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Biological features of cows with different levels of stress resistance

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The results of studies of biological features of 66 cows of Holstein breed with different reaction levels to stress have been quoted. The stress factor was the planned blood taking from them. In an hour after this procedure, blood was taken again from the jugular vein with the aim to determine the concentration of cortisol in experimental animals. Based on the different cortisol concentration in blood, the cows have been differentiated into three groups: I – with high stress resistance, II – with medium stress resistance and III – with low stress resistance. The aim of the studies was to establish the relationship between the different stress resistance in animals and the development and function of the udder, the milk productivity and the heat resistance of the organism under summer temperature loading in the conditions of Ukraine. It has been established that the animals of group I were milked in double-quick time (P <0.001), and their milk-ejection rate was 1.4 kg/min (P <0.001) compared to the third group. They also had a higher reaction rate at the beginning of milking (P <0.001). The parts of the udder in the cows of this group are more evenly developed, which serves as the best precondition for the implementation of the milk flow reflex by machine milking. Cows with low stress resistance have a well-defined prolonged latent period at the beginning of machine milking. This is explained by the delay in the reaction of their mammary gland in response to the beginning of milking. It has been suggested that this disadvantage can be mitigated by using a more improved milking equipment, with an udder massage regime, which is capable to compensate the disturbance of the milk yield reflex in cows, which are more sensitive to stress. The influence of different stress resistance on fat and protein content in the milk of Holstein cows has not been properly defined. But in comparison with low stress-resistant animals, milk yields for 305 days of the first lactation were higher in highly stress-resistant animals – by 639 kg (P <0.001), in cows of the middle group – by 216 kg (P <0.05). The lower resistance of cows to stress makes them vulnerable in conditions of temperature load during the hottest months (July, August) of the summer period in Ukraine. Their body reacts to such a load with sharper fluctuations in body temperature, pulse, respiration rate and thermal sensitivity index as compared to their peers with high level of stress resistance (P <0.01...0.001). To solve this problem with trans-humance grazing it is possible to use mobile canopies.
Key words: Holstein cows; cortisol; stress resistance; somatic development and udder function; milk production; heat resistance; Ukraine

Introduction

The most important property of living organisms is the ability to adapt to the influence of environmental factors, while maintaining homeostasis. Reasoning from this fact, life is a constant adaptation to changing environment conditions. Farm animals are influenced by a variety of stress factors: early weaning, permanent animal regrouping, high concentration of livestock on restricted areas, rank fighting, animal transportation, regular weighing, excessive noise from equipment and vibration, periodically changing technological regimes, planned veterinary and preventive measures, seasonal temperature fluctuations etc. It has been proved (Carroll et al., 2013; Chernenko et al., 2017; Hansen et al., 1998; Frank et al., 1989; Moberg et al., 2000; Roth et al., 1985), that under the influence of above mentioned and other factors, the organism leaves the state of equilibrium with the environment, and homeostasis is violated (Friend et al., 1977; Friend et al., 1980; Igono et al., 2008). It has also been established (Borell et al., 2007a; Chernenko, 2015; Lefcourt et al., 1982), that to the same stress factors, some of the animals react sedately, whereas others, on the contrary, react sharply. Researchers associate this with the ability of the body to adapt to operational stress (Salak-Johnson et al., 2006).
Some animals restore homeostasis faster, without a sharp deterioration in health, a decline in productivity and reproductive function abnormalities (Beilharz et al., 1982; Borell et al., 2007b). In others, the reaction to this stress factor leads to abrupt changes in the body (Giesecke et al., 1985; Wolfenson et al., 2000). It has been proved that the productivity of this process is

influenced by the intensity of stressor exposure (Lacetera et al., 2001), as well as individual adaptive abilities (Salak-Johnson et al., 2006), for which research is used a repeated blood sampling after a stressful load to study adrenocortical response. It has been established (Hopster et al., 1999) that the initial level of cortisol in cows was 0.5–15 ng/mL (1.4–41.4 nmol/L), and after repeated blood sampling from 4.5 to 22.6 ng/mL (12.4–62.3 nmol/L). And the maximum level of cortisol was observed after 30 and 60 minutes after re-taking blood.

In case of abrupt changes in the conditions of maintenance and operation, cows can face lactation disfunction (Gorewit et al., 1992; Wenzel et al., 2003). One of such visual displays is an inadequate reaction of the mammary gland in response to the beginning of milking, which may lead to mastitis, which result in significant economic losses, leads to a decrease in libido and a decrease in the quality of milk due to antibiotic therapy, as well as reduces the timing of cows' exploitation in herds (Heikkilä et al., 2012).

Climate change is a serious problem for the development of the livestock sector in Ukraine. Certain climatic conditions are typical for the steppe zone of Ukraine, with relatively warm and wet weather in winter, and quite hot and sometimes overly hot weather in summer. The problem of thermal stress is particularly relevant in regions where the weather is characterized by high summer temperatures and high humidity. In summer season, temperature indicators in Ukraine have only increased by 2–3 degrees in recent years (Martazynova et al., 2016). The researchers (Schüller et al., 2014; Bernabucci et al., 2014; Lalrengpuii Sailo et al., 2017) found that the temperature stress in dairy cattle every summer leads to the emergence of many diseases in dairy farms. According to their data, thermal stress reduces feed intake, reduces milk productivity, affects the content of fat and protein in milk, worsens the general health and disrupts the reproductive function of dairy cows. As a result, thermal stress reduces the achievements of genetics in increasing the milk yield of cows (Hammami et al., 2015). At the same time there is no data concerning how much overall stress resistance the heat resistance of cows, in particular the Holstein breed, the most highly productive in the world. In fact, different stress tolerance is caused by the manifestation of one or another type of higher nervous activity and is a hereditary conditioned symptom. Consequently, a gradual increase in the stress resistance of dairy cattle is possible by way of selection.

Thus, different body resistance to stress loads may have a negative influence on the thermal sensitivity of the body. The scientific novelty is represented by such studies of imported cattle, among which the most widespread breed is Holstein. Its thermal stability in Ukraine due to stress resistance remains an unexplored problem.

The purpose of this study is to establish a connection between the adaptive potential of the Holstein breed cows and the development and function of the udder, milk production, as well as the heat resistance of their organism in unfavorable climatic conditions during the summer period.

Material and methods

The researches have been carried out in the farming enterprise "Olympex-Agro" in Dnipropetrovsk oblast, where the stable-camp housing scheme of cows is used. It provides the housing scheme in the winter period in stationary premises for 200 heads. In the summer, animals are transferred to a summer camp, which is located near the fields of fodder crop rotation with the crops of the green forage chain. The milking of the cows was three-stage. At the summer camp, a passing through milking units has been used for this purpose.

The response of Holstein cows to stress has been based on the absolute values of the concentration of cortisol in the blood after the influence of technology stress factors on animals. Blood samples have been collected in 30 minutes after milking, according to the method (Hopster et al., 1999). Stress influence on animals has been provided by fixing them for collecting blood from the jugular vein. And the sharpest stress factor was the process of blood sampling and the contact with the veterinarian. The first blood collection was done because of the planned veterinary control of the physiological state of animals in the herd. And the second one in an hour after it. All this time the cows were in a captured position. Because of this, they did not have access to food and water. The concentration of cortisol has been found out in the blood of cows within an hour after stress load.

Blood serum has been used to determine cortisol concentrations in cows. The methodology provides that with certain amounts of antibody bonds, competition between unlabeled and enzyme-labeled antigens arises. Moreover, enzyme-labeled antigens have an inverse proportion with the concentration of the unlabeled sample. The study has been performed on a microplate enzyme immunoassay analyzer, *Stat Fax-2100*, with a configurable wavelength of 450, 600, and 620 nm. Diagnostic system laboratory reagents (USA) and semi-automatic dispensers for 5–50, 50–200 and 200–1000 µL have been used. The processing of the samples under investigation has been carried out on the *Stat Fax-2200* incubator shaker. Shaking samples took place at a frequency of 500–700 times per minute. For automatic washing out, the Stat Fax-2600 has been used. To measure the optical density of the solution, the instrument has been tuned to a wavelength of 450 and 620 nm. This has been done in an extension of 30 minutes after the stop solution had been added to the sample. Before the instrument readings recording, the instrument, with allowance for absorption, had been converted to "0", which corresponded to the zero standard (Blank). To determine the cortisol concentration, a standard curve based on the values of the standards of this hormone have been used. Repeated examinations were subjected those samples in which the value was found with the exceed of the highest standard. Prior to this, the samples have been re-diluted using the standard 0 µg/dL. Since the standards have units of measurement µg/dl, their values have been converted to nmol/l, multiplied by a coefficient 27.6.

For division of animals into groups, a frequency distribution testing ground of the variation series for cortisol has been constructed. The number of classes in the testing ground has been calculated using Sterjes's formula. The distribution of animals by cortisol in the blood corresponded to normal, so they have been divided into three groups, based on the statistical

regularity, according to which the largest group ($\overline{X} \pm \sigma$) included the largest number of individuals. Animals with cortisol concentrations less than this range have been allocated to a group, conventionally called "high stress resistance", and their peers with a hormone concentration exceeding this range – in the group with the conventional name "low stress resistance".
The functional properties of the udder in machine milking have been investigated by the intensity of milk yield, milking time, the amount of milk for the first minute of milking and the duration of the latent period at the beginning of milking. These studies have been carried out during the morning milking of the cows. Experimental animals have been in the third month of the first lactation.
The intensity of milk yield in cows has been determined by the ratio of single milk yield to the duration of milking in minutes. The milking time has been measured by the stopwatch. Seconds have been transformed in minutes based on the correspondence: 6 seconds is 0.1 minutes. Amount of milk for the first minute of milking has been expressed as a percentage of the one-time milk yield. This indicator has been used to characterize the timeliness of the reaction of the mammary gland at the beginning of milking. The latent period has been measured by a stopwatch from the attachment of the last milking cluster to the nipples of the cow's udder prior to the start of milk ejection. Comparatively with other animals, a relatively long latent period may indicate a delay in the reflux of milk deliveries. To study the uniformity of the development of the parts of the udder, they have been milked with a special milking unit.
In order to determine the adaptive ability of different stress resistance types of cows to the environment in the hottest summer months, the breathing time has been defined by calculating respiratory movements per minute, the rectal temperature – by an electronic thermometer of the TM Microlife and pulse – by palpation of the caudal vein. In summer season, all the measurements have been carried out with minimum air heating in the morning (400–600) and lunch (1300–1500) at maximum temperature in the shade +40°C and above.
Based on the evidence found, the heat tolerance coefficient (HTC) has been determined according to the formula (Benezra, 1954):

$$HTC = \frac{T_2}{38.3} + \frac{RR}{23},$$

Where:
HTC – Heat tolerance coefficient (*Benezra Coefficient of Heat Adaptability*); T_2 – body temperature at temperature load, *°C*; *RR* – respiratory rate per minute at temperature load; 38.3 и 23 – are the mean values of body temperature and respiration frequency under optimal environmental conditions.

All animal our studies have been performed in accordance with the requirements of the European Convention for the Protection of Vertebrates Used for Experimental and Scientific Purposes (Strasbourg, 1986). The experimental data analysis has been performed using *Statastica 6.1.* The data in the figures are presented as: average, mean ± standard error, mean ± standard deviation.

Results

In high stress resistant cows (group I), the concentration of cortisol after stress was in the range of 10.0 to 26.0 nmol/L, medium stress resistance (group II) – from 27.0 to 55.0 nmol/L, whereas low stress (group III) – from 56.0 to 72.0 nmol/L (Fig. 1).

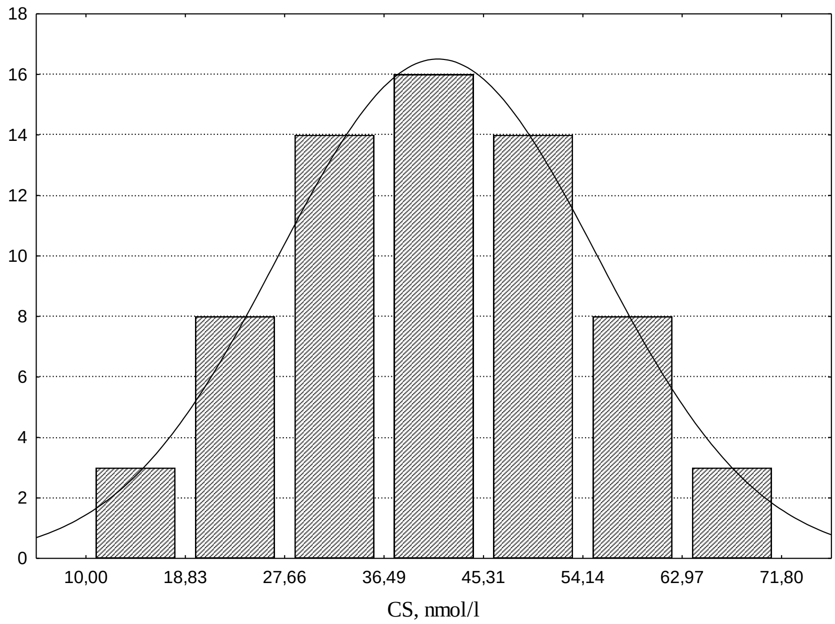

Fig. 1. Frequency distribution of the variation line for cortisol in 1 hour after stress load (n = 66)

The given data indicate that the experimental animals of the three groups are characterized by a certain variation in the concentration of cortisol (Fig. 2). In particular, in group III cows, the concentration of cortisol in the blood was 42.1 nmol/L (P <0.001), after an hour after the stress load, compared to the herd mates of group I. Animals of the II group took an intermediate position.

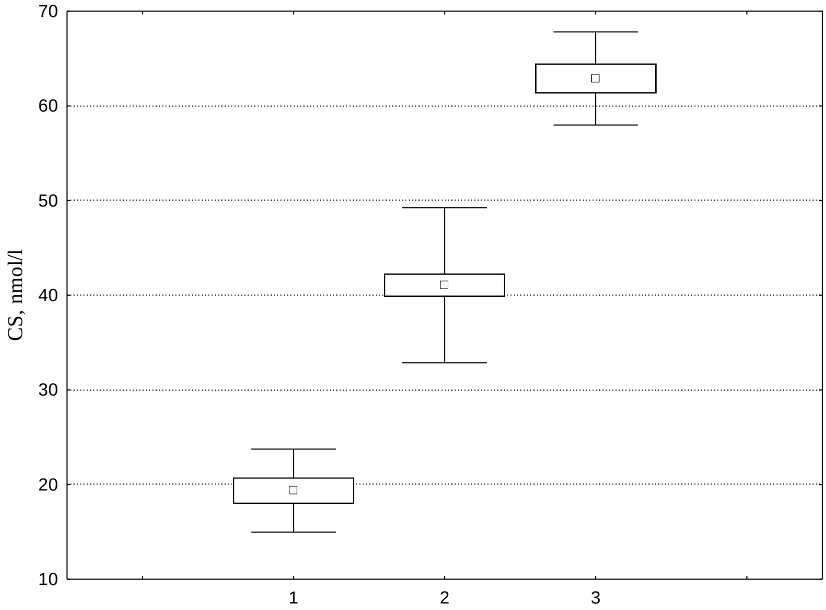

Fig. 2. Concentration of cortisol in blood of experimental animals after stress load: 1 – with high stress resistance (n = 11); 2 – with medium stress resistance (n = 44); 3 – with low stress resistance (n = 11); Friedman ANOVA method

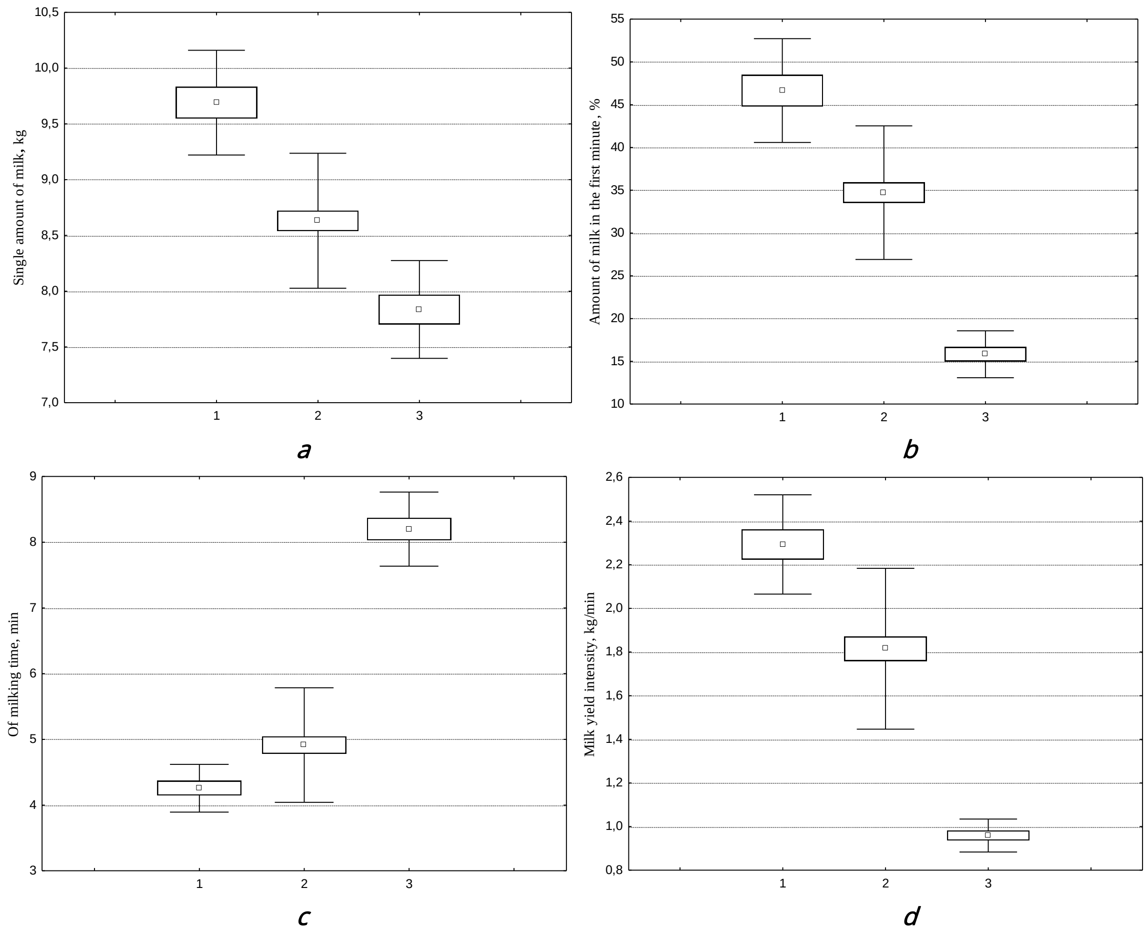

Fig. 3. Single amount of milk (a), amount of milk in the first minute (b) of milking time (c), milk yield intensity (d) in experimental cows with different stress response: 1 – with high stress resistance (n = 11); 2 – with medium stress resistance (n = 44); 3 – with low stress resistance (n = 11); Friedman ANOVA method

At one time, the cows of groups I and III significantly differed (Fig. 3a).
The difference was 1.7 kg (P <0.001) in favor of more stress resistant animals. In addition, they were milked more than twofold (P <0.001) (Fig. 3b). Accordingly, they had a higher milk-yielding intensity of 1.4 kg/min (P <0.001) (Fig. 3c).
Cows of group I better responded to the beginning of milking. The amount of milk during the first minute of milking exceeded the index of their herd mates of the third group by 36.4% (P <0.001) (Fig. 3d).
Animals of the II group took an intermediate position.
The amount of milk obtained from different parts of the udder in cows with different stress resistance is significantly different (Table 1).

Table 1. The amount of milk from individual parts of the udder and the duration of the latent period in cows with different stress resistance

Parts of the udder and latent period	Animal group		
	I, n=11	II, n=44	III, n=11
Right hand front	2.2±0.15	1.8±0.16	1.4±0.20**
Left hand front	2.2±0.17	1.7±0.17	1.5±0.19**
Right hand rear	2.7±0.15	2.5±0.18	2.3±0.19
Right hand rear	2.8±0.17	2.7±0.20	2.1±0.22
Front parts	4.4±0.21	3.5±0.20	2.9±0.22***
Rear parts	5.5±0.28	5.2±0.24	4.4±0.32*
Latent period, min	0.5±0.13	0.8±0.12	1.4±0.17***

* the difference from I group are valid at *P < 0.05*; ** – at *P < 0.01*; *** – at *P < 0.001*.

Compared to the peers of group I in the third group of cows, the front and rear parts of the udder have been developed significantly worse and the latent period was longer in 2.8 times (P <0.001), which indicates an inadequate response of their udder to the onset of machine milking.
We have also analyzed the milk yield of cows in the first 305 days of lactation (Fig. 4).

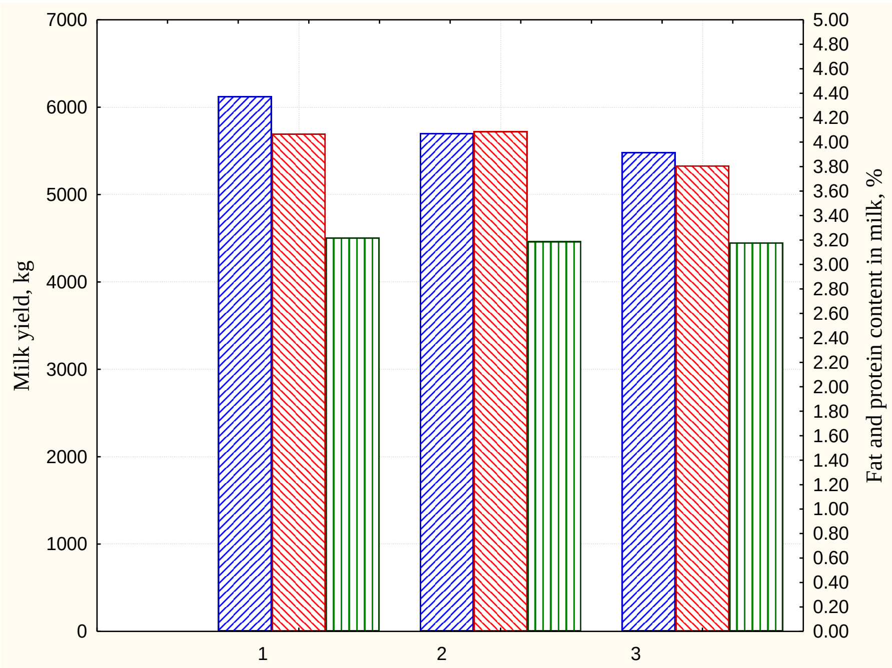

Fig. 4. Milk yield (left Y axis), fat and protein content in milk (right Y axis) in experimental cows with different stress resistance: 1 – with high stress resistance (n = 11); 2 – with medium stress resistance (n = 44); 3 – with low stress resistance (n = 11)

The obtained data (Fig. 4) indicate that according to the content of fat and protein in milk, the groups of animals almost have not difference. At the same time, in comparison with the third group, the milk yield was higher in cows of the group I – by 639 kg (P <0.001), in cows of group II – by 216 kg (P <0.05).
In recent years, climatic conditions have changed dramatically in Ukraine, especially in the summer. The periods with abnormal hot weather in the regions of Ukraine according to the data of hydrometeorological center (http://meteo.gov.ua/) are observed in July and August when the temperature of the air in the shadow reaches 35–38 °C and higher (Shevchenko et al., 2014). Therefore, in conditions of elevated temperature load, studies on the heat sensitivity of animals become relevant.

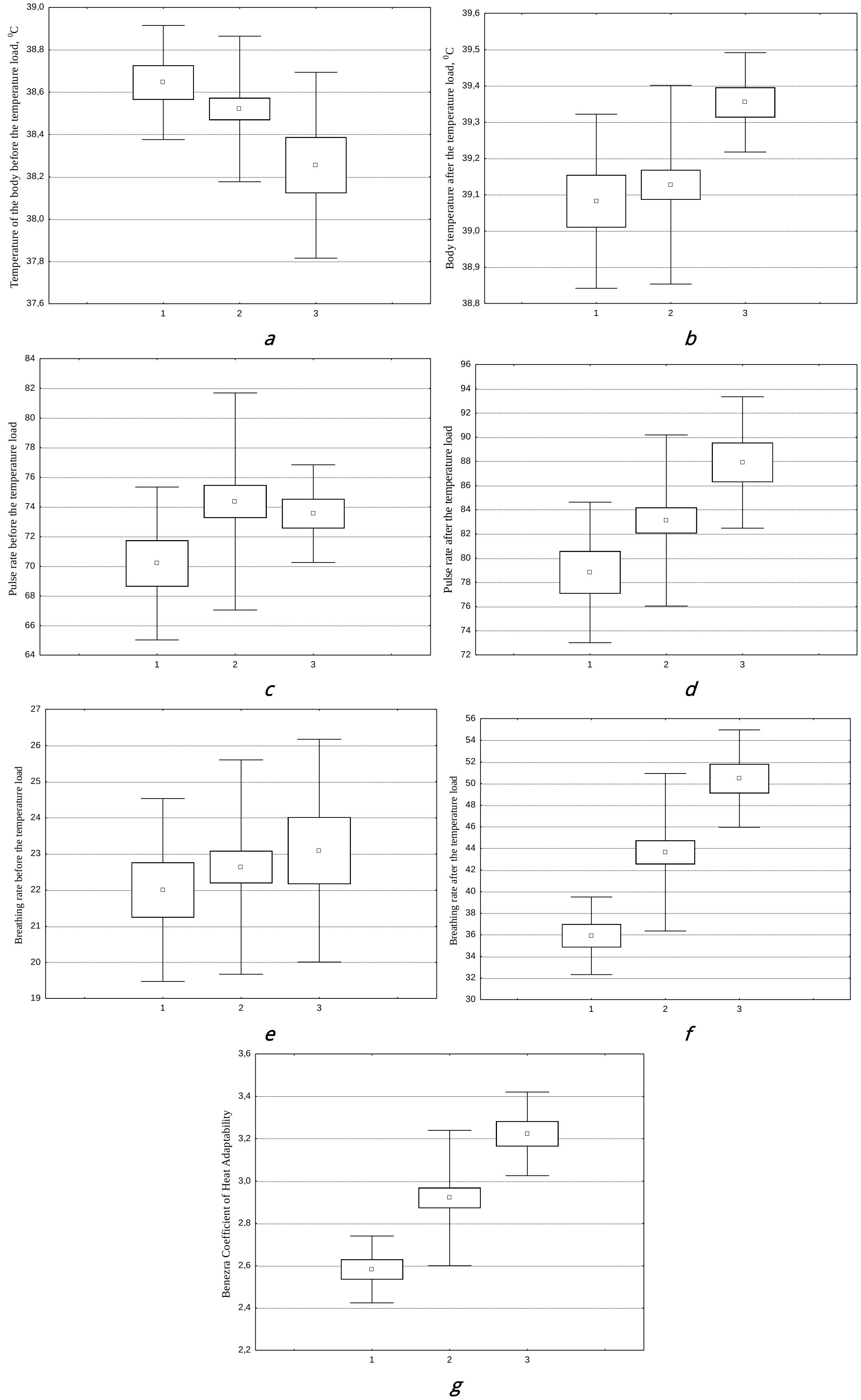

Fig. 5. Temperature of the body before the temperature load, °C *(a),* body temperature after the temperature load, °C *(b),* pulse rate before the temperature load *(c),* pulse rate after the temperature load *(d),* breathing rate before the temperature load *(e),* breathing rate after the temperature load *(f)* and Benezra Coefficient of Heat Adaptability *(g)* in experimental cows with different stress resistance: 1 – with high stress resistance (n = 11); 2 – with medium stress resistance (n = 44); 3 – with low stress resistance (n = 11); Friedman ANOVA method

From the data presented (Fig. 5a) it follows that, before the temperature load (in the morning after the night rest), the body temperature in cows of all groups was within the limits of physiologically permissible vibrations. In the peak period of daytime heat, it increased in cows of group I at 0.5 °C, and in cows of group III at 1.1 °C (P <0.001) in comparison with the index fixed in the morning. In cows of group III, it was still higher at 0.3 °C (P <0.01) compared with the peers of group I (Fig. 5b).
Similar dynamics have been observed according to the other factors, but under conditions of temperature load in cows of group III, compared with the peers of group I, the pulse increased by 9.1 (P <0.01) (Fig. 5d), the breathing rate – by 14.5 (P <0.001) (Fig. 5f), and the coefficient of thermal sensitivity by 0.6 units (P <0.001) (Fig. 5g). Cows of group II occupy an intermediate position.

Discussion

Cows with low adaptive capacity can experience stress immediately before milking, when they are in the milking area closely with other animals of higher rank. This results in a sharp ejection of adrenalin, which blocks the production of oxytocin by the pituitary gland – the evacuator of milk from the cow's udder at the machine milking (Moberg et al., 2000). At the same time, the ordinary milking machines are configured so that the cow, after connecting the udder with milking cluster, should almost immediately begin milk supply. If the milk release does not happen for a long time, there is a risk of penetration of the vacuum into the vena cysts and damage of blood capillaries. Subsequently, this may cause subclinical and clinical forms of mastitis (Giesecke et al., 1985).
Developers of milking equipment have created fundamentally new milk lines, which use a special electronic module designed to solve the problem of a prolonged latent period during milking. A special computer program transfers the pulsator to a ripple frequency, eight to ten times higher than with the usual milking mode, which achieves vibration and a peculiar massage of the nipples. This is how the standby mode starts before full milk delivery is started. But such technical characteristics possess exclusively electromagnetic pulsators of the new generation. Similar dairy lines using electromagnetic pulsators are present in Ukraine as an exception – only in single enterprises. At enterprises that use electromagnetic pulsators, a special milk line module is not always connected, without which the breast-massage mode is not active. Thus, such massage can help to smooth the inadequate response of low-stress tolerant cows to the onset of milking, due to a gentler mode.
In our studies, we have been confirmed that the different adaptive capacity of cows affects the duration of the latent period at the beginning of milking and other functional disorders of the udder, as well as leads to a reduction of milk yield. According to some data (Taponen et al., 2017), inadequate cow response to machine milking is also a factor which increases the risk of mastitis. Indeed, in the basis of the pathogenesis of mastitis, a fundamental role plays the innate immunity, as a natural reaction of the body to tissue trauma, which occurs under the influence of any damaging physical, chemical or biologic pathogen (Turk et al., 2017). At the same time, it has been proved that stress "breaks down" immunity (Salak-Johnson et al., 2006; Carroll et al., 2013). And the decrease in immunity leads to various diseases, in particular to mastitis. Such a fact is based on data of other authors (Negussie et al., 2008; Nyman et al., 2014; Rainard et al., 2006), which call this the main cause of the appearance of mastitis in cows. In addition, some scientists (Levison et al., 2016) associate mastitis not only with feeding and hygiene of cows, but also with the technology of machine milking (Leslie et al., 1997), which they believe is primarily associated with the possibility of bacteria penetration into the udder of cows. Of course, in the conditions of an increased latent period in machine milking and the uneven development of udder parts, in our opinion, such a risk increases significantly, and this may be the subject of further research.
Since the climatic conditions in Europe have been constantly changing, the study of heat sensitivity of animals is becoming more relevant not only in countries with traditionally hot climates (Barriopedro et al., 2011). In Ukraine, keeping cows on summer pastures and even in summer camps, animals often have no opportunity to escape from the sun under the sheds, since they have not been provided by a construction projects. After all, the problem was not so acute earlier, except for the South of the country.
In general, in our opinion, milk producers should be preferred only servicing bulls that have a genetic peculiarity to form a high adaptive capacity for their daughters, which in further generations can lead to the gradual displacement of animals with low stress resistance.

Conclusions

Low adaptive ability of cows can have a negative effect on the unevenness of the development of udder parts and the originating of an elongated latent period due to delays in the udder reaction to the onset of milking process. Functional dysfunctions of such animals' udder may be partially eliminated, due to the use of modern milking equipment capable of smoothing out the disadvantages of implementing the reflex of milk delivery at the beginning of milking.
Under low temperature stress conditions, cows with low-stress resistance are more vulnerable. This is proved by more sharp fluctuations in body temperature, pulse, respiratory rate and heat-sensitivity coefficient as compared with their high-stress resistance herd mates (P <0.01 … 0.001) and, therefore, leads to a decrease in milk yield. It is necessary to use modern mobile sheds to solve this problem in the conditions of grazing.
In our opinion, we must implement a planned standardization of dairy herds in terms of stress tolerance by means of selection. For this purpose, it is recommended to select for breeding flock those bulls which can model, in heritable way, at their daughters with high adaptive capacity. For this purpose, when selecting the bulls, it is important to apply a linear estimate of the exterior using those techniques in which there is a temper measure of their daughters.

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