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# BLOOD FATTY ACID COMPOSITION IN COWS DEPENDING ON THE TYPE OF AUTONOMIC REGULATION IN SUMMER PERIOD

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***Abstract.*** *Abstract. Coordination of physiological activity and intensity of metabolism in various organs and tissues of a productive animal depending on environmental conditions and own needs is provided by the regulatory activity of the corresponding nerve centers. However, the features of the autonomic regulation of the nervous system in this matter can have a significant impact not only on the physiological activity of animals but also on their productivity. Therefore, the question of studying the influence of autonomic regulation on the animal body in general and the interaction of the autonomic system and lipid metabolism is quite relevant.*

*Groups of animals were formed by determining the state of the cardiovascular system according to the Baevskyi method. Blood plasma was used for the study, lipid extraction was performed by the Folch method. Fatty acid analysis was performed on a Trace GC Ultra gas chromatograph (USA) with a flame ionization detector.*

*Studies have shown that the relative content of saturated fatty acids in blood of normotonics was the highest compared with other groups: sympathotonics – by 1.9%; vagotonics – 0.48%. Regarding the concentration of saturated fatty acids in sympathotonics, it should be noted that the content of stearic acid was the highest (18.07 ± 0.01; $P < 0.001$), and saturated fatty acids from C6 to C16 were characterized by the lowest values ($P < 0.01$–$0.05$) in comparison with other groups.*

*The total content of unsaturated fatty acids in blood plasma of sympathotonics and vagotonics was 1.19% and 0.49% higher, respectively, compared with normotonics. Quite interesting is the fact that sympathotonics were characterized by the highest content of polyunsaturated fatty acids, the content of which decreased in the range C18:2n6 > C20:4n6 > C18:3n3 > C22:6n3 > C22:5n3, a similar sequence we observed in other groups, although the relative concentration of acids could fluctuate. Normotonics were characterized by the highest levels of some polyunsaturated fatty acids (C18:3n3, C22:5n3, and C22:6n3; $P < 0.05$–$0.01$), while levels of some unsaturated fatty acids (C18:1n9 and C20:3n6) were the lowest.*

*Thus, the type of nervous activity has a significant effect on the ratio of fatty acids in blood plasma and autonomic regulation affects the metabolic processes in the body of cows.*

***Keywords:*** *cows, autonomic regulation, lipids, fatty acid composition*

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## Introduction

Currently, it is a very important issue to study the effect of autonomic regulation on lipid metabolism in animal (Martin et al., 2021). This is because the type of peripheral nervous system of the animal depends on the type of nervous activity and has a significant impact on the course of metabolic processes in its body (Chang et al., 2020). This question has a significant contribution to the understanding of what will depend on the productivity of the animal and its reproductive capacity (Carrell et al., 2021). Depending on the influence of autonomic regulation on the body, each environmental and external factor has a lever of influence on the course of internal processes (Bouffiou et al., 2020).

On the example of an animal with a predominance in the body of sympathetic nervous activity, each effect on it will be reflected in a significant reaction, which further negatively affects the normal course of metabolic processes in its body (Fernandez-Novo et al., 2020). It should also be noted that an animal that is more sensitive to changes in the environment will have increased production of cortisol, which will further increase the cost of energy reserves of nutrients and the use of nutrient-derived nutrients (Colditz, 2021). Lipids play an important role in the animal's body, they are carriers of high energy value, are used by the body as a structural element, are a part of the regulatory element in the system playing a role in influencing the life of individual cells and the body as a whole (Bionaz et al., 2020).

## Analysis of recent researches and publications

In the modern world, the study and understanding of the factors that affect the condition of the animal and its productivity play a significant role in creating highly productive livestock (Chen et al., 2021). Minor changes in the environment and the progression of stressors are strongly associated with the general condition of the animal (Estévez-Moreno, 2021). Many people face the fact that each animal responds differently to different circumstances and has different productivity (Hemphill et al., 2020). It is known that milk quality is affected by such indicators as genetic characteristics, the general physiological condition of the animal, it's feeding, exercise, type of keeping, and external conditions, etc. (Shanks, 2021). We understand these concepts and use them to improve them and get better results in our economy (Ujita et al., 2021). Quite a few issues have been studied and analyzed by scientists to obtain data that will help others to improve their work (Abdel-Hamid et al., 2017).

One such study is the study of the effect of autonomic regulation on lipid metabolism. Each animal has systems that control and maintain the mechanisms that regulate the body's constant homeostasis. Considering the nervous system, we understand that it has two – higher nervous and autonomic nervous systems. They play a role in controlling and regulating the stability of the body depending on the influence of both environmental and internal factors. These systems are strongly related to each other even though scientists describe them as higher nervous activity characterizing it as type of animal temperament, and the autonomic nervous system as a reflection of sympathetic or parasympathetic nervous systems that work in harmony or dominate each other. In this regard, we can conclude that everything is interconnected and if one part of the system has characteristic features, they will affect other systems, which will be reflected in the general condition of the animal's body, including lipid metabolism. Each animal has its own type of autonomous system, which plays the role of a mechanism that responds and acts depending on different stimuli (Lees et al., 2020). The whole organism has a whole system of nerve endings and analyzing organs that control the living organism and make changes in it (Danchuk et al., 2020).

The nervous system can affect almost all aspects of the development in cattle, including growth, reproduction, and immunity. For example, a sympathotonic animal gains weight more slowly than a normotonic animal (Parham et al., 2021). This is since animals with a predominance in the body of the sympathetic nervous system are more aggressive and react sharply to changes in the environment, they expend a lot of energy in this regard, deteriorating the development of this animal (Chang et al., 2020). It was also investigated that animals, in which excitation processes predominate, have worse insemination rates in contrast to animals with moderate nervous processes. Low carcass and fat content are observed in meat products, and bruises and injuries are noticeable on the carcass, which reduces the quality and cost of meat (Libis-Márta et al., 2021). Also, the fattening of animals for slaughter, depending on the autonomic regulation, will have a different result in the weight gain of the animal (Sant'Anna et al., 2019).

Types of nervous activity have a significant impact on animals. Considering the conditions of detention, studies have been conducted, determining that the strongest manifestation of milk production affects the nervous system in the first lactation, this is since the animal has significant life changes, in this regard, it is influenced by stress factors (Mincu et al., 2021). Over time, the animal gets used to this effect, and in the next lactation, there are no such active reactions of the body (Antanaitis et al., 2021).

Stress conditions have a very negative effect on animal health. A very influential stress factor for the animal is its transportation to another farm, slaughterhouse, etc. (Marçal-Pedroza et al., 2021). During this load, the animal will not have a positive result of any type of autonomic regulation. But here the role is that depending on what type of nervous process predominates, the animal will react differently to the influencing factor, and in the future, it will affect the acclimatization and quality of animal raw materials (Melendez et al., 2021).

Immunity also depends on the type of nervous activity, as calves with weak nervous processes have a low immune response to vaccines (Smith et al., 2021). In the study of the immune system comparing the signs of autonomic processes, it was found that bulls with aggressive temperament had a lower proliferation of lymphocytes *in vivo* and lower concentrations of IgG specific for vaccines *in vivo*, compared with calmer bulls (Altman, 2019).

Many studies have been conducted to investigate the metabolism in the body. The path of lipid conversion from food and their circulation in the body has been well studied (Antanaitis et al., 2021). With feed, cattle receive relatively few lipids, but many polyunsaturated fatty acids, especially linolenic acid. In the rumen, lipids are hydrolyzed and released fatty acids are bio-hydrogenated by a microbial population, with 90% of linolenic acid being lost and increasing the amount of stearic acid. Free fatty acids are adsorbed in the rumen and turn into the abomasum, their absorption is almost absent in the rumen (Alexandre et al., 2020).

Microorganisms in the rumen are also involved in the synthesis of lipids and when moving them with food into the abomasum, they are broken down by digestive enzymes, releasing fatty acids (Zeineldin et al., 2018). All fatty acids enter the duodenum, where they mix with the secretions of pancreatic acid (lipase and phosphatase) and bile containing lipid (phosphatidylcholine) and bile acids (taurine conjugates) (Vinyard et al., 2021). Lipases and phospholipases partially hydrolyze microbial lipids and any food lipids that do not hydrolyze in the rumen. The contents of the duodenum move to the distal part of the small intestine, where absorption occurs (Fortin et al., 2017). Most fatty acids are absorbed in the form of free fatty acids (Bernard et al., 2017). Free fatty acids are esterified, using the glycerol-3-phosphate pathway and partially by the monoacylglycerol pathway (Brake & Swanson, 2018). Before esterification, there is some desaturation of stearic acid to oleic, this process is characteristic only for ruminants (Xin et al., 2020). Then triacylglycerols, phospholipids, and cholesterol esters are created, which are used for the synthesis of chylomicrons, which are then secreted into the lymph (Kairenius et al., 2018). Triacylglycerols are the major lipids of lymph, accounting for 70–80% of the total weight of lipids in ruminants and playing the role of a major source of fatty acids for tissues (Danchuk, 2006). Once triacylglycerols enter the bloodstream, they are hydrolyzed by lipoprotein lipase, which is synthesized by body tissues such as muscle, fat, and the mammary gland, which subsequently releases fatty acids for tissue use (Li et al., 2018), for the synthesis of structural components such as cell membranes, biologically active substances such as prostaglandins, as a source of energy and to create a reserve for the future, or for the synthesis of milk (Toral et al., 2018).

***The purpose of the study*** is to investigate the effect of autonomic regulation on blood fatty acid composition in cows.

## *Materials and methods of research*

The research was carried out on cows of the Ukrainian, black-spotted breed of the 3rd–4th lactation. Types of the autonomic regulation were determined by the state of the cardiovascular system according to the Baevskyi method, the essence of which is that an electrocardiogram is recorded in the animal and the mode in the range of cardio intervals is determined. Then we formed 3 groups of animals: sympathotonic – a type of the nervous activity where the sympathetic nervous system predominates over the parasympathetic; vagotonic – a type of the nervous activity where parasympathetic nervous activity predominates over the sympathetic; normotonic – a type of the nervous activity with balanced parasympathetic and sympathetic nervous activity. The material for the study was blood samples obtained from the jugular vein in the morning before feeding. Blood was stabilized with heparin, plasma was obtained by centrifugation. In addition, information was taken on the daily productivity of the animals that they had on the day of blood sampling.

Extraction of lipids from blood plasma was performed by the Folch method (Folch et al., 1957). The next step in sample preparation was the hydrolysis and methylation of fatty acids in lipids derived from blood plasma. To do this, 4 $cm^3$ of methyl sodium hydroxide solution was added to 100 mg of the obtained fat, the reflux condenser was connected to the flask containing it and boiled until the fat droplets disappeared, stirring the contents of the flask at intervals of 30–60 seconds. To the contents of the flask, 5 $cm^3$ of boron methyl fluoride solution was added, continuing to boil for up to 1 hour. Three $cm^3$ of hexane was added to the boiling mixture through the top of the reflux condenser and removed from the heating element. To a still hot solution, 20 $cm^3$ of saturated sodium chloride solution was added and stirred for 15 seconds. The upper (hexane) layer was selected for study (Sinyak et al., 1976). Analysis of methyl esters of volatile fatty acids was performed on a Trace GC Ultra gas chromatograph (USA) with a flame ionization detector. Chromatography conditions: column temperature 140–240 ºC, detector temperature 260 ºC. The sample was introduced into the chromatograph using a TriPlus autosampler at a dose of 1 μl. The duration of the analysis was 65 minutes.

Identification of fatty acids was performed using a standard sample Supelco 37 Component FAME Mix. Quantitative evaluation of the fatty acid spectrum of lipids was performed by the method of internal normalization, determining their content in percent. The study was performed in 3 parallels.

The following fatty acids were detected by gas chromatography: caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), myristoleic (C14:1), pentadecanoic (C15:0), palmitic (C16:0), palmitoleic (C16:1n9), stearic (C18:0), oleic (C18:1n9), linoleic (C18: 2n6), arachidic (C20:0), linolenic (C18:3n3), cis-11-eicosenoic (C20:1n9), cis-8,11,14-eicosatrienoic (C20:3n6), arachidonic (C20:4n6), docosapentaenoic (C22:5n3), and cis-4,7,10,13,16,19-docosahexaenoic (C22:6n3).

Statistical processing of experimental data was performed by conventional methods of variation statistics. The probability of the difference in indicators was assessed by Student's t-test. Differences between the compared indicators were considered significant at the level of significance $P < 0.05$, $P < 0.01$, $P < 0.001$.

## *Results of the research and their discussion*

It is known that the fatty acid composition of blood in animals depends on many factors, among which the main ones are the type of productive animal, age, the composition of the diet, the intensity of rumen digestion, and its physiological state.

According to the results of research, the absence of clinical signs of pathology is not a guarantee of similarity of the main characteristics of the fatty acid composition of blood plasma in dairy cows (Fig. 1) under the same conditions of feeding and keeping. One of the physiological factors that should be considered in the chromatography of fatty acids in blood plasma of lactating cows is the types of autonomic regulation (normotonic; sympathotonic; and vagotonic).

According to Fig. 1, the type of nervous activity of a productive animal has a significant impact on their daily milk productivity. At this stage of our study, it is difficult to fully judge how close the relationship is between blood fatty acid composition in cows (Table 1), the type of their nervous activity, and the level of productivity. However, a superficial analysis of the results shows that normotonics were characterized by the highest level of daily milk yield, and the total content of saturated fatty acids (SFA) in this experimental group was also the highest. It is difficult to say how much this is related to the level of motor activity and stress resistance, but we can safely say that the increase in motor activity is provided by increasing the intensity of ATP synthesis and β-oxidation of fatty acids. However, this issue needs further study.

Animals-normotonics have a type of nervous activity, where the sympathetic and parasympathetic nervous systems

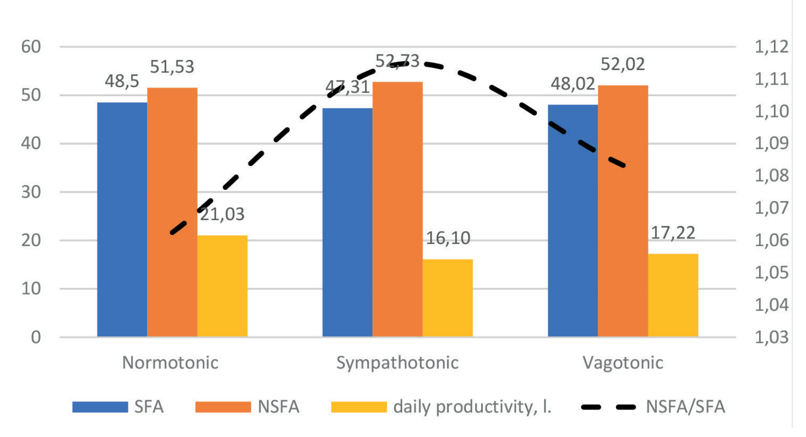


**Fig. 1. The ratio of blood fatty acids and daily milk productivity in cows with different types of autonomic regulation**

have a balanced effect on the animal's body, so we chose it as a guide to compare the features of lipid metabolism in other types. Thus, as can be seen from Fig. 1, the content of SFA was the highest in blood of normotonics in comparison with other groups – sympathotonics (by 1.9%) and vagotonics (by 0.48%).

According to the results, the relatively high content of SFA was provided by C6:0, C10:0, C15:0, C16:0, and C20:0.

Regarding the concentration of SFA in sympathotonics, it should be noted that the content of stearic acid in comparison with others was the highest (18.07 ± 0.01) and 0.89% higher than that observed in normotonics ($P < 0.001$). SFA from C6:0 to C16:0 in sympathotonics were characterized by the lowest relative content compared with other experimental groups (C6:0, $P < 0.05$; C10:0, $P < 0.01$; C16:0, $P < 0.05$).

It is known that the content of fatty acids with an odd number of carbon atoms in blood may depend on grazing time and the botanical composition of the pasture. However, even under the same feeding conditions of lactating cows, as shown by chromatographic studies presented in Table 1, it may depend on the type of autonomic regulation of nervous activity. We are talking about pentadecanoic acid (C15:0), the relative content of which in blood of animals decreased in the range: normotonics > sympathotonics > vagotonics, with significant differences in vagotonics both in comparison with normotonics ($P < 0.01$) and sympathotonics ($P < 0.05$).

Dairy cows, the intensity of nervous activity of which is characterized as vagotonic, in terms of the relative content of SFA in blood, occupied mainly an intermediate position between the values observed in sympathotonics and normotonics.

The total plasma content of unsaturated fatty acids (UFA) in animals classified as sympathotonics and vagotonics was 1.19% and 0.49% higher, respectively, compared with normotonics. Quite interesting is the fact that sympathotonics were characterized by the highest content of polyunsaturated fatty acids (PUFA), the content of which decreased in the range of C18:2n6 > C20:4n6 > C18:3n3 > C22:6n3 > C22:5n3, a similar sequence was observed in other groups, although the relative concentration of acids could fluctuate.

**1. Fatty acid content (%) in blood plasma of cows according to autonomic regulation (M ± m; n = 5)**

| Fatty acid | Groups of cows | | |
|---|---|---|---|
| | Normotonics | Sympathotonics | Vagotonics |
| Caproic, C6:0 | 1.11 ± 0.06 | 0.82 ± 0.04* | 0.98 ± 0.02 |
| Caprylic, C8:0 | 0.61 ± 0.04 | 0.69 ± 0.01 | 0.61 ± 0.01 |
| Capric, C10:0 | 1.00 ± 0.05 | 0.76 ± 0.02** | 0.81 ± 0.01** |
| Lauric, C12:0 | 0.42 ± 0.03 | 0.43 ± 0.01 | 0.54 ± 0.02* |
| Myristic, C14:0 | 3.10 ± 0.04 | 2.67 ± 0.06 | 2.86 ± 0.03 |
| Myristoleic, C14:1 | 0.64 ± 0.04 | 0.56 ± 0.01 | 0.67 ± 0.01 |
| Pentadecanoic, C15:0 | 0.45 ± 0.02 | 0.42 ± 0.01 | 0.37 ± 0.01** |
| Palmitic, C16:0 | 24.48 ± 0.44 | 23.30 ± 0.21* | 23.82 ± 0.04 |
| Palmitoleic, C16:1n9 | 2.14 ± 0.14 | 2.19 ± 0.01 | 2.46 ± 0.03 |
| Stearic, C18:0 | 17.18 ± 0.23 | 18.07 ± 0.01** | 17.91 ± 0.02* |
| Oleic, C18:1n9 | 23.15 ± 0.16 | 23.39 ± 0.18 | 24.51 ± 0.28** |
| Linolenic, C18:2n6 | 15.33 ± 0.37 | 16.21 ± 0.14 | 15.45 ± 0.27 |
| Arachidic, C20:0 | 0.15 ± 0.01 | 0.15 ± 0.01 | 0.12 ± 0.01 |
| α-Linolenic acid, C18:3n3 | 1.33 ± 0.01 | 1.04 ± 0.01*** | 0.89 ± 0.01*** |
| Cis-11-eicosenoic, C20:1n9 | 0.42 ± 0.02 | 0.45 ± 0.01 | 0.40 ± 0.01 |
| Cis-8,11,14-eicosatrienoic, C20:3n6 | 0.10 ± 0.01 | 0.14 ± 0.01* | 0.12 ± 0.01 |
| Arachidonic, 20:4n6 | 7.24 ± 0.18 | 7.74 ± 0.13 | 6.43 ± 0.15*** |
| Docosapentaenoic, C22:5n3 | 0.31 ± 0.01 | 0.30 ± 0.01 | 0.27 ± 0.01* |
| Cis-4,7,10,13,16,19-docosahexaenoic, C22:6n3 | 0.88 ± 0.01 | 0.71 ± 0.01*** | 0.82 ± 0.03 |

**Note:** * P < 0.05, ** P < 0.01, *** P < 0.001 – compared with normotonics; data are presented as the mass fraction of fatty acid in % of the sum of fatty acids.

Normotonics, even though they had the lowest content of UFA, compared to other experimental groups, still for some PUFAs (C18:3n3, C22:5n3, and C22:6n3) were characterized by the highest values (P < 0.05–0.01) compared with other groups, while the SFA (C18:1n9 and C20:3n6) were the lowest compared with other experimental groups.

As shown in Fig. 2, normotonics were characterized by the highest concentration of omega-3 fatty acids in blood. Sympathotonics have a higher concentration of omega-6 fatty acids by 1.42% and 2.1% compared with vagotonics and normotonics, respectively.

Vagotonics were characterized by the lowest concentration of linolenic acid (P < 0.001), eicosenoic acid (P < 0.05), arachidonic acid (P < 0.001), and docosapentaenoic acid (P < 0.05) compared with animals with other types of the nervous system.

It is known that both the types of the nervous system and the intensity of motor activity can affect the performance of dairy animals, regardless of their level of nutrition. Obviously, increasing physical activity can only to some extent ensure stable productivity, on the other hand, overuse of energy due to β-oxidation of

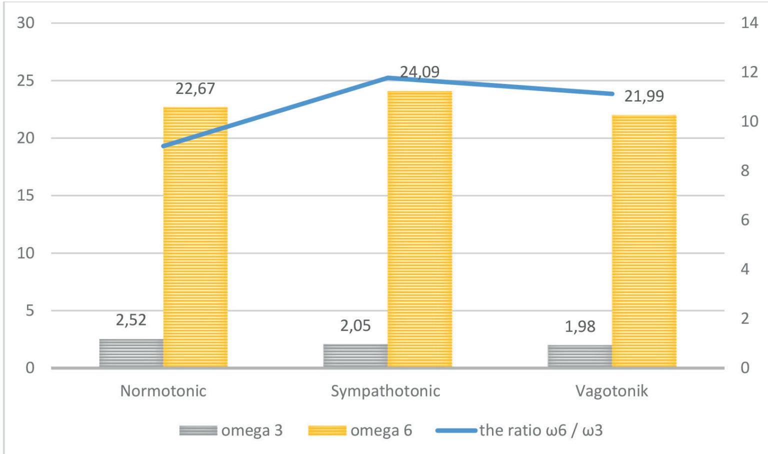


**Fig. 2. The ratio of ω6/ω3 fatty acids in blood of cows with different types of autonomic regulation**

saturated fatty acids can significantly affect not only their concentration in blood but also obviously their content in milk. This is what we associate with a decrease in the relative content of SFA in sympathotonics, which may be one of the hallmarks of this type of nervous activity.

## *Conclusion*

The ratio of fatty acids in blood plasma of lactating cows depends to some extent on the type of autonomic nervous regulation and may be related to the level of their daily milk productivity. Normotonics are characterized by the highest level of daily milk productivity, relatively high content of saturated fatty acids and slightly lower level of unsaturated fatty acids. Sympathotonics and vagotonics were characterized by a lower level of daily milk productivity and a higher percentage of unsaturated fatty acids in blood. The content of fatty acids in blood with an odd number of carbon atoms may depend on the type of autonomic nervous regulation: the relative content of pentadecanoic acid (C15:0) in blood of animals decreased in a number: normotonics > sympathotonics > vagotonics, differences in vagotonics were observed both in comparison with normotonics ($P < 0.01$) and sympathotonics ($P < 0.05$).

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***Анотація.*** *Координація фізіологічної діяльності та інтенсивності обміну речовин у різних органах і тканинах продуктивної тварини залежно від умов середовища та власних потреб забезпечується регуляторною діяльністю відповідних нервових центрів. Проте особливості автономної регуляції нервової системи в цьому питанні можуть суттєво впливати не тільки на фізіологічну діяльність тварин, а й на їхню продуктивність. Тому досить актуальним є питання вивчення впливу автономної регуляції на організм тварин загалом та взаємодії автономної системи та ліпідного обміну.*

*Групи тварин формували за допомогою визначення стану серцево-судинної системи за Баєвським. Для дослідження використовували плазму крові, екстракцію ліпідів проводили за методом Фолча. Аналіз жирних кислот проводили на газовому хроматографі Trace GC Ultra (США) з полум'яно-іонізаційним детектором. Дослідженнями встановлено, що відносний вміст насичених жирних кислот був найвищим у крові нормотоніків: на 1,9%, ніж у симпатотоніків і на 0,48%, ніж у ваготоніків. Що стосується концентрації насичених жирних кислот у симпатотоніків, то треба зазначити, що вміст стеаринової кислоти в крові був найвищим (18,07 ± 0,01; $P < 0,001$) порівнюючи з іншими групами, а насичені жирні кислоти від С6 до С16 характеризувалися найнижчими значеннями в порівнянні до інших дослідних груп (С6:0, $P < 0,05$; С10:0, $P < 0,01$; С16:0, $P < 0,05$).*

*Загальний вміст ненасичених жирних кислот у плазмі крові тварин, які належать до симпатотоніків і ваготоніків, був відповідно на 1,19% та 0,49% вищим порівнюючи з нормотоніками. Досить цікавим є факт, що симпатотоніки характеризувалися найвищім вмістом поліненасичених жирних кислот, вміст яких знижувався в ряді С18:2n6 > С20:4n6 > С18:3n3 > С22:6n3 > С22:5n3; аналогічну послідовність ми спостерігали і в інших групах, хоча відносна концентрація кислот могла коливатися. Нормотоніки, не дивлячись на те, що мали найнижчий вміст ненасичених жирних кислот, порівнюючи з іншими дослідними групами, однаково за деякими поліненасиченими жирними кислотами (С18:3n3, С22;5n3, С22:6n3) характеризувалися найвищими рівнем ($P < 0,05–0,01$) порівнюючи з іншими групами, тоді як вміст ненасичених жирних кислот (С18:1n9, С20:3n6) був найнижчим.*

*Отже, тип нервової діяльності має значний вплив на співвідношення жирних кислот у плазмі крові й автономна регуляція впливає на обмінні процеси в організмі корів.*

***Ключові слова:*** *корови, автономна регуляція, ліпіди, жирнокислотний склад*