animals with high-stress resistance, medium, and low. According to the rennet fermentation test, the animals were selected, which were most characteristic of their type. The study was carried out in duplicate. The duration of milk clotting was determined after milk-clotting enzyme application by a mug with a calibrated hole.

We have determined the best milk for cheese making from cows with high and medium stress resistance. It was rennet active and required moderate consumption of rennet for clotting (Table 2).

Table 2. Rate of coagulation of bulk milk under the influence of rennet fermenter, min

Stress resistance	Phases of curdling milk		The total duration of coagulation	Rennet consumption, g
	coagulation	gelatination		
High, (1 group)	2.45	0.20	3.05	2.50
Middle (2 group)	2.55	0.45	3.40	2.80
Low (3 group)	5.50	1.00	6.50	5.60

When casein combines with calcium, phosphorus, and other milk components, the coagulation phase passed twice as fast during clotting of milk from cows of the first two groups. When the casein complex begins to precipitate in the form of flakes and a clot forms, the gelation phase was also shorter during clotting of milk from cows with high and medium stress resistance by 0.8 and 0.55 minutes milk from cows with low-stress resistance. Also, milk from low-stress cows required twice the amount of rennet. It is recognized as rennet flabby, as evidenced by the somewhat elongated phases of coagulation. It should be noted that the milk of cows with low-stress resistance with an average amount of added rennet did not curdle at all. Furthermore, only with its double introduction is milk coagulation achieved, which is not economically viable. If for the coagulation of 50 quintals of milk from highly stress-resistant cows 125 g of rennet (50 quintals x 2.5 g) is needed, then to coagulate such an amount of milk from low-stress cows – 280 g (50 quintals x 5.6 g), which is 155 g more, or it is 124%.

According to the organoleptic research data (Table 3), the tasting commission made the following assessments: the first and second samples of "Lyubitelsky" cheese comply with DSTU 4395: 2005 Soft cheeses. General Specifications.

Table 3. Organoleptic properties of cheese "Lyubitelsky"

Index	Stress resistance		
	high	middle	low
Taste and smell	pure, fermented milk		fodder taste, bitter
Consistence	gentle, homogeneous		
Color	light, cream, homogeneous throughout the mass		

The third sample does not meet the requirements of DSTU in taste; that is, the cheese obtained from the milk of cows with low-stress resistance does not meet the standard's requirements. It has an off-taste and is bitter. To determine the value of the yield of the finished product, produced from the milk of cows with extreme types of stress resistance, the cheese was weighed. The weight of cheese made from milk of highly stress-resistant animals is 4.4 kg, and from the milk of low-stress animals – 4.2 kg. That is, no significant difference has been established.

Discussion

Recently, small processors' interest in producing various dairy products, including cheese, has increased. However, the manufactured products are examined mainly for organoleptic characteristics and indicators that determine dairy products' conformity and safety. Considering that the processing of products plays a significant role in the dairy industry, the efficiency of which mainly depends on the quality and technological properties of raw materials (Bergamaschi et al., 2016), a comprehensive study of milk as raw material for the production of rennet cheeses, depending on the breed of animals, feeding rations, housing conditions, the welfare of animals and their type of nervous system, is relevant.

Cheese-makers-practitioners and scientists explain the disadvantages of cheese, such as a bitter taste, by the action of various factors. Holt (2004) noted that cheese tastes bitter most often due to increased calcium chloride doses and rennet when the milk is flabby. Scientists report that the bitter-tasting cheese has a score reduced to 30-36 points, affecting its cost. If we take into account that the milk that we used to make cheese was tested for mastitis, like each animal in general throughout lactation, the flasks for transporting milk were boiled in advance, and the cheese was made three times, then for this reason, it remains to adhere to this very idea. Since, indeed, milk from low-stress cows for clotting in our studies with fermentation test required an increased introduction of calcium chloride and rennet, which are part of the rennet composition that explains the taste flaws of cheese made from milk of low-stress type cows (the third group).

Many factors of various origins determine cheese suitability of milk, but it has the most significant dependence on the content of casein, phosphorus, and calcium in milk. Thus, with an increase in gamma-casein content, the process of milk coagulation under the influence of rennet lengthens, and an increase in the size of casein micelles and alpha-casein fraction decreases (Bergamaschi et al., 2016). Our experiments failed to cover such studies. However, we explain milk's low cheese suitability from stress-sensitive cows by the different content of such vital minerals like calcium and phosphorus in the raw material. The calcium and phosphorus content in the milk of the third group's cows was increased relative to the first and second groups, respectively, by 18.0 and 10.6, by 12.0 and 5.0 mg%. Besides, milk from cows of the third group had a high calcium content of 17.8% relative to the average values for the red steppe breed (121.0 mg%, Davydov, 1958); the Ukrainian red dairy breed was developed. De Kort et al. (2011) and Bergamaschi et al. (2016) established that the effect of rennet is weakened not only due to a reduced, but also due to an increased content of ionized calcium in milk. They noted that it was difficult to obtain a good-quality clot.

Conclusions

A high level of sensitivity to stress negatively affects milk's composition and technological properties during its processing into cheese. Our data on the cheese suitability allows us to assert that milk obtained from cows with high and medium stress resistance can be used to produce cheese using a shortened technological process. As a result, energy costs for production can be reduced, and the price of cheese can be droped.

To reduce the mass fraction of flabby rennet milk and improve the quality of dairy products, farms must form productive groups of cows depending on their resistance to stress and reject animals with increased sensitivity to stress in time. To improve the coagulation properties of milk and its cheese suitability, we suggested studying the influence on these characteristics of the breed, genetic and paratypic factors, and the type of cow nervous systems.

In the production of cheese suitability milk, the problem of stress must be addressed comprehensively, while improving the feeding and living conditions of animals, as well as selectively – in the selection of breeding stock, sire bulls should be used, which, based on the results of a linear assessment of the exterior, form a balanced type of the nervous system in the daughters.

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