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Determination of heart rate variability as an indicator of the influence of autonomic nervous system tone in cows

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Abstract. The relevance of the subject is the significance of exploring the role of the autonomic nervous system in regulating the cardiovascular system to improve the welfare of productive animals. Heart rate variability is a non-invasive research method that can be useful in exploring the health status of an animal and analysing its psychophysiological state in farm conditions. The purpose of the research – to determine the effect of the tone of autonomic nervous regulation on the cow's body, which is reflected in changes in the sympathovagal balance. Experimental research was conducted on Ukrainian Black-and-White dairy cows. To explore the variability of heart rate, an electrocardiograph was used, followed by the determination of the main indicators according to the Baevsky method, which included the determination of mode, mode amplitude, variation range, autonomic balance index, autonomic rhythm index and stress index. Based on the results of the study, three experimental groups of animals were established: normotonics, vagotonics, and sympathotonics. Considering the results obtained, cows, depending on the influence of the tone of the autonomic nervous system, have differences in the activity of the cardiovascular system. It will result in different responses to stress, which in turn will affect their productivity. Determination of heart rate variability can be one of the indispensable indicators in analysing the health of an animal on a dairy farm. This issue is a promising area of research, especially when exploring the metabolic processes of high-yield cows to improve productivity while maintaining the physiological state of the animal

Keywords: sympathovagal balance; normotonics; vagotonics; sympathotonics; animal health; cattle

Introduction

Many techniques have been developed to improve the welfare of modern dairy farms producing animal products. One of the most frequently asked questions is the impact of stress on animals. It is explained by the fact that cows are kept on farms in a relatively isolated environment, with no possibility of migration outside the paddock (Periyanayagi *et al.,* 2022). The animal's motility is stable and consistent with familiar places, surroundings and climatic conditions. When the environment in which a cow lives changes, stress occurs in its body under the influence of a new factor. Depending on individual characteristics, animals react differently to these changes (Kremer *et al.,* 2022). One of the key factors in the mechanism of response to this stress is changes in the autonomic nervous system. It is involved in the regulation of homeostasis along with other structures of the body through its departments (Mulkey & du Plessis, 2019; Jänig, 2022). Under the influence of the sympathetic nervous system, the activity of the cardiovascular system increases, the accumulation of nutrients in the bloodstream increases, and they are distributed to all organs and tissues of the animal's body. Due to this, the body has the necessary energy to ensure a proper response to the stressor (Bellato *et al.,* 2020; Weber *et al.,* 2021). The parasympathetic nervous system performs the opposite functions: it reduces the activity of the cardiovascular system and activates the processes of digestion and synthesis of nutrients to establish a reserve for immediate use (Gibbons, 2019; Benarroch, 2020).

Cardiovascular studies provide informative indicators that reflect the health and welfare of many animals. The researchers initially analysed heart rate to reflect the activity of the sympathetic and parasympathetic nervous systems. However, the results were mixed

(Quevedo *et al.,* 2019; Turini *et al.,* 2022). Changes in heart rate fluctuated greatly under the influence of physical and psychological factors. It could include a change in the animal's environment, pain, for example, as a result of medical procedures, or a stranger in the cows' location. To obtain more accurate data, they began to measure cardiac intervals based on cardiological and psychophysiological diagnostic methods. It allowed for a better interpretation of cardiac activity in terms of the activity of the autonomic nervous system. Heart rate intervals are frequently referred to as heart rate variability, which reflects the sympathetic-vagal balance. Considering this indicator, it allows for a better analysis of animal health, welfare, and psychophysiological state (Kovács *et al.,* 2019; Grelet *et al.,* 2022).

Electrocardiography is one of the most effective and non-invasive methods of exploring heart rate variability. During the recording of the heart's electrical potentials, fluctuations in the variability of successive heartbeat intervals can be recorded. Depending on the individual characteristics of the animal organism, the activity of the autonomic nervous system varies. It is reflected in an increase or decrease in the heart rate variability. These differences are explained by the cow's different perception of the environment and adaptation to the changes it encounters throughout its life. Most scientists, based on their research, emphasise the multidimensionality of individual animal characteristics (Hunter *et al.,* 2021; Kitajima *et al.,* 2022). Reactivity is one of the most common signs to measure the state of a cow's body. It is frequently replaced by behavioural reactivity in a person. Since each animal develops in a specific familiar environment, any change can cause stress (Emelyanova *et al.,* 2020). The reason for this may be a stranger in the cow's resting area or milking parlour. Depending on its reactivity, the animal may try to increase its distance to interact less with the stressor or, on the contrary, demonstrate interest and activity in the opposite area (La Maestra *et al.,* 2021; Devi *et al.,* 2022). When analysing the activity of the cardiovascular system, certain groups of animals can be identified based on the sympathetic-vagal balance. Normotonics will have a balanced equilibrium of both systems, sympathotonics – with a predominance of the sympathetic nervous system and vagotonics – with a greater influence of the parasympathetic nervous system. Accordingly, cows will have different responses to the factors (Gibbons, 2019; Cheshire *et al.,* 2020).

The purpose of the research – to determine the effect of the tone of autonomic nervous regulation on the body of cows, which is reflected in changes in sympathovagal balance.

Materials and Methods

The research was conducted based on the dairy farm of "Obriy" LLC, the breed of cows is Ukrainian black-and-white dairy. The research was conducted according to the ARRIVE guidelines for reporting experiments using live animals. 100 clinically healthy cows of 3-4 lactation were selected for the variation-pulse oximetry study. Electrocardiographic examination was performed using a single-channel electrocardiograph Heart Mirror IKO (Hungary, Innomed). An electrocardiograph – is a voltmeter or galvanometer that records changes in the electrical potential of the heart. The animal should be sufficiently rested before electrocardiography. The cow is then kept in a standing position. Electrocardiological gel was applied to the electrode attachment sites after pretreating the skin with alcohol. Three sites were used for the research. The first is located between 3-5 intercostal spaces on the left, behind the elbow. The electrode of the right hand was attached to it. The second one is located to the caudal 1/3 of the jugular sulcus, where the left-hand electrode was placed. The third – in the withers of the animal, where a neutral lead was placed. During the research, a "crocodile" clamp was used. These clips have a sharp mouth with serrated ends to securely hold them in place on thick cattle skin. The toothed ends are very sharp, thus, they need to be dulled beforehand to avoid injuring the animal. During the recording of changes in the electrical potentials of the heart occurring during each cardiac cycle, the corresponding indicators were recorded by a galvanometer. The data was recorded using a stylus on cardiac examination paper. The stylus deflects according to the intensity of the heart's electrical activity (Jackson & Cockcroft, 2008). The tape pulling speed during cardiac signal recording was 50 mm/s. The results of the cardiac examination were calculated using Microsoft Excel.

According to the variational pulse oximetry study, the main indicators were determined, such as mode (Mo) - the interval that most often occurs in the R-R interval of the heartbeat, i.e. the duration of the heartbeat recorded by the cardiograph on paper; mode amplitude (AMo) – the percentage value of the mode that develops the mode, i.e. the percentage value of the most frequent value; variation range (Δx) – the difference between the maximum and minimum value of the mode; autonomic balance index (ABI) – an indicator that reflects the influence of the sympathetic and parasympathetic nervous system on the body, determined by the difference between the amplitude of the mode and the variation range; autonomic rhythm index (ARI) – an indicator that reflects the influence of the sympathetic nervous system on the body, determined by formula 1:

$$ARI = 1 \div (\text{Mo} \times \Delta\text{x}); \quad (1)$$

stress index (SI) – an indicator that reflects the state of stress of the body, i.e. reflects the stress index characterising the tone of the autonomic nervous system, is determined by formula 2:

$$SI = \text{AMo} \div (2 \times \text{Mo} \times \Delta\text{x}). \quad (2)$$

Analysing the results by the indicators obtained during the research, research groups were established. The first indicator that helped to determine the tone of the autonomic nervous system was the tension index, which reflected the sympathetic-vagal balance. Animals with low scores belonged to the group of vagotonics, those with high scores belonged to the group of sympathotonics, and cows with intermediate scores belonged to the group of normotonics. Out of 100 cows, 5 animals from each experimental group were selected for a detailed examination of the tone of autonomic nervous regulation. Statistical processing of the experimental data was performed by conventional methods of variation statistics. The significance of the difference was assessed by the Student's t-test. Differences between the compared indicators were considered probable at the level of significance $P<0.05$, $P<0.01$, $P<0.001$.

Results and Discussion

During the research of Ukrainian Black-and-White dairy cows of 3-4 lactation, it was established that the vast majority of animals were characterised by the predominant influence of the sympathetic division of the autonomic nervous system and belonged to the sympathotonic group (Fig. 1). The proportion of cows with sympathotonia was 50%. In contrast, the proportion of cows with normotony and vagotony was 28% and 22%, respectively.

According to the results of the electrocardiographic study, differences in the variational and pulse rate indices in cows depending on the tone of the autonomic nervous system were obtained (Table 1).

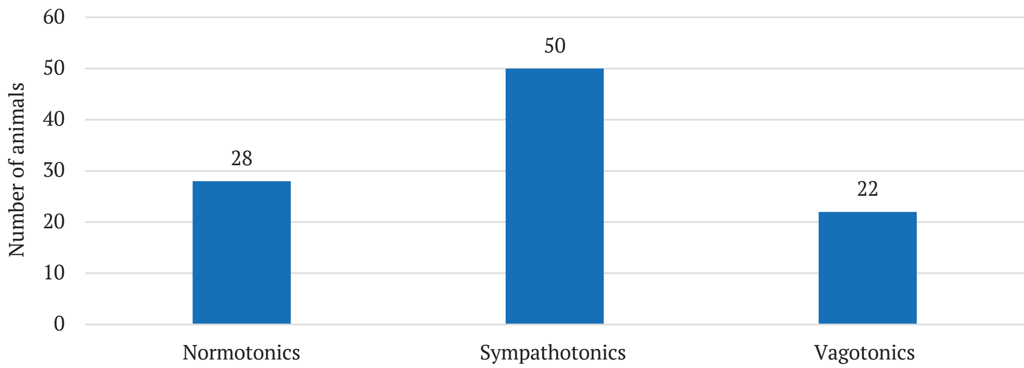

Figure 1. Number of animals depending on the tone of the autonomic nervous system (n=100)

Table 1. Indicators of heart rate variability depending on the tone of the autonomic nervous system in cows (M±m; n=5)

Indicator	Normotonics	Sympathotonics	Vagotonics
Heart rate, beats/min	62.0±3.74	78.0±3.04	50.0±1.60
Mo, c	1.0±0.05	0.78±0.03*	1.20±0.04*
AMo, %	20.40±1.04	32.18±0.89**	11.69±0.38**
Δx, c	0.13±0.01	0.07±0.01*	0.25±0.01**
ABI	153.31±8.06	437.0±22.05	47.93±1.44
ARI	7.95±0.87	18.0±0.89**	3.45±0.15**
SI	79.85±7.90	284.0±19.11	20.0±0.96

***Note**: *P<0.01, **P<0.001 – relative to the normotonic group*
***Source:** author's development*

In sympathotonic animals, the modal parameters of 0.78±0.03 s were the lowest (P<0.01), indicating the predominance of the sympathetic nervous system. The decrease in this value is explained by the fact that the cardiovascular system is dominated by excitation processes. It increases the heart rate. The heart rate increases within physiological standards. In vagotonics, the mode index (1.20±0.04 s) is the lowest compared to other groups (P<0.01) due to the action of the parasympathetic nervous system. Under the influence of a stress factor on the animal's body, the autonomic nervous system is dominated by the phenomenon of vagotonia. The cardiovascular system reduces its excitability, which helps to reduce the heart rate and, accordingly, the pulse rate. There was an inverse correlation between pulse rate and mode, which was r=-0.99 for the experimental groups. Accordingly, the animals of the experimental groups demonstrated a dependence between heart rate and mode index.

The amplitude of the mode characterises the percentage of fashion indicators, reflects the degree of mobilisation influence of the sympathetic nervous system. If sympathotonia prevails in the body, the AMo values will be high. It is explained by the fact that with a high intensity of excitation of the cardiovascular system by the sympathetic nervous system, the interval of repetitions of the cardiac cycle increases. It increases the amplitude of the mode. Accordingly, high values of indicators were noted in cows of the experimental group of sympathotonics 32.18±0.89% and in small cows of vagotonics 11.69±0.38% (P<0.001). A straight correlation between the mode and the mode amplitude was found in the animals of the experimental groups, namely: normotonics – r=0.92, sympathotonics – r=0.82 and vagotonics – r=0.96.

The variation range describes the difference between the maximum and minimum values of a mode. This indicator describes the influence of the vagus nerve. An increase in this value reflects the predominance of the parasympathetic nervous system over the sympathetic one. Accordingly, the experimental group of vagotonics is defined by vagotonia due to the largest variation range of 0.25±0.01 s (P<0.001).

The autonomic balance index reflects the ratio of the influence of the sympathetic and parasympathetic nervous systems. An animal with sympathotonia has an increase in the amplitude of the mode and a decrease in the variation range. As a result, the value of the autonomous equilibrium index will increase. Therefore, the experimental group of sympathotonics had the highest index (437.0±22.05) due to the superiority of the influence of the sympathetic nervous system over the parasympathetic one. An indirect correlation between the index of autonomous balance and the variation range in animals of the experimental groups was found, namely: normotonics – r=-0.73, sympathotonics – r=-0.80 and vagotonics – r=-0.74. A straight correlation between the autonomic balance index and the mode amplitude was established: in normotonics – r=0.82, sympathotonics – r=0.52, vagotonics – r=0.66.

The autonomic rhythm index reflects the influence of the parasympathetic nervous system on the body. As its activity increases, the indicator decreases. In the experimental group, vagotonics had the lowest value of the autonomous rhythm index – 3.45±0.15 (P<0.001). It indicates the predominance of the parasympathetic nervous system in the animal's body. An indirect correlation of the autonomous rhythm index and the variation range was identified in the animals of the experimental groups, namely: normotonics – r=-0.57, sympathotonics –r=-0.76 and vagotonics – r=-0.77. An indirect correlation between the autonomous rhythm index and mode was determined: in normotonics – r=-0.65, in sympathotonics – r=0.70, in vagotonics – r=-0.58.

The tension index characterises the tone of the autonomic nervous system. Its main value – the determination of the stress index, which indicates the predominance of the sympathetic or parasympathetic nervous system. Animals with high stress index values demonstrate increased aggressiveness and reduced stress resistance, resulting in increased nervous fatigue. Considering this, the experimental group of sympathotonics was distinguished by high values of the tension index (284.0±19.11), which indicates the predominance of the sympathetic nervous system, and vagotonics had the lowest values (20.0±0.96), which indicates the influence of the parasympathetic nervous system.

When considering the variation histogram of sympathicotonic animals (Fig. 2), some features should be noted. First of all, they had small R-R intervals. It, in turn, reflects the increased activity of the cardiovascular system. As the processes of excitation of the sympathetic division of the autonomic nervous system prevailed, the heart rate increased accordingly. Thus, the mode indicators demonstrated slight differences in the size of the interval but varied in the number of intervals. This feature of the variation histogram reflects the predominance of the sympathetic nervous system over the parasympathetic one.

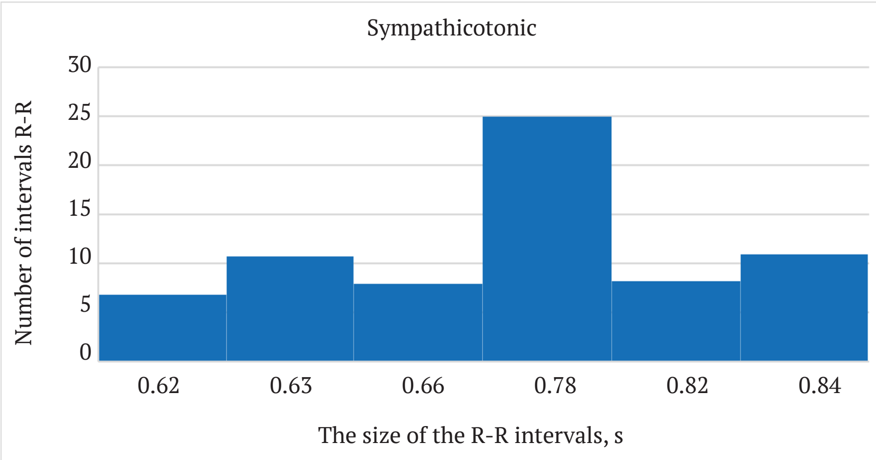

Figure 2. Variational histogram of sympathotonics in the experimental group

The variation histogram of the vagotonics of the experimental group (Fig. 3) demonstrated an increase in the size of the R-R intervals. This feature is explained by a decrease in the activity of the cardiovascular system against the background of vagotonia. The cardiac muscle contracted less actively and at different intervals, which in turn was reflected in the high values of the mode and the value of the R-R interval. It resulted in a decrease in the number of R-R intervals compared to that of animals in other experimental groups. It reflects the predominance of the parasympathetic nervous system over the sympathetic one in the animal's body, as demonstrated by the histogram above.

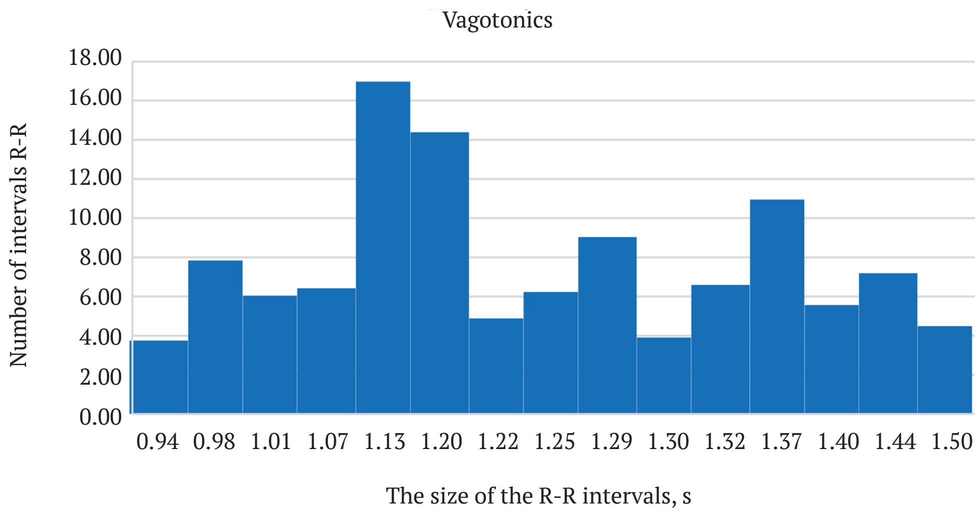

Figure 3. Variational histogram of vagotonics of the experimental group

The experimental group of normotonics on the variation histogram, compared to the others, was distinguished by a uniform ratio of the size of the R-R interval and its number (Fig. 4). This experimental group of animals was characterised by a balanced sympathetic-vagal balance, which in turn affected the cardiovascular system. If the sympathetic and parasympathetic nervous systems have a uniform effect on the cow's body, the heart muscle has a uniform R-R interval frequency. It has a corrective effect on the amount of the R-R interval. Considering the above, normotonics were distinguished by a balanced histogram of heart rate compared to animals of other experimental groups.

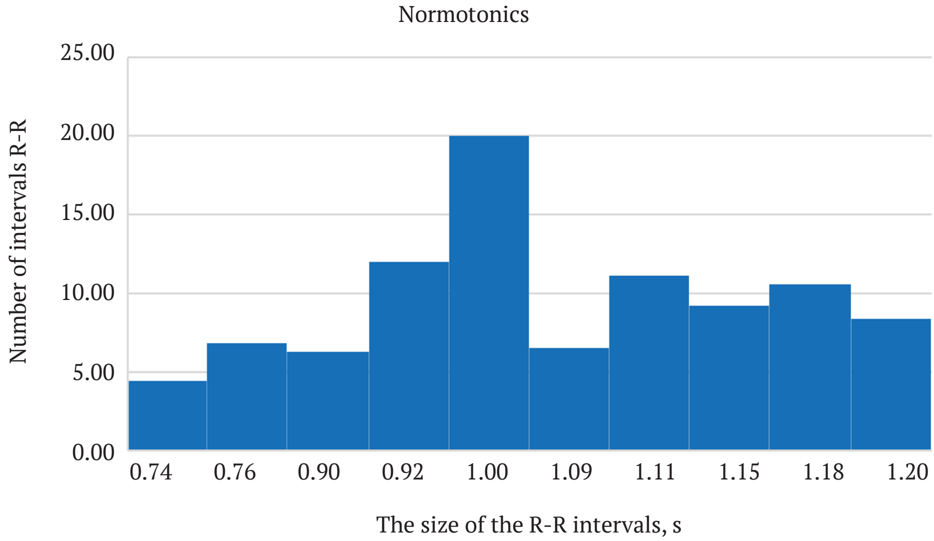

Figure 4. Variation histogram of normotonics of the experimental group

As is known, the issue of assessing the impact of stress on the body of cows is actively developing. The analysis of heart rate during milking or pain factor was frequently used to diagnose the condition of the animal. Over time, the question of better analysis of the cardiovascular system to explore the effects of stress on cows has arisen (Kovács *et al.,* 2019; Scoley *et al.,* 2019). Heart rate variability reflects the activity of the autonomic nervous system in the body. Depending on the influence of the sympathetic or parasympathetic nervous system, an animal responds differently to a stressor. Measurement of these indicators is successfully used to diagnose the health status of an animal and the effectiveness of treatment. It is necessary for a better understanding of the role of the autonomic nervous system in the body, as its influence significantly affects cow productivity (Quevedo *et al.,* 2019; Scoley *et al.,* 2019).

The results obtained in the variational pulse oscillometric test may vary from one researcher to another. It is explained by the fact that depending on the chosen methodology and using electrocardiographs of different models and years, different digital data can be obtained. Despite this, there is a certain regularity in the determination of sympathetic-vagal balance and heart rate. Thus, the experiments of Grelet *et al.*

(2022) noted that animals exposed to stress factors had a disorder of sympathovagal balance, as evidenced by the results of the variational pulse examination. Notably, at week 0, no differences in heart rate were observed between the groups of cows. At the last stage of the research, the animals exposed to the stress factor had a heterogeneous heart rate and a decrease in the values of sympathovagal balance. When comparing the results obtained with the studies of Grelet *et al.* (2022), notably, the animals exposed to stress had low cardiovascular activity. The researchers noted that the changes in the data obtained between the control and experimental groups during the four weeks of stress changed by only 8.2%. It indicates that the distribution of animals without consideration of the tone of the autonomic nervous system does not give a significant result when assessing the effect of a stressor. Therewith, it is important to consider the factor of fluctuations in sympathetic-weight balance, and the data of variability-pulse oximetry, which, according to the results of the research, corresponded to fluctuations between the groups within 21%.

Kovacs *et al.* (2015) used a variational pulse oscillometric examination as an indicator of the effect of chronic stress on the body of cows. In animals with a predominance of the parasympathetic nervous system over the sympathetic nervous system, a decrease in heart rate and high values of sympathovagal balance were observed, which confirmed vagotonia in the experimental groups. The research on the activity of the autonomic nervous system in cows before and during milking identified some differences in the indicators. The researchers note that animals with high activity of the sympathetic nervous system have the highest heart rate and low levels of variation. According to the results of the author's research, the difference in values between the study groups was 21 and 22%, respectively, in contrast to Kovacs *et al.* (2015), who obtained differences in values between the groups of 9 and 14%.

Jurkovich *et al.* (2017) used a variation-pulse study to assess the stress state of cows by changing the type of milking. It appeared that the connection of animals to automated milking machines was manifested by a decrease in heart rate and an increase in sympathetic-vagal balance, unlike those in conventional milking parlours. In cows that had more contact with humans, increased activity of the sympathetic nervous system was observed, which was reflected in aggressive behaviour and an increase in the content of glucocorticoids in faeces. To assess the state of the autonomic nervous system, the cardiovascular system was assessed, and the sympathetic-weight balance was determined. In particular, the authors noted that the cows of the experimental groups demonstrated an increase in the value of the examined indicators during prolonged exposure to a stress factor on the body. Therewith, an increase in pulse rate by 15% and sympathetic-weight balance by 35% was recorded. The experimental group of sympathotonic cows in research was a good reflection of the high activity of the sympathetic nervous system, which allowed obtaining slightly higher values of pulse rate – up to 25% and sympathetic weight balance – up to 38%, compared to other experimental groups of animals.

When analysing the variability of the heart rate of cows, an increase in the value of its indicators was observed in sympathotonics and a decrease in vagotonics. Therewith, normotonics occupied an intermediate position compared to the results typical of animals in other experimental groups. Considering the already-known results of Jurkovich *et al.* (2017) and their comparison with the data of the author's research, similar patterns were noted. When evaluating the results of other researchers, notably, the results of their studies vary due to different methods of determining the tone of autonomic nervous regulation. When calculating the initial data, most scientists use the software installed in the cardiograph to calculate the parameters of the heart's electrical potentials. Accordingly, the units of measurement will be various, but there is a particular pattern in determining the activity of the autonomic nervous system. The opposite results were observed in vagotonic cows compared to sympathotonic cows. Notably, regardless of the breed of animal and the size of the farm, there is a distribution of animals according to the tone of the autonomic nervous system. In addition, it reflects the individual characteristics of each cow (Kovács *et al.,* 2019). The established patterns, in turn, will serve as a reflection of the animal's health status and the reaction of its body to stress factors that will arise during milking, treatment, regrouping, etc.

After analysing the results, it can be concluded that among the animals of the experimental groups, sympathotonics demonstrated the highest level of nervous tension. It was confirmed by the indicators of the variational pulse study, which provide the initial data describing sympathotonia in such cows. As a result, the cows demonstrated constant nervous tension, which was expressed in a decrease in the heart rate interval and an increase in its frequency. In this regard, the activity of the cardiovascular system increased. During such loads, the animal expended more energy under the influence of external stress factors, which can adversely affect the health of its body and milk production.

Conclusions

According to the results of the studies, it was found that the tone of the autonomic nervous system affects the cardiovascular system, which is confirmed by the results of a variation pulse examination. Therewith, animals with sympathotonia had high values of heart rate – 78.0±3.04 beats/min, R-R interval intensity – 32.18±0.89 s and low values of R-R interval – 0.78±0.03 s ($P<0.01$; $P<0.001$). Animals with vagotonia demonstrated the opposite features, which was characterised by a decrease in heart rate to 50.0±1.60 beats/min, intensity of the R-R interval to 11.69±0.38 s and an increase in the R-R interval to 1.20±0.04 s ($P<0.01$; $P<0.001$). Animals with a balanced influence of the sympathetic and parasympathetic nervous systems occupied an intermediate position in terms of the examined indicators compared to animals of other experimental groups. In addition, it was established that the values of the variational pulse examination are a reliable reflection of the influence of the autonomic nervous system on the entire organism. Depending on the influence of the sympathetic or parasympathetic nervous system, a prediction of the animal's reaction to a stressor is possible. In addition, it allows determining the reaction of the cow's body to changes introduced to its environment, for example, new milking machines, regrouping into new housing units, etc. This method allows for the analysis of changes in animal metabolism under the influence of various exogenous factors and can be used to further explore the effect of new factors in improving the productivity of the dairy cow herd and their welfare.

In the future, as a continuation of experimental research in this area, it is planned to explore the influence of the tone of autonomic nervous regulation, which is determined using a variation-pulse study, on the homeostasis of the body of cows with different indicators of their productivity.

Acknowledgements

None.

Conflict of Interest

None.

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Визначення варіабельності серцевого ритму як показника впливу тонусу автономної нервової системи в корів

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Анотація. Актуальність теми полягає у важливості вивчення ролі автономної нервової системи в регулюванні діяльності серцево-судинної системи для покращення благополуччя продуктивних тварин. Варіабельність серцевого ритму є неінвазійним методом дослідження, що може бути корисним під час дослідження стану здоров'я тварини та аналізу її психофізіологічного стану в умовах господарства. Мета дослідження – встановити вплив тонусу автономної нервової регуляції на організм корови, що відображається в змінах симпато-вагального балансу. Експериментальні дослідження проводили на коровах породи українська чорно-ряба молочна. Для дослідження варіабельності серцевого ритму використовували електрокардіограф із подальшим визначенням основних показників за методикою Баєвського, що включало визначення моди, амплітуди моди, варіаційний розмах, індекс автономної рівноваги, автономний показник ритму та індекс напруги. За результатами дослідження було сформовано три дослідні групи тварин: нормотоніки, ваготоніки та симпатотоніки. З огляду на отримані результати, корови, залежно від впливу тонусу автономної нервової системи, мають відмінності в діяльності серцево-судинної системи. За рахунок цього буде різнитися їх відповідь на дію фактору стресу, що у свою чергу відображатиметься на їхній продуктивності. Визначення варіабельності серцевого ритму може стати одним з незамінних показників під час аналізу стану здоров'я тварини на молочнотоварних фермах. Це питання є перспективним напрямом досліджень, особливо при вивченні метаболічних процесів організму високопродуктивних корів для покращення ефективності продуктивності зі збереженням фізіологічного стану тварини

Ключові слова: симпато-вагальний баланс; нормотоніки; ваготоніки; симпатотоніки; здоров'я тварин; велика рогата худоба