



## Autonomic regulation of unsaturated fatty acid content in cow's milk

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**Abstract.** Investigating the role of the autonomic nervous system, as one of the key regulatory systems controlling metabolism in animals, is essential for assessing individual differences in the intensity and direction of metabolic processes. Therefore, studying the influence of the

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parasympathetic and sympathetic nervous systems on lipid metabolism, including fatty acids, is a relevant issue for ensuring dairy productivity in cows. This study aimed to provide a comparative assessment of the content of unsaturated fatty acids in cow's milk under varying types of autonomic regulation during the summer period. Experimental groups of Ukrainian Black-and-White dairy cows were formed based on sympathovagal balance, determined using a variation-pulsometry method. Gas chromatography was used to determine the fatty acid composition of the milk. The results revealed that the level of myristoleic acid in the milk of cows with vagotonia was 22% higher ( $P < 0.001$ ) compared to that in cows with normotonia, and 7% higher ( $P < 0.05$ ) compared to cows with sympathotonia. Palmitoleic acid levels in the milk of cows with vagotonia were 21% lower ( $P < 0.001$ ) compared to those with normotonia, and 16% lower ( $P < 0.001$ ) compared to cows with sympathotonia. In the milk of cows with sympathotonia, a 3.6% reduction ( $P < 0.05$ ) in oleic acid content was recorded relative to cows with normotonia. The level of linoleic acid in cows with vagotonia was 19% lower ( $P < 0.05$ ) than that in cows with normotonia, whereas in those with sympathotonia, it was 6% higher ( $P < 0.05$ ). The content of  $\alpha$ -linolenic acid in the milk of cows with vagotonia was 46% lower ( $P < 0.05$ ) compared to normotonic cows, while in those with sympathotonia, it was 30% higher ( $P < 0.05$ ). A relationship was established between autonomic nervous system activity and the content of unsaturated fatty acids in milk. The findings highlight the importance of exploring the regulatory mechanisms involved in lipid metabolism, particularly in dairy farming, where the individual productivity of cows is determined by the levels of fats consumed, absorbed, synthesised and metabolised within the body

**Keywords:** ruminants; gas chromatography; lipids; variation pulsometry; nervous system

## Introduction

Examining and analysing the factors that influence fatty acid metabolism in cows' milk is a primary concern when evaluating the quality of dairy production. These factors include: the animal's genetics, environmental conditions (such as feeding regime, season, and housing), and physiological aspects (age, lactation stage, health status, and the functioning of systems responsible for metabolism). With a focus on the functioning systems within the animal's body, it is important to consider the nervous system, particularly the autonomic nervous system, which plays a significant role in regulating the body's metabolic processes. F. Di Gregorio *et al.* (2024) highlighted that each organism has individual differences in the way their central and peripheral nervous systems function. Consequently, the activity of different

parts of the autonomic nervous system will vary, and this will be reflected in the metabolic processes occurring within the animal's body.

Milk synthesis is a rather energy-intensive process for high-producing livestock, as secreting a significant volume of milk requires a substantial amount of organic matter. To create these compounds, the animal's body undergoes biochemical processes of feed absorption and digestion, followed by the formation of complex molecules from the metabolic products. A. Zsombok *et al.* (2024) demonstrated that these biochemical processes within the body demand considerable energy expenditure and are maintained by the stability of homeostasis, which is regulated by the nervous system. It therefore follows that, due to the individual characteristics of cows' bodies, metabolic

processes occur differently from one animal to another, and this is particularly evident when the animal is under stress.

Z. Liu & S. Rochfort (2023) pointed out that for the synthesis of high-quality milk, the required lipid content falls within the range of 3% to 5%, which provides significant nutritional value for humans. Achieving this percentage necessitates a considerable number of fatty acids, which, according to these researchers, amounts to over 400 different types. These are present as triacylglycerols, phospholipids, and sphingolipids and constitute a significant portion of milk. K.E. Mitchell *et al.* (2023) demonstrated that the total number of lipid varieties exceeds 4,000, and this number continues to grow annually due to advancements in technologies for identifying these substances. Consequently, the cow's body expends a considerable amount of energy to produce the fat content necessary for milk synthesis, a process regulated by the nervous system to maintain homeostatic balance.

Scientists R. Gervais *et al.* (2023) found that investigating the structure and diversity of lipids in milk is a common aspect of assessing its quality, particularly concerning the composition of fatty acids, the levels of which are identified using mass spectrometry. G. Celano *et al.* (2022) reported on the use of methods for the quantitative or semi-quantitative determination of milk lipids. These methods rely on the application of multiple standards and their comparison with a reference sample.

In their research, L. Grille *et al.* (2023) found that saturated fatty acids are the most prevalent type of fatty acid in milk, with monounsaturated and polyunsaturated fatty acids present in smaller amounts. Milk fat content is the component that varies the most. One of the most significant factors affecting the amount of fat in milk is the cows' feed. Allowing animals to graze in pastures or including green forage in the diet of dairy cows kept

indoors improves the fatty acid composition of their milk, which subsequently has a beneficial impact on human health. Under such conditions, when cows are on pasture, the levels of saturated fatty acids (C12:0, C16:0, C14:0) and the ratio of omega-6 to omega-3 fatty acids (n-6/n-3) are lower. Simultaneously, the levels of certain polyunsaturated fatty acids are significantly higher, such as C18:3 (n-3) and C18:2 (conjugated linoleic acid; cis9-trans11), which are important for human health. According to S.K. Gulati *et al.* (2022), allowing dairy cows to graze on pastures enhances the fatty acid profile of their milk by increasing the content of some health-promoting fatty acids, like n-3, and consequently reducing the n-6/n-3 ratio.

B. Walther *et al.* (2022) highlighted that the external environment is the primary and most significant factor influencing the milk production of cows, particularly in regions with seasonal climate changes. Consequently, the animal's body begins to adapt to these shifts by increasing the activity of its nervous system, especially the autonomic nervous system. The authors also noted that cow productivity tends to be more stable in areas with consistently temperate climates, as these animals can graze on pastures year-round, unlike those forced indoors during severe frost or intense heat. However, with changing climate patterns, more extreme weather conditions are emerging, which significantly impact the health and productivity of cows. Furthermore, considering the individual biological characteristics of each animal, their ability to adapt to such environmental changes varies, which is reflected in differences in the activity of their autonomic nervous systems. A straightforward example of this is the increased energy expenditure in cows during winter to maintain their body temperature, as well as the reduced intensity of their metabolic processes in summer to minimise heat production within their bodies. L. Grille *et al.* (2022)

demonstrated variations in the levels of fatty acids, specifically C18:2, C18:1, and C18:3 (n-3), in the milk of cows between the summer and winter seasons. Furthermore, the breakdown (catabolism) of fatty acids in cows experiencing heat stress led to an increase in the concentration of fatty acids in their blood plasma (particularly oleic and linoleic acids). This also resulted in a higher overall content of long-chain fatty acids and a decrease in the levels of short- and medium-chain fatty acids in their milk.

Research by O.V. Zhurenko *et al.* (2023) revealed that the presence of specific minerals in animal feed significantly influences cow productivity. Moreover, considering how the autonomic nervous system and its branches – the sympathetic and parasympathetic nervous systems – adjust metabolic processes within the animal, fundamentally alters the understanding of metabolic pathways and their impact on milk production in the mammary gland.

Scientists have extensively investigated the relationships between the levels of fatty acids in cows' milk and the influence of the autonomic nervous system. This study aimed to determine the impact of the autonomic nervous system tone as a factor regulating fatty acid metabolism, as reflected in the levels of unsaturated fatty acids in cows' milk.

### Literature Review

The autonomic nervous system is a part of the peripheral nervous system. It has two main branches: the sympathetic and parasympathetic nervous systems. The sympathetic nervous system stimulates the cardiovascular system, increasing the force of the heartbeat. As T. LeBouef *et al.* (2023) identified that, by triggering the release of adrenaline, it activates the breakdown of nutrients, particularly carbohydrates and especially lipids. This generally promotes the mobilisation of nutrients and their active circulation throughout the body.

This branch of the autonomic nervous system also inhibits digestion in the gastrointestinal tract, specifically the movement of the intestines. Conversely, the parasympathetic nervous system has the opposite effect, promoting the synthesis of nutrients and increasing the activity of the digestive system. It is important to note that these two systems constantly interact with each other. However, each living organism is unique and exhibits different levels of activity in the components of its autonomic nervous system. As a result, one might observe a greater influence of either the parasympathetic or the sympathetic nervous system. In other animals, these two branches function in a more balanced manner. Due to these individual characteristics, any changes in the cow's external or internal environment will affect its regulatory systems in different ways, and the resulting responses will also vary.

A.H. Rezakhani *et al.* (2024) established that the start of lactation in cows is significantly influenced by the activity of their regulatory systems and their individual ability to maintain the body's homeostasis, as well as the animal's overall health. According to these researchers, a normal lactation period relies on sufficient energy reserves built up during the dry period. The beginning of lactation is a substantial metabolic stress, controlled by the central nervous system through the action of the autonomic nervous system. A. Veshkini *et al.* (2024) emphasised the need to manage these energy shifts and develop strategies for controlling these processes. Currently, this is partly addressed by adjusting feed rations, but the effectiveness of this approach does not always yield the desired outcome.

The metabolism of lipids in cows and the development of balanced diets to improve their energy profile, thereby ensuring a positive lactation period, have been studied by W.B. Gallardo & I.A. Teixeira (2023) and C.D. Camell (2022). These studies highlighted the

importance of monitoring lipid concentrations in the animal's body, as lipids not only provide energy but also perform crucial bioactive physiological functions. Consequently, evaluating this aspect remains a highly relevant topic in scientific publications.

T.M. Knutsen *et al.* (2022) demonstrated that significant challenges exist in accurately measuring lipid concentrations in milk. Firstly, milk fat originates from two primary sources: *de novo* fatty acids, synthesised within the mammary gland from acetate and beta-hydroxybutyrate, and fatty acids derived from the bloodstream, which are obtained from the cow's diet and mobilised from the body's fat reserves. R.W. Shephard & S.K. Maloney (2023) highlighted the importance of proper energy metabolism and evaluating the sources of lipids entering the animal's system, as these factors will influence the fatty acid profile of the milk. Research into the essential components regulating lipid metabolism can help in predicting the optimal formulation of the animal's diet.

The importance of developing a strategic approach to feeding and managing cows during the transition period, which is crucial for maintaining the animals' health, productivity, and the farm's profitability, was emphasised in the study by S. Lashkari *et al.* (2024). Their findings indicate that the most critical challenge is meeting the cow's increasing energy demands for milk production at the start of lactation. To compensate for the high energy requirement in early lactation, the body mobilises fat reserves as an adaptive metabolic mechanism to prevent an energy deficit, leading to an increased concentration of non-esterified fatty acids in the blood plasma. The authors also noted the necessity of evaluating the systems that control lipid metabolism.

A.M. Bales *et al.* (2024) investigated the potential to adjust the fatty acid content of cows' milk by using feed supplements containing

fatty acids such as palmitic and oleic acid in varying proportions. Three experimental groups of cows were established: the first was a control group, consuming a standard diet; the second group received a feed supplement composed of 80% palmitic acid and 20% oleic fatty acid; and the third group consumed a feed supplement containing 60% palmitic acid and 40% oleic fatty acid. The authors found that these feed supplements did not affect the cows' milk yield. However, they observed an increase in the fat content of the milk in the experimental groups where the modified feeding regime was implemented. Changes in the concentration of milk fatty acids were not significantly different when a low concentration of fatty acids was used in the animals' feed.

The factors that stimulate lipolysis were investigated by M.B. Oquendo *et al.* (2022). They found that some of the main factors in the release of lipids from storage are adrenergic agonists: isoprenaline and adrenaline. This variable also indirectly stimulates the synthesis of insulin by releasing lipids into the bloodstream. The primary driver for the release of adrenergic antagonists is the activity of the sympathetic nervous system, which is one of the first systems to respond to changes in the environment and helps the body adapt to unexpected alterations. M.A. North *et al.* (2023) noted significant changes in climatic conditions in the regions where cows are kept. These changes had a considerable negative impact on the animals' bodies, subsequently causing metabolic shifts under the influence of the sympathetic nervous system.

An analysis of the literature led to the conclusion that investigating the factors influencing the correction of the fatty acid composition of milk is an important issue. This is because the levels of fatty acids are crucial indicators when assessing milk quality and its nutritional benefits for humans. Adjusting the fatty acid

composition of milk, particularly the unsaturated fatty acids, is achieved through key factors such as a balanced nutrient content in the cows' feed rations, the state of their gut microbiota, and especially through neurohumoral regulation. Therefore, studying this topic is highly relevant in contemporary research.

## Materials and Methods

The research was conducted in 2023 at the dairy farm of the LLC Obriy (Vyshneve Village, Orzhytskyi District, Poltava Region, Ukraine) using Ukrainian Black-and-White dairy cows. The analysis of the milk's fatty acid profile from the experimental cows was carried out at the interdepartmental educational and scientific laboratory for veterinary diagnostic research (Faculty of Veterinary Medicine, National University of Life and Environmental Sciences of Ukraine, Kyiv)

The animal-based experimental studies described in this research, specifically all procedures involving the cows, were conducted following the fundamental principles of bioethics. This adhered to Article 26 of the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986), Directive 2010/63/EU (2010), and the "General Ethical Principles of Experiments on Animals" adopted by the First National Congress on Bioethics (Law of Ukraine No. 249, 2012).

The animal groups were established using variation-pulseometric research (according to Bayevsky's method) and employing a Heart Mirror IKO electrocardiograph (Innomed, Hungary). Based on the electrocardiogram readings, a measure known as the heartbeat interval between the R-R wave segments on the recorded trace was determined, and this was expressed as the mode (*Mo*). The percentage value of

the mode, which was most frequently observed over a sequence of 100 heartbeats, and represented as the amplitude of the mode (*AMo*), was then calculated. The difference between the maximum and minimum mode values within the selected readings was also calculated, which corresponded to the variation range ( $\Delta x$ ) indicator. Following this, indices reflecting the activity of the autonomic nervous system were computed, including the autonomic balance index (ABI), the autonomic rhythm index (ARI), and the tension index (TI).

After conducting the variation-pulseometric study, three experimental groups of cows were formed: sympathotonics, vagotonics, and normotonics. Each experimental group consisted of five animals. Milk samples were collected from the animals in the experimental groups using individual milking machines during the morning milking session. The collected milk samples were transported using insulated containers.

Lipid extraction was carried out using the method developed by J. Folch *et al.* (1957). Milk was placed in a glass container with a ground glass stopper, and then a mixture of methanol and chloroform in a 2:1 ratio was added, with a volume ratio of 1:20 relative to the milk. The resulting mixture was thoroughly mixed and left to stand for 24 hours. The following day, the mixture was filtered, and a 0.74% potassium chloride solution was added to the filtrate in a 1:5 ratio. After settling, the solution was separated into two layers. The lower layer was collected, and the chloroform was evaporated off using a rotary evaporator. The remaining lipids were then used to analyse their fatty acid composition.

Chromatographic analysis was performed using a Trace GC Ultra gas chromatograph (USA) equipped with a flame ionisation detector. The chromatography of the samples was conducted under the following conditions: detector temperature +260°C, column temperature

+140-240°C. To identify the fatty acid components in the cow milk lipid samples obtained from the chromatography, a Supelco 37 Component FAME Mix standard (USA) was used.

Statistical analysis of the research findings was performed using the Microsoft Excel software package, with the probability of significant differences between the measured values assessed using Student's t-test ( $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ ).

## Results and Discussion

Based on the results of the chromatographic analysis of milk from cows predominantly showing normotonia, the levels of the following unsaturated fatty acids were determined: myristoleic acid, palmitoleic acid, oleic acid, linoleic acid, and  $\alpha$ -linolenic acid. According to the statistical analysis, the results obtained from five cows were compiled based on key descriptive statistics and are presented in Table 1.

**Table 1.** Unsaturated fatty acid content (%) in the milk of the normotonic experimental group of cows during the summer period

| Indicator                          | NVO | SE   | M     | SD   | Asym. | Min.  | Max.  |
|------------------------------------|-----|------|-------|------|-------|-------|-------|
| Myristoleic acid (C14:1)           | 5   | 0.02 | 1.35  | 0.05 | -0.11 | 1.29  | 1.42  |
| Palmitoleic acid (C16:1)           | 5   | 0.05 | 1.89  | 0.11 | -1.58 | 1.70  | 1.99  |
| Oleic acid (C18:1n9c)              | 5   | 0.19 | 23.44 | 0.43 | -1.54 | 22.71 | 23.86 |
| Linoleic acid (C18:2n6c)           | 5   | 0.07 | 4.56  | 0.17 | -0.08 | 4.39  | 4.73  |
| $\alpha$ -Linolenic acid (C18:3n3) | 5   | 0.04 | 0.61  | 0.08 | -0.28 | 0.49  | 0.72  |

**Note:** NVO – number of valid observations; SE – standard error; M – mean value; SD – standard deviation; Asym. – asymmetry; Min. – minimum value; Max. – maximum value

**Source:** authors' development

Based on the statistical analysis (Table 1) of the fatty acid composition of milk lipids from the normotonic experimental group of cows, the myristoleic acid content in the cows' milk showed a low standard error, indicating minimal variability between the values. The standard deviation also indicated a low level of variability in the data. Furthermore, the asymmetry measure reflected a near-perfect symmetrical distribution of the data points, and the difference between the maximum and minimum values for this fatty acid was 0.13%, suggesting a very small range in the obtained results.

The palmitoleic acid content also exhibited a low standard error, again indicating little variability in the measured values. The standard deviation value pointed to a non-significant level of data variability, and the asymmetry measure demonstrated a reasonably symmetrical

distribution of the data. The difference between the maximum and minimum values for this fatty acid was 0.29%, indicating a small range in the obtained results.

For the oleic acid content in the milk of the five cows, a low standard error with minimal variability in the values was observed. The standard deviation indicated a small degree of variability in the data. The asymmetry measure revealed a slight leftward skew, reflecting a small negative asymmetry. The difference between the maximum and minimum values for this fatty acid was 1.15%, reflecting a small range in the obtained data.

Regarding the linoleic acid content, a low standard error was found, suggesting little variability in the values, and the standard deviation indicated a negligible level of data variability. Conversely, the asymmetry measure showed a normal distribution of the obtained data,

indicating no significant asymmetry. The difference between the maximum and minimum values for this fatty acid was 0.34%, suggesting a small range in the obtained data.

For the  $\alpha$ -linolenic acid content among the five animals, a standard error with minimal variability in the values was established. The standard deviation reflected slight variability in the data for this fatty acid. The asymmetry measure indicated a small negative skew, describing a relatively even distribution of the data. The difference between the maximum and

minimum values was 0.23%, confirming a small range in the numerical data.

Based on the chromatographic analysis of milk lipids from cows predominantly showing sympathotonia, the levels of the following unsaturated fatty acids were determined: myristoleic acid, palmitoleic acid, oleic acid, linoleic acid, and  $\alpha$ -linolenic acid. According to the statistical analysis conducted, the results obtained from the five cows involved in the study were processed based on key descriptive statistics and are presented in Table 2.

**Table 2.** Unsaturated fatty acid content (%) in the milk of the sympathotonic experimental group of cows during the summer period

| Indicator                          | NVO | SE   | M     | SD   | Asym. | Min.  | Max.  |
|------------------------------------|-----|------|-------|------|-------|-------|-------|
| Myristoleic acid (C14:1)           | 5   | 0.02 | 1.44  | 0.05 | -0.13 | 1.38  | 1.49  |
| Palmitoleic acid (C16:1)           | 5   | 0.02 | 1.58  | 0.04 | -0.40 | 1.52  | 1.62  |
| Oleic acid (C18:1n9c)              | 5   | 0.07 | 22.60 | 0.17 | 0.28  | 22.41 | 22.81 |
| Linoleic acid (C18:2n6c)           | 5   | 0.05 | 4.85  | 0.11 | 0.08  | 4.71  | 4.98  |
| $\alpha$ -Linolenic acid (C18:3n3) | 5   | 0.05 | 0.79  | 0.12 | 0.60  | 0.64  | 0.97  |

**Note:** NVO – number of valid observations; SE – standard error; M – mean value; SD – standard deviation; Asym. – asymmetry; Min. – minimum value; Max. – maximum value

**Source:** authors' development

According to the statistical evaluation of the results from the chromatographic analysis of the cows' milk, the standard error for myristoleic acid was low, indicating negligible variability in the data. The standard deviation pointed to slight variability in the obtained values. The asymmetry measure was insignificant, indicating no data asymmetry, thus reflecting a normal distribution. The difference between the maximum and minimum values for this fatty acid was 0.11%, signifying a small range in its measured levels.

For palmitoleic acid, the standard error values were relatively small, indicating negligible variability in the measurements. The variability of the results was within acceptable limits, based on the standard deviation. The asymmetry measure indicated a slight negative skew, suggesting that the results approximated a normal

distribution. The difference between the maximum and minimum values for this fatty acid was 0.10%, confirming a small range in its levels.

The oleic acid levels showed slight variation in the data and their variability, which is supported by the low standard deviation and standard error values. The asymmetry showed moderately small positive skew values, suggesting the distribution of the values was reliable. The difference between the maximum and minimum values for this fatty acid was 0.40%, further supporting the normal distribution of the obtained results.

The values for linoleic acid were characterised by low standard error and standard deviation, indicating minimal variability and fluctuations in the results. The asymmetry further confirmed a normal distribution for this fatty acid's levels, implying a virtual absence of

asymmetry. This was consistent with the difference between the maximum and minimum values, which was 0.27%.

For  $\alpha$ -linolenic acid, the statistical analysis indicated slight variability and fluctuations in the data, which were supported by the standard error and standard deviation values. The asymmetry reflected a small positive skew, indicating a reasonable approximation to a normal distribution. The difference between the highest and lowest values for this

fatty acid was 0.33%, corresponding to a small range in the raw data.

Based on the chromatographic analysis of milk lipids from cows predominantly showing vagotonia, the levels of the following unsaturated fatty acids were determined: myristoleic acid, palmitoleic acid, linoleic acid, oleic acid, and  $\alpha$ -linolenic acid. According to the statistical analysis conducted, the results obtained from five cows were processed based on key descriptive statistics and are presented in Table 3.

**Table 3.** Unsaturated fatty acid content (%) in the milk of the vagotonic experimental group of cows during the summer period

| Indicator                          | NVO | SE   | M     | SD   | Asym. | Min.  | Max.  |
|------------------------------------|-----|------|-------|------|-------|-------|-------|
| Myristoleic acid (C14:1)           | 5   | 0.04 | 1.65  | 0.09 | -0.50 | 1.53  | 1.75  |
| Palmitoleic acid (C16:1)           | 5   | 0.04 | 1.49  | 0.09 | 0.41  | 1.37  | 1.62  |
| Oleic acid (C18:1n9c)              | 5   | 0.04 | 23.57 | 0.10 | 0.24  | 23.45 | 23.70 |
| Linoleic acid (C18:2n6c)           | 5   | 0.04 | 3.70  | 0.09 | 0.28  | 3.59  | 3.82  |
| $\alpha$ -Linolenic acid (C18:3n3) | 5   | 0.04 | 0.43  | 0.09 | 0.66  | 0.32  | 0.57  |

**Note:** NVO – number of valid observations; SE – standard error; M – mean value; SD – standard deviation; Asym. – asymmetry; Min. – minimum value; Max. – maximum value

**Source:** authors' development

According to the statistical analysis of the results from the chromatographic analysis of the cows' milk, the standard error for myristoleic acid was low, characterising a small variability in the data. The standard deviation indicated slight variability in the obtained values. The asymmetry showed a small negative skew, characterising a reliable symmetry in the data. The difference between the maximum and minimum values for this fatty acid was 0.22%, signifying negligible differences in their levels.

For palmitoleic acid, the standard error values were quite small, indicating negligible variability in the measurements. The variability of the results was within acceptable limits, based on the standard deviation values. The asymmetry value indicated a slight positive skew, suggesting that the results approximated a normal distribution. The difference between the maximum

and minimum values for this fatty acid was 0.25%, confirming a small range in its levels.

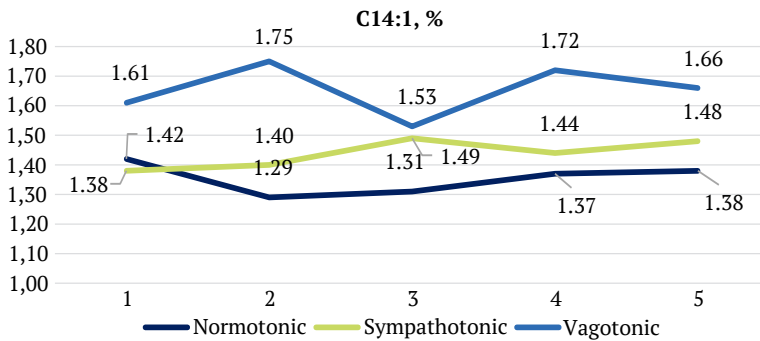
The values for linoleic acid were characterised by low standard error and standard deviation, indicating minimal variability and fluctuations in the data. The asymmetry further confirmed a normal distribution for this fatty acid's levels, implying a virtual absence of asymmetry. This was consistent with the difference between the maximum and minimum values, which was 0.23%.

For  $\alpha$ -linolenic acid, the statistical analysis indicated slight variability and fluctuations in the data, which were supported by the standard error and standard deviation values. The asymmetry reflected a small positive skew, indicating a reasonable approximation to a normal distribution. The difference between the maximum and minimum values for this fatty acid

was 0.25%, corresponding to a small range in the original data.

According to the obtained results regarding the content of unsaturated fatty acids in the cows' milk, it was found that the myristoleic acid content in the milk of the vagotonic experimental group increased by 22% ( $P < 0.001$ ), and in the sympathotonic group by

7% ( $P < 0.05$ ), when compared to the normotonic experimental group. It is worth noting that, among the three experimental groups of animals, the vagotonic group exhibited the highest levels of myristoleic acid. The lowest relative content of the fatty acid C14:1 was observed in the milk of the sympathotonic experimental group (Fig. 1).



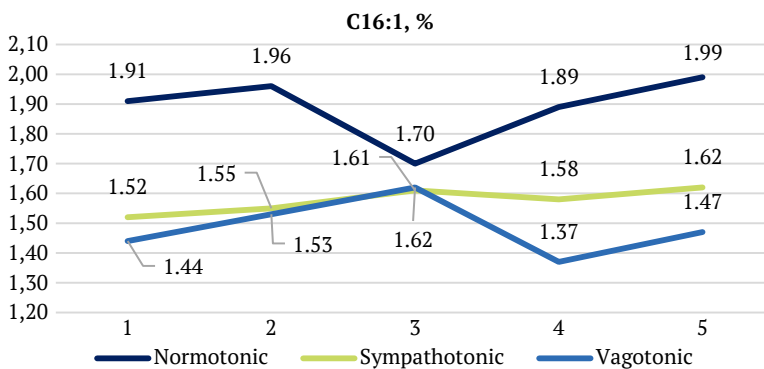
**Figure 1.** Myristoleic acid content in the milk of experimental groups of cows

*Note:* colours represent the experimental groups of animals

*Source:* authors' development

The palmitoleic acid content in the milk of cows in the vagotonic experimental group decreased by 21% ( $P < 0.001$ ), and in the sympathotonic group by 16% ( $P < 0.001$ ) compared to the normotonic group. Among the animals in the experimental groups, the highest

relative content of this fatty acid (C16:1) was found in the normotonic cows. The animals in the sympathotonic and vagotonic experimental groups showed only slight differences in the levels of palmitoleic acid in their milk lipids (Fig. 2).



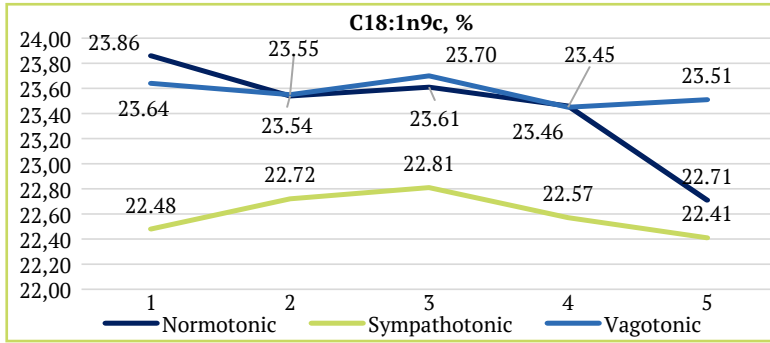
**Figure 2.** Palmitoleic acid content in the milk of experimental groups of cows

*Note:* colours represent the experimental groups of animals

*Source:* authors' development

The oleic acid content in the milk of cows in the sympathotonic experimental group decreased by 3.6% ( $P < 0.05$ ) compared to the normotonic group. It is worth noting that, among the cows in the three experimental groups, the

sympathotonic group had the lowest levels of oleic acid. The experimental groups of cows with a predominance of vagotonia and normotonia showed only slight differences in the levels of oleic acid in their milk lipids (Fig. 3).



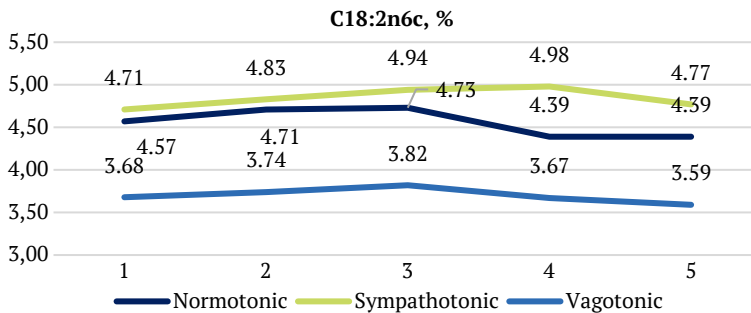
**Figure 3.** Oleic acid content in the milk of experimental groups of cows

*Note:* colours represent the experimental groups of animals

*Source:* authors' development

The linoleic acid content in the milk of cows in the vagotonic experimental group decreased by 19% ( $P < 0.05$ ), while in the sympathotonic group, it increased by 6% ( $P < 0.05$ ) compared to the normotonic group. Among the cows in the experimental groups, the lowest relative content of the fatty acid C18:2n6c was observed in the vagotonic animals. The cows in the sympathotonic and normotonic experimental groups showed only slight differences in the levels of linoleic acid in their milk lipids (Fig. 4).

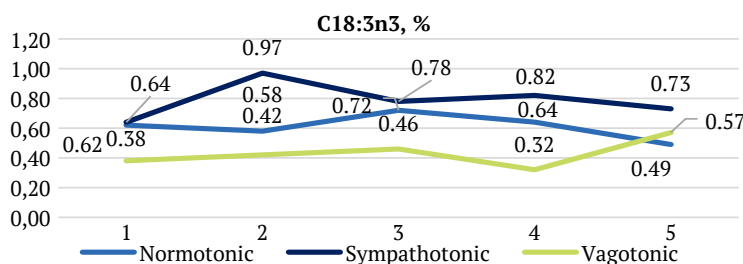
The  $\alpha$ -linolenic acid content in the milk of cows in the vagotonic experimental group decreased by 46% ( $P < 0.05$ ), while in the sympathotonic group, it increased by 30% ( $P < 0.05$ ) compared to the normotonic group. It is worth noting that, among the cows in the three experimental groups, the sympathotonic group had the highest levels of  $\alpha$ -linolenic acid. The lowest relative content of the fatty acid C18:3n3 in the milk of cows was observed in the vagotonic experimental group (Fig. 5).



**Figure 4.**  $\alpha$ -Linolenic acid content in the milk of experimental groups of cows

*Note:* colours represent the experimental groups of animals

*Source:* authors' development



**Figure 5.**  $\alpha$ -Linolenic acid content in the milk of experimental groups of cows

**Note:** colours represent the experimental groups of animals

**Source:** authors' development

A.M. Wanderley *et al.* (2022) demonstrated that the lipid content in milk constantly fluctuates within a defined range. These variations are due to several influencing factors, such as diet, housing conditions, the animal's genetic makeup, and physiological characteristics like neurohumoral regulation. The combination of these elements forms a list of important indicators that should be considered when raising cows. Indeed, without accounting for and adjusting these fundamental factors, the normal development of the animal will be compromised, leading to reduced milk yield and energy imbalances during lactation. Neglecting the control systems governing metabolic processes in cows can also undermine the effectiveness of any attempts to correct them.

Lipids are crucial for maintaining high productivity in dairy farming, as a cow's body consumes a significant amount of energy-rich compounds during milk synthesis. M.S. Gold *et al.* (2022) in their research highlighted the significant role of diet in maintaining the animals' energy metabolism. However, it is also necessary to investigate the influence of additional factors on lipid synthesis, with a focus on how this impact affects productivity. This study, therefore, highlights the importance of studying the role of autonomic regulation in fat metabolism to improve milk production in cows.

J. Shpirer *et al.* (2023) investigated the influence of diet on lipid metabolism in cows and

the resulting changes in the milk's lipid profile. As previously mentioned, several factors regulate metabolism within an animal's body. The digestion, absorption, and utilisation of nutrients represent fundamental physiological processes. The authors demonstrated that a higher concentration of specific fatty acids in the diet leads to an improved lipid profile in the milk. For instance, increasing the levels of palmitic and oleic acids in the feed ration positively impacted the percentage of fat in the milk, and an increase in milk production was observed. This study emphasised the factors controlling the metabolic transformations of substances within the animals' bodies, which was evident in the differences in unsaturated fatty acid content in the milk, depending on the activity of the autonomic nervous system.

The specifics of fatty acid digestibility in the digestive tract and their subsequent production in the mammary gland under varying feed rations were investigated by A. Irawan *et al.* (2024). These researchers explored how the fatty acid composition of milk changes in response to physiological processes involved in the digestion and absorption of lipids within cows. They found that the inclusion of clover in the diet resulted in minor changes to milk composition, with increases observed in linoleic,  $\alpha$ -linolenic, and stearic acid levels. When sainfoin silage was present in the diet, the levels of  $\alpha$ -linolenic acid and linoleic acid improved. In the current

study, the impact on the fatty acid composition of milk was achieved through differing autonomic regulation, meaning variations in the activity of systems controlling metabolic intensity.

G. Satir *et al.* (2023) examined the role of palm oil in the diet of cows on fatty acid metabolism and their concentration in milk. Their analysis of milk showed an increase in palmitic acid content and a slight rise in the overall level of saturated fatty acids. The author highlighted the importance of studying lipid metabolism to enhance the fatty acid profile of milk, particularly in increasing the levels of essential omega-6 and omega-3 fatty acids. The present study established the influence of autonomic regulation on the content of omega-6 and omega-3 fatty acids, resulting from changes in their synthesis and breakdown processes.

E. Vargas-Bello-Pérez *et al.* (2022) investigated alterations in the lipid composition of milk when sunflower and rapeseed oil were included in the animals' diet. Evaluating the feed digestion processes, with the key aspects outlined, they found minor changes in the levels of butyric acid,  $\alpha$ -linolenic acid, and linoleic acid. This indicated a dietary dependence on the feed consumed and its digestibility within the digestive system. The authors' own research focused on analysing changes in lipid concentration in cows' milk under the influence of the autonomic nervous system, which consequently showed significant alterations based on the chromatographic analysis data.

Having analysed the obtained results, including a comparative perspective with existing knowledge, conclusions can be drawn regarding the factors that influence the fatty acid composition of cows' milk. The overwhelming majority of research by other scientists studying lipid metabolism in the mammary gland focuses on the dietary dependence of consumed feed and its composition on the concentration of lipids in milk. However, their findings when assessing

factors influencing lipid metabolism in the mammary gland often reveal only minor correlations. A possible explanation for this is the exclusion of systems responsible for metabolic processes within the animal's body, particularly neurohumoral regulation. By evaluating only a small part of this system, specifically the autonomic nervous regulation, significant differences in the lipid composition of milk in cows were revealed according to the study results. When analysing lipid metabolism in lactating cows, it is also important to consider individual differences within the animals' bodies. Categorising them according to their autonomic regulation will improve both the general understanding of metabolic processes and the development of methods for adjusting the intensity and direction of lipid metabolism in lactating cows.

## Conclusions

It has been established that autonomic regulation influences the levels of unsaturated fatty acids in cows' milk. This is supported by the data from the chromatographic analysis of the lipid composition of milk from animals exhibiting normotonia, vagotonia, and sympathotonia. It was determined that a higher level of sympathotonia in cows is a contributing factor to increased levels of myristoleic, linoleic, and  $\alpha$ -linolenic acids in their milk, compared to the group of animals with a balanced sympathovagal system. Specifically, the myristoleic fatty acid content, according to the chromatographic analysis, increased in the milk of sympathotonic cows by 7% ( $P < 0.05$ ) and in vagotonic cows by 22% ( $P < 0.001$ ) when compared to the normotonic experimental group. Conversely, the level of palmitoleic acid decreased in the milk of vagotonic cows by 21% ( $P < 0.001$ ) and sympathotonic cows by 16% ( $P < 0.001$ ), relative to its levels in the milk of the normotonic experimental group. The oleic acid content in the milk of sympathotonic cows decreased by 3.6% ( $P < 0.05$ )

compared to the normotonic experimental group. The level of linoleic fatty acid in the milk of sympathotonic cows increased by 6% ( $P < 0.05$ ), while in vagotonic cows it decreased by 19% ( $P < 0.05$ ) compared to the animals in the normotonic experimental group. The  $\alpha$ -linolenic acid content in the milk lipids of sympathotonic cows increased by 30% ( $P < 0.05$ ), while in vagotonic cows it decreased by 46% ( $P < 0.05$ ) compared to the normotonic animals. Thus, the autonomic nervous system, depending on individual differences in the cows' physiology, such as varying activity of the sympathetic and parasympathetic nervous systems, has a distinct influence on fatty acid metabolism. This manifests as either an increase or decrease in lipolysis and lipogenesis, particularly during periods when external environmental factors exert their influence. This is evidenced by the differing levels of unsaturated fatty acids in the cows' milk, the percentage ratios of which depend on the intensity of lipid metabolism. This metabolism is controlled by the body's regulatory systems, which include

the autonomic nervous system. Therefore, individual physiological characteristics of the animals, expressed as different tones of the autonomic nervous system – normotonia, sympathotonia, vagotonia – are an important indicator when analysing and adjusting lipid metabolism.

Further research will focus on the application of nanoaquachelate bioactive substances to high-yielding cows, with mandatory consideration of their individual characteristics, specifically their autonomic regulation. This will involve developing a methodology to enhance metabolic intensity within the body to improve overall cow productivity.

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### Conflict of Interest

None.

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## **Вегетативна регуляція вмісту ненасичених жирних кислот у молоці корів**

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**Анотація.** Вивчення ролі автономної нервової системи як однієї з провідних систем контролю за метаболізмом в організмі тварин важливе для оцінки індивідуальних відмінностей інтенсивності і напряму перебігу обміну речовин. Тому дослідження впливу парасимпатичної і симпатичної нервової системи на обмін ліпідів, у тому числі жирних кислот, є актуальним питанням для забезпечення молочної продуктивності корів. Метою цього дослідження було порівняльне оцінювання вмісту ненасичених жирних кислот у молоці корів за різної вегетативної регуляції у літній період. Формування дослідних груп корів породи українська чорно-ряба молочна здійснювалося відповідно до симпто-вагусного балансу шляхом проведення варіаційно-пульсометричного дослідження. Для ідентифікації жирнокислотного складу молока використовувався метод газової хроматографії. В результаті встановлено, що рівень мірістоолеїнової кислоти у молоці корів із ваготонією виявлявся вищим на 22 % ( $P < 0,001$ ) порівняно з її вмістом у молоці корів із нормотонією, а із симпатотонією – на 7 % ( $P < 0,05$ ). Пальмітолеїнова кислота у корів із ваготонією відмічався менше на 21 % ( $P < 0,001$ ) порівняно з таким у молоці корів із нормотонією, а із

симпатотонією – на 16 % ( $P < 0,001$ ). У молоці корів із симпатотонією зафіксовано знижений на 3,6 % ( $P < 0,05$ ) вміст олеїнової кислоти порівняно з її концентрацією у корів із нормотонією. Вміст лінолевої кислоти у корів із ваготонією відзначався значеннями меншими на 19 % ( $P < 0,05$ ) порівняно з її рівнем у молоці корів із нормотонією, а із симпатотонією – більшими на 6 % ( $P < 0,05$ ). Вміст  $\alpha$ -ліноленової кислоти у молоці корів із ваготонією виявлявся меншим на 46 % ( $P < 0,05$ ) порівняно з таким у молоці корів із нормотонією, а у симпатотоніків – більшим на 30 % ( $P < 0,05$ ). Виявлено залежність між активністю автономної нервової системи та вмістом ненасичених жирних кислот у молоці. Отримані результати підтверджують важливість вивчення питання ролі регуляторних механізмів обміну ліпідів, особливо у молочному скотарстві, де індивідуальна продуктивність корів визначається рівнем жирів, які споживаються, засвоюються, синтезуються та метаболізуються в організмі

**Ключові слова:** жуйні; газова хроматографія; ліпіди; варіаційна пульсометрія; нервова система