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## Research Article

### Study of the influence of the type of higher nervous activity on the secretion of organic components of milk

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#### Abstract

The purpose of the experiment was to study the influence of the type of higher nervous activity (HNA) on the secretion of organic components of milk in various milking conditions. Under the influence of the unconditioned stimulus "inadequate udder preparation" in the milk of machine milk in cows in the morning milking: I HNA type - secretion and percentage of milk fat and the ratio of percentage of fat to percentage of lactose decreases, while in all other types there is a decrease in the percentage of fat and protein. In the unbalanced (II) type, the average duration of the milk flow cycle decreases; in the inert (III) and weak (IV) type, the concentration of fat and lactose decreases, and the ratio of fat to lactose in the latter. It is advisable to characterize the stress resistance of animals based on knowledge of their types of HNA. At the same time, the complexity of their determination by known methods should be noted. In dairy farming, it is necessary to provide for the presence of all types of HNA among the cows and take into account their individual characteristics in terms of resistance to stress, the level of milk production and milk yield. It is recommended to apply the model of dairy cows developed by us to objectively determine the type of their stress resistance in production conditions. The presented patterns of changes in the secretory function of the mammary gland under the influence of tested stress factors on dairy cows of different types of higher nervous activity should be used to develop express methods for determining the types of stress resistance in animals and completing the herds of dairy farms.

**Keywords:** cows, milking, higher nervous activity, milk productivity, lactation, milk composition

**Abbreviations:** HNA – higher nervous activity; ACTH – adrenocorticotrophic hormone

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## **Introduction**

During the synthetic activity of the mammary gland, hormones are inactivated. A decrease in their concentration in the blood is perceived by various receptors. In response to this, the central nervous system stimulates the secretion of new amounts of hormones that ensure milk synthesis at a high level.

So, it was found that sucking sharply reduces the content of prolactin in the pituitary gland of animals, degranulation of acidophilic cells of the adenohypophysis, hypertrophy of the Golgi apparatus, and, as is known, it is acidophilic cells that secrete prolactin. Sensitive impulses from the mammary gland, caused by milking or sucking, stimulate not only the secretion of prolactin, but also thyroid-stimulating hormone, and change the secretion of gonadotropins. There is evidence of a change in the secretion of adrenocorticotrophic hormone (ACTH) and somatotrophic hormone under the influence of impulses from the mammary gland. In confirmation of this, we can cite experiments in which udder massage caused the growth of the mammary gland and the secretion of milk in non-lactating animals and increased milk production in lactating animals. The reflex connection between the mammary gland, digestive, respiratory and cardiovascular systems of the body, as well as the genitals is shown. Therefore, under the influence of milking incentives, deep changes occur in the body. Irritation of the udder receptors in animals can cause continuous secretion of milk (Kézér et al. 2015; Vedovatto et al, 2021; Wenzel et al. 2003).

It has been proven that irritation of the udder receptors causes increased blood flow to it. This promotes milk excretion and stimulates the secretory process by increasing the supply of milk precursors and hormones. Synthetic activity of the mammary gland is also closely related to its motor function.

Thus, under the influence of sensitive impulses from the mammary gland, a multilateral restructuring occurs not only in the hormone-forming activity of the pituitary gland, but also in other internal organs that are closely related to the function of the mammary gland.

Of great value for the physiology of lactation is information on the influence of higher parts of the

brain in the regulation of milk secretion. Experiments have shown this when the functional state of the cerebral cortex changes. Especially when the cortical processes of excitation and inhibition collide, significant changes in the secretion of milk and milk fat are observed (Hemsworth 2003; Marçal-Pedroza et al. 2020; Silva and Passini, 2017).

Regulation is understood as a continuous physiological process that ensures the adaptation of the mammary gland, as an executive organ, and the entire animal body to perform the function of formation, accumulation and excretion of milk in a continuously changing external and internal environment. This regulation proceeds in the form of reflex acts. The reflex is based on the reflex arc. The initial stage of the reflex is a nerve impulse from the receptor, which transforms the energy of mechanical, chemical, and other stimuli. The nerve impulse reaches the regulatory centers, from which the corresponding reactions emanate. Moreover, both the afferent and efferent links can be represented by the nervous and neurohumoral pathways. The reflex depends not only on the strength and nature of the stimulus, but also on the state of the structural elements of the reflex arc (Marçal-Pedroza et al. 2021; Stephansen et al. 2018).

The highest department of regulation of the function of the mammary gland is the cerebral cortex. The latter, through subcortical, brain stem formations, the hypothalamus and other links, regulates the secretory function of the mammary gland.

Impulses from the udder receptors are addressed not only to the lactation center, but also to the centers that regulate the functions of the endocrine, digestive, respiratory, cardiovascular and other body systems. Therefore, the initial nerve impulse from the mammary gland, as it were, turns into a volley of impulses that has a powerful effect on the entire body.

In the process of reflex regulation of lactation, an important place belongs to humoral substances. The introduction of the extract of the posterior pituitary gland leads to the release of milk. This effect is due to the action of the oxytocin hormone, which is normally released in response to suckling and milking stimuli (Mincu et al. 2021; Sutherland et al. 2012).

The neurohormonal nature of the milk ejection reflex in different types of higher nervous activity (HNA) leads to a clear synchrony of milk ejection from the quarters, therefore, the study of the milk ejection reflex in one quarter of the udder characterizes milk ejection in general.

The purpose of the experiment was to study the influence of the type of higher nervous activity (HNA) on the secretion of organic components of milk in various milking conditions.

## Materials and Methods

**Experimental design.** The experiment was carried out on 37 first-calf heifers of Black-and-White breed. In all animals, the types of higher nervous activity were determined by the method of

conditioned reflexes to food stimuli. Cows in the first half of lactation were divided into groups according to the type of HNA. Nine animals were assigned to the 1st type of HNA (strong balanced mobile), 8 animals - to 2nd (strong unbalanced), 11 animals - to 3rd (strong inert), and 9 animals - to 4th (weak inhibitory), i.e. cows of different types were approximately equal in number. The first group of type I was the control group.

Cows were fed according to zootechnical standards, kept tethered in cowsheds for 200 heads, milked 3 times a day with an interval of 12.6 and 6 h. The animals belonged to the Rassvet experimental farm, Krasnodar Research Centre for Animal Husbandry and Veterinary Medicine.

The scheme of experiment is given in Table 1.

**Table 1.** Scheme of the experiment to study the effect of the type of HNA of cows on the secretion of milk under the influence of stimuli

Period of experiment	Period duration, d	Milking technology	Stimulus	
			conditioned	unconditioned
1	10	Standard	-	-
2	5	Standard	Presence of an outsider	-
3	1	Inadequate preparation of the udder (10 s), delay putting on the milking machine for 30 s	1. Presence of a stranger. 2. Milking delay, 30 s	Reduced irritation of udder receptors in preparation for milking

*Compiled by the authors*

In the first period, the amount of milk and its chemical composition were determined under standard conditions for 10 d. In the next two periods, a batch analysis of the composition of a single milking was carried out: the first streams of milk, machine milk and milk obtained by manual after-milking, performed after machine milking and removal of the milking machine. Moreover, in manual after-milking, only a portion of milk of  $20 \pm 5$  g was milked evenly from all quarters.

In the second period, cows were exposed to a conditioned stimulus - the presence of a stranger (experimenter). In the third period, an inadequate 10-second preparation of the udder for milking and its delay by 30 s were used.

**Studied parameters.** The effect of the influence of the nervous system of cows on the concentration of lactose, fat and protein in milk was studied. At the same time, it was taken into account that protein is of the greatest importance, followed by fat and the carbohydrate lactose as a plastic substance for building the body of a calf. As an energy source, fat comes first, followed by lactose and then protein. In terms of ease of digestion, lactose comes first, then fat, and then protein. Consuming an equal amount of milk, but of different composition of nutrients, the offspring of animals of different types becomes in unequal conditions. More plastic substances and energy will be received by those descendants who receive milk with an increased ratio of protein to

lactose and fat to lactose. Therefore, for the convenience of reflecting this phenomenon, we propose a unit of measurement: the coefficient of the ratio of the concentration of protein in milk to the concentration of lactose or the protein-lactose coefficient (C p/ l) and the coefficient of the ratio of fat concentration in milk to the concentration of lactose or fat-lactose coefficient (C f/ l).

## Results

In cows with I HNA type under standard conditions, the ratio of the mass fraction of fat to lactose was  $0.68 \pm 0.04$  in the morning, and  $0.91 \pm 0.06$  at lunch time; protein to lactose:  $0.75 \pm 0.03$  and  $0.85 \pm 0.03$ , respectively.

In the first period of the experiment with the standard milking technology, cows of I HNA type 1

were at  $114.6 \pm 12.4$  day of lactation. The stage of lactation in the experimental groups practically did not differ.

The indicators characterizing the function of the mammary gland in the morning and lunch milking were, respectively, the following: the amount of secreted milk -  $8.88 \pm 0.48$  kg and  $5.42 \pm 0.36$  kg; mass fraction in milk: fat -  $3.06 \pm 0.20\%$  and  $4.06 \pm 0.18\%$ ; protein -  $3.36 \pm 0.12\%$  and  $3.41 \pm 0.11\%$  and lactose -  $4.52 \pm 0.12\%$ .

In order to enhance the contrast and reliability of the analysis, to identify the distinctive features in the secretion of milk of various types of HNA, a comparison was made with I HNA type, and not with the average indicators for the sampling, since in the latter case differences would be levelled by including numbers of the type in the average data, with which the comparison is being made.

**Table 2.** Characteristics of the composition of milk and its organic components under standard milking conditions,  $\pm$  difference with I HNA type in absolute terms (First period of experiment)

Indicators	HNA type								
	II			III			IV		
	Milking: M (Morning), L (Lunchtime)								
	M	L	M+L	M	L	M+L	M	L	M+L
	Amount								
Milk, kg	-1.42 <sup>a</sup>	-0.66	-0.95	-0.02	+0.10	+0.11	-0.68	-0.99	-0.84
Fat, g	-42.7 <sup>a</sup>	-18.4	-29.6	-20.5	+34.5	+7.0	-54.0 <sup>a</sup>	-59.9 <sup>b</sup>	-57.0 <sup>b</sup>
Protein, g	-25.4	-38.1	-28.3	-9.6	-38.9	-18.7	-41.3	-62.6 <sup>b</sup>	-51.9 <sup>a</sup>
Lactose, g	-63.3 <sup>a</sup>	-18.5	-37.2	+12.4	+7.2	+13.3	-12.6	-33.6	-23.1
	Mass fraction in milk, %								
Fat	+0.05	-0.03	-0.02	-0.12	+0.36	+0.09	-0.37	-0.53 <sup>b</sup>	-0.45 <sup>a</sup>
Protein	+0.30	+0.14	+0.22 <sup>a</sup>	-0.01	-0.16	-0.08	-0.28 <sup>a</sup>	-0.21	-0.24
Lactose	+0.02	-0.15	-0.06	+0.15	+0.07	+0.11	+0.25 <sup>a</sup>	+0.26 <sup>a</sup>	+0.25 <sup>b</sup>
	The ratio of the mass fraction in milk								
Fat to lactose	+0.01	+0.02	+0.01	-0.05	+0.05	+0.04	-0.11 <sup>a</sup>	-0.17 <sup>b</sup>	-0.14 <sup>b</sup>
Protein to lactose	+0.06	+0.05	+0.06 <sup>a</sup>	-0.02	-0.05	-0.04	-0.10 <sup>b</sup>	-0.09 <sup>b</sup>	-0.09 <sup>b</sup>

Note: <sup>a</sup> – P < 0.05, <sup>b</sup> – P < 0.01.

Compiled by the authors

The main features of II HNA type cows were that the amount of secreted milk, fat and lactose was significantly lower in the morning milking. This amounts to 15.8-15.9% in relative terms. In absolute terms, the amount of milk is lower by

1.42 kg, fat - by 42.7 g, protein - by 25.4 g and lactose - by 63.3 g. In the lunchtime milking there is a tendency to lag behind, as in general for two milkings up to 13.3% relative to animals of I HNA type (Table 2).

The mass fraction of protein in milk of cows of this type tended to increase separately for each milking and a significant difference for two milkings together by 0.22%. The ratio of the mass fraction of protein to the percentage of lactose for two milkings was significantly higher by 0.06.

In cows of III HNA type, compared with similar indicators of cows of I HNA type, there were only weak stable trends in increasing the mass fraction of lactose in milk up to 0.15% and reducing the protein content, as well as the ratio of protein to lactose (especially at lunch) up to 0.05% ( $P < 0.10$ ).

In cows of the IV HNA type, there was a tendency or a significant decrease in the morning and lunchtime of milk secretion to 18.3%, fat and protein to 30.6%. In these animals, compared with animals of the I HNA type, the mass fraction of fat in milk was lower up to 0.53% ( $P < 0.05$ ) and protein up to 0.28%, but lactose in milk, on the contrary, was consistently higher in both milkings by 0.25-0.26% ( $P < 0.05$ ).

The coefficients of the ratio of the mass fraction of fat and protein in milk to lactose were, both separately and together for two milkings, significantly lower in cows of IV HNA type, respectively, to 0.14 and 0.09, compared with cows of type I.

Thus, the amount of the main components of milk, their concentration and relationships under normal conditions are associated with the type of HNA. Under standard milking conditions, I HNA type cows outperformed type II animals in the amount of secreted milk, fat and lactose in the morning milking by 15.7-16.0%, type IV cows - in the amount of fat and protein per morning and lunch milking by 20.1-23.2% and in the mass fraction of fat - by 0.45%, protein - by 0.24%, but had a lower mass fraction of lactose in the secret by 0.25% from type IV cows.

Type II cows had a higher protein-lactose ratio by 0.06%, and type IV cows had a lower fat-lactose and protein-lactose ratio by 0.09-0.14 on average for two milkings, compared with type I cows. Trends were noted in this experiment, but no significant difference was found between animals of the I and III types of HNA in the secretory function of the mammary gland.

During the 2nd period of the experiment, the secretion of milk was studied during milking with a conditioned stimulus - the presence of a stranger, who was the experimenter. The technology of milking differed from the usual one in that for analysis, samples of milk were taken from the first streams (1 portion), machine milk (2 portion) and a limited ( $20 \pm 5$  g) portion of milk, manually milked after removing the milking machine, called "manual after-milking" (3 portion).

The first portion of milk was obtained before the milk ejection reflex, the second under conditions of prolonged action of this reflex, and the third portion at its final stage. The manifestation of the milk ejection reflex depends on the type of nervous system, and the composition of milk is associated with its characteristics. In cows of the I type of HNA, the average indicators for  $132 \pm 19$  day of lactation are presented. In cows of other types, the stage of lactation was almost the same. Type I cows secreted more machine milk by 10-20.6%, fat - by 7.7-23%, protein - by 10.6-19.6% and lactose - by 12.1-23.4%, compared with other types of HNA. They had machine milk yield with a mass fraction of fat and lactose at the level or higher than in animals of other types of HNA and protein relative to IV HNA type (Table 3).

In cows of I HNA type, from 1 to 3 portions of milk, the percentage of fat increases from 1.22 to 8.17 in the morning and from 2.05 to 7.35 - at lunchtime. Protein and lactose concentrations are more stable. In cows of I HNA type, the milk of the first streams is characterized by a low ratio of the mass fraction of fat to lactose: in the morning  $0.26 \pm 0.03$ , at lunchtime -  $0.46 \pm 0.07$ . This indicator for machine milk increases to  $0.74 \pm 0.04$  and  $0.86 \pm 0.05$ , respectively; and for manual after-milking - to  $1.77 \pm 0.11$  and  $1.58 \pm 0.21$ . The ratio of the mass fraction of protein in milk to lactose is more stable. In the studied portions, both in the morning and at lunchtime, it fluctuates in a limited range from 0.74 to 0.78.

In cows of the II HNA type in the milk of the first streams, there is a tendency or a significant increase both in the morning and at lunchtime, the mass fraction of fat - up to 0.99% ( $P < 0.05$ ), protein - up to 0.33% and, conversely, a decrease to 0.71% of mass fraction of lactose compared to type I.

Therefore, for milk of the first streams, the ratio of the mass fraction of fat to lactose is higher by 0.28 (P<0.05), and protein - by 0.11-0.13 (P<0.05). Cows of the II HNA type in terms of the amount of synthesized milk, fat, protein and lactose of the second portion tend or are significantly inferior to animals of the I type by 12.1-23%, especially in the morning milking (P<0.05).

**Table 3.** Characteristics of milk and its organic components when milking cows in the presence of a stranger (Second period of experiment)

Indicators	HNA type							
	I		II		III		IV	
	M ± m		± difference with type I					
Milking: M (Morning), L (Lunchtime)								
	M	L	M	L	M	L	M	L
Milk of the first streams (1st portion). Mass fraction, %								
Fat	1.22±0.12	2.05±0.37	+0.13	+0.99 <sup>a</sup>	+0.11	+1.34 <sup>a</sup>	-0.04	+0.84 <sup>a</sup>
Protein	3.57±0.05	3.25±0.17	+0.26 <sup>a</sup>	+0.33	+0.04	+0.15	-0.26 <sup>a</sup>	0
Lactose	4.70±0.06	4.28±0.12	-0.27 <sup>a</sup>	-0.71	+0.09	-0.75	+0.02	-0.92
The concentration ratio in milk								
Fat to lactose	0.26±0.03	0.46±0.07	+0.05	+0.28 <sup>a</sup>	+0.02	+0.29 <sup>a</sup>	-0.05	+0.10
Protein to lactose	0.76±0.03	0.74±0.07	+0.11 <sup>a</sup>	+0.13 <sup>a</sup>	-0.01	+0.01	-0.10 <sup>b</sup>	-0.07
Machine milk, (2nd portion). Amount								
Milk, kg	8.69±0.29	5.0±0.33	-1.65 <sup>b</sup>	-0.71	-1.79 <sup>a</sup>	-0.75	-0.89	-0.57
Fat, g	308.0±16	207.4±13	-70.8 <sup>b</sup>	-32.4	-62.1 <sup>a</sup>	-16.0	-83.3 <sup>b</sup>	-30.6
Protein, g	310.0±5.7	181.0±11.3	-42.0 <sup>a</sup>	-22.5 <sup>a</sup>	-32.8 <sup>a</sup>	-35.4 <sup>a</sup>	-62.0 <sup>a</sup>	-30.0 <sup>a</sup>
Lactose, g	414.0±12.3	226.0±20.6	-96.9 <sup>b</sup>	-27.3	-66.3 <sup>a</sup>	+42.0	-84.2 <sup>a</sup>	-51.0
Machine milk Mass fraction, %								
Fat	3.55±0.18	4.17±0.24	-0.15	-0.20	-0.22 <sup>a</sup>	-0.06	-0.65 <sup>b</sup>	-0.22
Protein	3.57±0.07	3.62±0.07	+0.26 <sup>a</sup>	+0.09	+0.02	-0.14	-0.28 <sup>a</sup>	-0.26 <sup>a</sup>
Lactose	4.77±0.22	4.58±0.09	-0.26 <sup>b</sup>	-0.44 <sup>b</sup>	-0.10 <sup>a</sup>	-0.14	-0.04	-0.16
The concentration ratio in milk								
Fat to lactose	0.74±0.04	0.86±0.05	+0.01	+0.04	-0.03	+0.01	-0.16 <sup>b</sup>	-0.12 <sup>b</sup>
Protein to lactose	0.75±0.03	0.75±0.03	+0.10 <sup>b</sup>	+0.10 <sup>b</sup>	+0.02	-0.01	-0.11 <sup>b</sup>	-0.08 <sup>b</sup>
Hand after-milking milk, (3rd portion) Mass fraction, %								
Fat	8.17±0.61	7.35±0.93	-0.16	-0.43	-0.90	-0.66	-1.14	-0.99
Protein	3.55±0.12	3.62±0.09	+0.32 <sup>a</sup>	+0.07	+0.10	+0.14	+0.05	-0.21 <sup>a</sup>
Lactose	4.60±0.12	4.50±0.08	-0.18 <sup>a</sup>	-0.32 <sup>a</sup>	-0.13	-0.09	-0.16	+0.02
The concentration ratio in milk								
Fat to lactose	1.77±0.11	1.58±0.21	+0.06	+0.02	-0.15	-0.11	-0.47	-0.53 <sup>a</sup>
Protein to lactose	0.77±0.04	0.78±0.03	+0.11 <sup>a</sup>	+0.08 <sup>a</sup>	+0.01	-0.01	+0.01	-0.04

Note <sup>a</sup> – P < 0.05, <sup>b</sup> – P < 0.01.

Compiled by the authors

In cows of the II HNA type in the milk of machine milking and manual after-milking, especially in the morning milking, the mass fraction of protein was higher up to 0.26-0.32% ( $P<0.05$ ) and, conversely, significantly lower - up to 0.44% ( $P<0.05$ ) of lactose. Milk of 2 and 3 portions is characterized by an increased ratio of the mass fraction of protein to lactose by 0.08-0.11 ( $P<0.05$ ), compared with type I of HNA.

Cows of the III HNA type are characterized in machine milk yield by a reduced level of secretion of milk, fat, protein and lactose, compared with animals of the I type of HNA by 7.7-20.2%, especially manifested in the morning milking. At lunchtime milking, there was an increase in the fat content of the milk of the first streams by 1.34% in absolute terms, as well as the ratio of the mass fraction of fat to lactose to 0.29 ( $P<0.05$ ). Other indicators changed insignificantly relative to I HNA type.

Cows of IV HNA type differed from animals of type I in machine after-milking with a reduced level of fat, protein and lactose secretion, especially in the morning milking, from 10.2 to 27.0%. The milk of the first streams is characterized by a reduced mass fraction of protein in the morning milking (up to 0.25%), its ratio to the mass fraction of lactose (up to 0.10%) and a slightly increased mass fraction of fat, especially in the lunchtime milking. In machine milk, these animals had lower mass fractions: fat up to 0.65% and protein up to 0.28% ( $P<0.05$ ) in absolute terms; the fat-lactose and protein-lactose coefficients in the morning and lunchtime milkings were also lower to 0.16 ( $P<0.01$ ) relative to I HNA type.

The milk of the third portion in cows of type IV is characterized by a reduced coefficient of the ratio of the mass fraction of fat to lactose (up to 0.53 at  $P<0.05$ ), compared with I HNA type.

In the third period of the experiment, the following were additionally applied: a conditioned stimulus - a delay in milking and an unconditioned stimulus - a decrease in irritation of the udder receptors before milking, compared with the previous one. The milking technology differed from the usual one in that the preparation of the udder was incomplete - for 10 s and included rubbing the udder with a damp towel and milking off the first streams. This was followed by abstinence with putting teat cups on the nipples for 30 seconds. From the beginning of the preparation of the udder to the beginning of milking,

50 seconds passed, as in previous periods of experiment. Also, portioned selection of milk and its analysis were carried out. This technology is the same as in practice.

The average indicators for the third period in cows of the I HNA type are presented in Table 4. Animals showed a tendency or significantly secreted more milk by 6-24.5%, fat by 10.3-50.0%, protein by 12.8-36, 1% compared with other types of HNA, and lactose - up to 28.9% compared with type II. In type I animals, the mass fraction of fat increases: in the morning from  $1.15\pm 0.09$  in the first portion of milk to  $2.97\pm 0.29$  in the second and up to  $5.15\pm 0.24$  in the third. At lunch, it increases from  $2.77\pm 0.54$  in the first portion of milk to  $5.03\pm 0.35$  in the second and up to  $8.47\pm 1.4$  in the third. In the morning, the mass fraction of fat was at a lower level compared to the previous period, and at lunchtime, on the contrary, it was higher. The ratio of the mass fraction of fat to lactose increases in the morning from  $0.24 \pm 0.02$  in the milk of the first streams to 1.2 in the milk of the third portion. And in lunch milking, respectively, it increases from  $0.58 \pm 0.13$  to  $1.8 \pm 0.34$ . The mass fraction of protein and lactose in different portions of milk changes to a lesser extent than its fat content. The ratio of the mass fraction of protein to lactose varies within a limited range from 0.74 to 0.79.

It is noteworthy that cows of the II type of HNA, in comparison with animals of the I type, in machine milk yielded significantly less milk in the morning and afternoon milking up to 24.5%, fat, protein and lactose from 12.8 to 43.7%. At the same time, in both milkings in all portions of milk, a reduced concentration of lactose to 0.69% was observed. In the second portion of milk, the fat content in the lunch milking decreased to 1.45% ( $P<0.05$ ).

A distinctive feature of cows of II HNA type is also that in all three portions of milk there is a significant increase in the protein-lactose ratio, especially in the morning milking, up to 0.17 relative to I HNA type (Table 4).

The fat-lactose coefficient for machine milk in the first milking was higher by 0.14, and in the lunch, on the contrary, lower by 0.21% ( $P<0.05$ ), compared with I HNA type.

Cows of III HNA type tended to reduce the synthesis of milk and its components, compared with type I, up to 25%. In the milk of all three portions, there was a

trend or a significant decrease in the mass fraction of lactose and protein. The fat concentration was significantly reduced in the second portion of the lunch milking to 0.83% compared with I HNA type.

A sharp increase in milk fat content up to 3.68% ( $P < 0.01$ ) in absolute terms in morning milking and fat-lactose coefficient up to 0.64 was noted in the third period, which is characteristic of the III type of HNA, in manual milking milk, compared with I type of HNA (Table 4).

**Table 4.** Characteristics of milk secretion in case of inadequate udder preparation with a delay in milking (Third period)

Indicators	Type of HNA							
	I		II		III		IV	
	Milking: morning (M), Lunchtime (L)							
	M	L	M	L	M	L	M	L
	M±m		M±m		± differences with type I			
Milk of the first streams (1st portion).								
Mass fraction, %								
Fat	1.15±0.09	2.77±0.54	+0.03	-0.01	+0.04	+0.28	+0.29	+0.13
Protein	3.77±0.09	3.77±0.09	0	-0.01	-0.21	-0.18	-0.38 <sup>a</sup>	-0.27
Lactose	4.82±0.05	4.80±0.12	-0.68 <sup>c</sup>	-0.39 <sup>a</sup>	-0.23	-0.26	-0.07	-0.33
The ratio of the mass fraction in milk								
Fat to lactose	0.24±0.02	0.58±0.13	+0.05	+0.05	+0.02	+0.10	+0.08	+0.24
Protein to lactose	0.78±0.03	0.79±0.04	+0.13 <sup>b</sup>	+0.07	0	+0.01	-0.12 <sup>c</sup>	+0.26
Machine milk yield, (2nd portion). Amount:								
Milk, kg	8.79±0.25	5.02±0.34	-2.15 <sup>c</sup>	-1.01 <sup>a</sup>	-1.30	-0.41	-0.53	-1.38 <sup>a</sup>
Fat, g	263.0±3.2	254.4±33.6	-61.2 <sup>a</sup>	-11.12 <sup>b</sup>	-43.6	-6.36 <sup>a</sup>	-27.1	127.3 <sup>b</sup>
Protein, g	284.6±2.64	189.1±14.5	-36.4	-39.0 <sup>a</sup>	-60.0	-24.5	-89.0	-68.3 <sup>c</sup>
Lactose, g.	383.6±38.3	344.2±38.3	-110.9 <sup>a</sup>	67.8 <sup>b</sup>	-13.1	-80.5	+32.1	-67.82
Mass fraction, %								
Fat	2.97±0.29	5.03±0.35	+0.12	-1.45 <sup>b</sup>	-0.06	-0.83 <sup>a</sup>	-0.10	1.45 <sup>c</sup>
Protein	3.57±0.10	3.77±0.09	+0.18	0	-0.16	-0.20	-0.26	-0.29
Lactose	4.80±0.06	4.87±0.09	-0.69 <sup>c</sup>	-0.50 <sup>c</sup>	-0.5 <sup>c</sup>	-0.29 <sup>a</sup>	-0.13 <sup>a</sup>	-0.21
The ratio of the mass fraction in milk								
Fat to lactose	0.62±0.06	1.03±0.06	+0.14 <sup>a</sup>	-0.21 <sup>a</sup>	+0.02	-0.11	-0.03	-0.33 <sup>c</sup>
Protein to lactose	0.74±0.02	0.78±0.03	+0.17 <sup>c</sup>	+0.09 <sup>a</sup>	+0.03	+0.01	-0.1 <sup>c</sup>	-0.09 <sup>a</sup>
Hand after-milking milk, (3 <sup>rd</sup> portion)								
Mass fraction, %								
Fat	5.15±0.24	8.47±1.4	+0.47	-2.71	+3.68 <sup>b</sup>	-1.65	-1.16	-4.45 <sup>c</sup>
Protein	3.40±0.26	3.63±0.12	+0.36	+0.13	0	-0.22	+0.1	-0.01
Lactose	4.35±0.22	4.73±0.12	-0.23	-0.33 <sup>a</sup>	-0.03	-0.31	+0.2	-0.08
The ratio of the mass fraction in milk								
Fat to lactose	1.20±0.10	1.80±0.34	+0.17	-0.47	+0.64 <sup>b</sup>	-0.24	-0.04	-0.95 <sup>a</sup>
Protein to lactose	0.78±0.03	0.77±0.04	+0.13 <sup>c</sup>	+0.09	+0.01	0	-0.02	-0.01

Note: <sup>a</sup> –  $P < 0.05$ . <sup>b</sup> –  $P < 0.01$ ; <sup>c</sup> –  $P < 0.001$ .

Compiled by the authors



Cows of the IV type of HNA, under conditions of inadequate udder preparation, secreted a smaller amount of milk and its organic components in machine milk in the morning milking, up to 31.1%, and at the lunchtime, up to 50%, compared with type I. At the same time, in animals of a weak type, the mass fraction of protein and lactose in the milk of the first portion was lower, and in the second portion of milk, fat was also added, both in the morning and in the afternoon. In cows of the weak type, in the third portion of milk at lunch, the mass fraction of fat dropped sharply (up to 4.45% at  $P < 0.001$ ) and, accordingly, the fat-lactose coefficient - by 0.95, compared with the strong balanced mobile type. For type IV cows, it is characteristic that their machine milk has a reduced protein-lactose ratio to 0.10 in both milkings. There is also a sharp decrease in the mass fraction of fat in this portion of milk and in the milk of manual milking at lunch milking to 4.45% and a decrease in the fat-lactose coefficient to 0.33-0.95, compared with type I of HNA (Table 4). According to the complexity of synthesis, organic components can be conditionally distributed as follows: milk protein comes first, then fat, then lactose disaccharide. Increased protein synthesis under normal conditions occurs in cows of types I and II of HNA, worse in cows of types III and IV. This is accompanied by an increased protein-lactose ratio in strong types of cows and especially in type II cows. This means a direct connection with the process of growth of the protein-lactose ratio. Fat synthesis is most successful in type I cows in absolute terms, compared to other types, regardless of milking conditions. Moreover, in strong cows of types II and III, the mass fraction of fat is also generally maintained at a high level. The lowest mass fraction of fat is in type IV cows. Moreover, the ratio of the percentage of fat to lactose is also higher in type I cows and lower in type IV animals, that is, there is a direct connection between the excitation process and the synthesis and mass fraction of fat and fat-lactose coefficient. Fluctuations in the fat-lactose ratio directly copied the absolute percentage of fat in milk, showing the biochemical regularity of the use of glycerides, monosaccharides as a "building material" for fat. The ratio of the mass fraction of fat and protein in milk shows the relationship between the processes of protein synthesis, as a plastic material in the life of the species, and fat, as the most important source of energy and building material, to a lesser extent. Moreover, in cows of weak type IV,

relative to types I, II and III of HNA, the synthesis is decided by the management systems of the animal body in favor of protein synthesis, compared with fat.

Consequently, there are distinctive features of the secretion of the main organic components of milk in cows, due to the types of HNA. The well-known pattern of genetic determination of the concentration of protein, fat and sugar in milk should be supplemented by a mechanism for its implementation with the inclusion of the type of HNA. Moreover, the genetic code, according to our hypothesis, is implemented through a physiological mechanism with the inclusion of the type of nervous system. Further experiments are required to decipher it.

As an example of intermediate links between the higher parts of the nervous system and the mammary gland, one can, in particular, take the insular activity of the pancreas, which is involved in the regulation of carbohydrate metabolism in the body and the mammary gland.

Type IV HNA cows have an increased reserve of sugar or its precursors, which is not used as intensively for fat and protein synthesis under normal conditions as in other types. In stressful situations, the amount of secreted lactose in cows of types II, III and IV and its concentration in milk decrease.

Thus, with inadequate preparation of the udder for milking in type I cows, secretion and the mass fraction of fat in milk and the ratio of its concentration to lactose decrease to 17% in the first (morning) milking. Moreover, in the milk of manual after-milking, the last two indicators are reduced to 36%, compared with the previous day. Other indicators did not change significantly; however, secretion is completely restored in the second milking. Under these conditions, in all cows of other types of HNA, in the first milking in the milk of the first streams, the mass fraction of protein and lactose decreases by 5-10%, and in the milk of machine milk - lactose by 3-9% compared with I HNA type. Additionally, in type II cows in the first milking, the protein-lactose ratio increases by 7%, and the fat-lactose ratio - by 14%. But the latter in the second milking is reduced to 11%. In cows of the III and IV types of HNA in the first milking in the milk of manual after-milking, the concentration of fat and the ratio of fat to lactose increase by 31-51%, respectively. And in type IV cows, in the first milking, the fat concentration and the ratio of fat to

lactose concentrations increase by 16%, and in the second milking, on the contrary, they decrease to 21% compared to type I.

Type I cows show increased resistance to stressors, followed by type III and II animals. This feature of the types shows the flexibility of nature in terms of life support options: the lower limit of adaptability to the environment is provided with a reduced level of fat and protein secretion in milk, giving preference to protein synthesis. At the same time, the proportion of lactose, and not fat, increases from the energy material. Therefore, a more economical way to ensure the vital activity of the organism of highly productive animals with a weak excitation process is a reduced content of fat and protein in milk.

The above material shows nothing more than the presence of four types of HNA, qualitatively differing in the synthetic activity of the mammary gland. In a broader sense, this reflects no less clear differences in physiological, biochemical, and other differences in the animal body, due to the properties of the nervous system as a whole and reflected in the genetic code inherent in the reproductive cell. These data can be used in deciphering the mechanism of transmission by inheritance of the HNA type and the role of the quality of milk fed to the calf.

Thus, according to the adequacy of the reaction, the stability of the secretory function and the speed of adaptation to the applied stimuli, type I HNA is in the first place, followed by type II and then type III, and finally, at the lowest level, type IV HNA.

Using the term "stress resistance" it is possible to conditionally classify animals as follows: I HNA type - stress-resistant, type II and III - medium stress-resistant, type IV - non-stress-resistant, inverse.

In the following experiment, the influence of the type of higher nervous activity on the lactation of cows in the production conditions of the Rassvet experimental farm, Krasnodar Research Centre for Animal Husbandry and Veterinary Medicine, Krasnodar was studied on an experimental breeding farm. It was carried out throughout lactation on 39 Black-and-White cows (taken without exception as they calved). Of these, 9 heads were assigned to type I HNA, 8 heads - to type II, 13 heads - to type III, and 9 heads to type - IV.

The technology of milk production on the Rassvet experimental farm of the Krasnodar Research Centre

for Animal Husbandry and Veterinary Medicine was described earlier.

The secretory function of the mammary gland was studied by control milking two times a month.

An analysis of daily productivity by months of lactation showed that in cows of type I HNA, the amount of secreted milk per day in the first month of lactation was  $20.3 \pm 1.51$  kg, in the second -  $19.9 \pm 1.78$  kg, in the third -  $21, 3 \pm 1.73$  kg. The difference in daily milk yield in favor of I HNA type was indicated from the first month of lactation to the third month. It increased to a clear trend or significant level compared to other types of HNA.

The milking of type I cows was more successful than others. Moreover, the differences in productivity were clearer in absolute terms of milk mass between types than in percentage of the level of the first month of lactation, which characterizes a stable retention of the level of secretion. As a result, already in the first hundred and two hundred days of lactation, trends or significant differences in the secretion of the amount of milk and fat between types of HNA were established. These differences with other types increased over 305 days in terms of the amount of secreted milk and fat to significant.

The coefficient of lactation stability (the ratio of milk secretion for the second 100 days of lactation to the first) in cows of I HNA type was  $0.92 \pm 0.07$ ; II type -  $0.86 \pm 0.05$ ; III type -  $0.92 \pm 0.04$ ; IV type -  $0.90 \pm 0.02$ . The difference between the coefficients is not significant. The difference in the duration of the service period in cows of different types is also not significant. From cows of the It type of HNA, 5557 kg of milk containing 210.5 kg of fat were milked for the first lactation, which is more than in animals of the II type, respectively, by 1037.7 and 42.4 kg; III type - for 1257.7 and 43.8 kg; IV type - for 650.2 and 34.6 kg. The fat content of milk in cows of type I was 3.78% and did not significantly differ from cows of types II and III, but was higher by 0.19% compared to animals of IV HNA type.

An analysis of the level of productivity of the three closest ancestors of cows of various types of HNA shows that mothers, mothers of mothers and mothers of fathers of cows of I HNA type do not have an excess in milk secretion compared to other types.

Thus, the manifestation of milk productivity by cows in our experiment is not associated with the high milk

yield of ancestors, but is associated with their types of higher nervous activity.

Based on the studies carried out on the systematization of individual differences in animals of different types of higher nervous activity, we propose a model of indicators of the desired type of

Black-and-White cow with increased adaptation to violations of the milking stereotype and keeping that occur in production conditions when milked by serial machines. It can be used in the development and implementation of breeding programs and in the acquisition of modern animal farms with increased resistance to intensive technology (Table 5).

**Table 5.** Model of Black-and-White breed cows with different resistance to stress, compared with average indicators (In the morning milking under normal production conditions at 2-5 month of lactation)

Index	Stress resistance type		
	steady	average	weak
	Secretory function for machine milk yield: amount, kg		
Milk	higher	average, lower	lower, average
Fat	higher	average	lower
Protein	higher	average	lower
Lactose	average, higher	average	average
	Mass fraction, %		
Fat	higher	average	lower
Protein	average	higher, average	lower
Lactose	average	higher, average	higher
	Ratio		
Fat % to lactose %	higher	average	lower
Protein % to lactose %	higher	average, higher	l

*Compiled by the authors*

## Discussion

The experiment was carried out on first-calf heifers of Black-and-White breed. In all animals, the types of higher nervous activity were determined by the method of conditioned reflexes to food stimuli. Cows in the first half of lactation were divided into groups according to the type of higher nervous activity (HNA).

The amount of the main components of milk, their concentration and relationships under normal conditions are associated with the type of HNA (Stephansen et al. 2018).

The bioactive components contained in milk have antibacterial, antiviral and antifungal effects, as well as anti-inflammatory and antioxidant properties. The growing demand for the so-called "clean label" foods is the driving force for many of the conducted researches in the last two decades (Khalid et al. 2022; Studenica et al. 2022; Zheleva 2022).

Under standard milking conditions, I HNA type cows outperformed type II animals in the amount of secreted milk, fat and lactose in the morning milking

by 15.7-16.0%, type IV cows - in the amount of fat and protein per morning and lunch milking by 20.1-23.2% and in the mass fraction of fat - by 0.45%, protein - by 0.24%, but had a lower mass fraction of lactose in the secret by 0.25% from type IV cows.

Type II cows had a higher protein-lactose ratio by 0.06%, and type IV cows had a lower fat-lactose and protein-lactose ratio by 0.09-0.14 on average for two milkings, compared with type I cows. Trends were noted in this experiment, but no significant difference was found between animals of the I and III types of HNA in the secretory function of the mammary gland.

According to the adequacy of the reaction, the stability of the secretory function and the speed of adaptation to the applied stimuli, type I HNA is in the first place, followed by type II and then type III, and finally, at the lowest level, type IV HNA. Using the term "stress resistance" it is possible to conditionally classify animals as follows: I HNA type - stress-resistant, type II and III - medium

stress-resistant, type IV - non-stress-resistant, inverse.

An analysis of daily productivity by months of lactation showed that in cows of type I HNA, the amount of secreted milk per day in the first month of lactation was  $20.3 \pm 1.51$  kg, in the second -  $19.9 \pm 1.78$  kg, in the third -  $21.3 \pm 1.73$  kg. The difference in daily milk yield in favour of I HNA type was indicated from the first month of lactation to the third month. It increased to a clear trend or significant level compared to other types of HNA. This is consistent with the findings of other studies (Hemsworth 2003; Marçal-Pedroza et al. 2020).

Based on the studies carried out on the systematization of individual differences in animals of different types of higher nervous activity, we propose a model of indicators of the desired type of Black-and-White cow with increased adaptation to violations of the milking stereotype and keeping that occur in production conditions when milked by serial machines. It can be used in the development and implementation of breeding programs and in the acquisition of modern animal farms with increased resistance to intensive technology.

## Conclusions

Based on the experiments performed in a series of tests, we came to a number of general conclusions:

1. From first-calf heifers of I type of HNA, 5557 kg of milk was produced for lactation, containing 210.5 kg of fat, which is more milk by 650-1257 kg, milk fat - by 35-43.8 kg than from animals of other types.
2. Under conditions of an intensive production method in the morning milking, cows of the I HNA type, the most resistant to stressors, have higher milk yield and fat secretion, milking time, the number of milk flow cycles and the ratio of percentage of fat to percentage of lactose, relative to the average level. Cows of unbalanced and inert types of HNA, as cows of the average type of stress resistance, show average milk productivity, higher milk flow rate at milking of the first 50 (100) g of milk and in the first minute. In cows of a weak type of HNA, secretion and concentration of fat and protein in milk, the ratio of the percentage of fat and protein to the percentage of lactose, the rate of milk flow, the number of milk flow cycles are lower, compared with cows of type I HNA.

3. The conditioned stimulus, the presence of an "experimenter" during morning milking, practically does not affect the function of the mammary gland in cows of I HNA type. In cows of other types, the concentration of lactose decreases. At the same time, in animals of an unbalanced type of HNA, relative to the standard level, the number of milk flow cycles increases with a decrease in the duration of one cycle. In inert type cows, the secretion of fat, protein and lactose is sharply reduced, and in weak type cows, lactose secretion is reduced.

4. Under the influence of the unconditioned stimulus "inadequate udder preparation" in the milk of machine milk in cows in the morning milking: I HNA type - secretion and percentage of milk fat and the ratio of percentage of fat to percentage of lactose decreases, while in all other types there is a decrease in the percentage of fat and protein. In the unbalanced (II) type, the average duration of the milk flow cycle decreases; in the inert (III) and weak (IV) type, the concentration of fat and lactose decreases, and the ratio of fat to lactose in the latter.

5. In an experiment on a breeding farm, we found an approximately equal number of all four types of HNA among cows. Consequently, the transmission of HNA types by inheritance did not actually lead to the predominant representation of I type in the enterprise: all types are present in the offspring.

6. It is advisable to characterize the stress resistance of animals based on knowledge of their types of HNA. At the same time, the complexity of their determination by known methods should be noted.

7. In dairy farming, it is necessary to provide for the presence of all types of HNA among the cows and take into account their individual characteristics in terms of resistance to stress, the level of milk production and milk yield.

8. It is recommended to apply the model of dairy cows developed by us to objectively determine the type of their stress resistance in production conditions.

9. The presented patterns of changes in the secretory function of the mammary gland under the influence of tested stress factors on dairy cows of different types of higher nervous activity should be used to develop express methods for determining the types of stress resistance in animals and completing the herds of dairy farms.

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