



## Influence of feeding with fatty acids emulsions on their content in sheep wool

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The feeding of sheep has a significant impact on the processes of wool formation. This especially applies to the fat components of the diet, and in particular, fatty acids. In this regard, the aim of our work was to determine the effect of feeding a water-soluble complex of fatty acids on their content in the internal lipids of the wool of Prekos ewes and lambs obtained from them. The ewes of the experimental group were fed an emulsion of fatty acids consisting of: (9E,12E)-octadeca-9,12-dienoic (C18:2 $\omega$ 6), (9Z)-octadec-9-enoic (C18:1 $\omega$ 9), hexadecanoic (C16:0), (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic (C20:4 $\omega$ 6), octadecanoic (C18:0) and (9Z,12Z,15Z)-octadeca-9,12,15-trienoic (C18:3 $\omega$ 3). After the removal of surface lipids with carbon tetrachloride, free internal lipids of wool fibers were obtained by extraction with a chloroform/methanol mixture (2:1) in a Soxhlet apparatus, and the bound ones were obtained after preliminary alkaline hydrolysis. Their fatty acid composition was studied using a gas-liquid chromatograph, after converting lipids into methyl esters by direct transesterification of fatty acids. It was established that feeding ewes with a water-soluble complex of fatty acids leads to a significant increase in free internal lipids of hexadecanoic (C16:0) acid, both in the wool of ewes and in lambs obtained from them. In the covalently bound internal lipids of wool, the content of hexadecanoic (C16:0), octadecanoic (C18:0), and (9Z)-octadec-9-enoic (C18:1 $\omega$ 9) acids increased. Although the content of (9Z)-octadec-9-enoic (C18:1 $\omega$ 9) acid only tended to increase, the amount of hexadecanoic (C16:0) acid significantly increased in ewes from 4.29% to 6.78%, and that of octadecanoic (C18:0) increased in ewes from 10.62% to 17.35%, and in lambs from 15.20% to 19.04%. This, in turn, led to an increase in the total amount of saturated bound acids in lambs' wool, from 84.49% in the control group to 88.37% in the experimental group. The inclusion of fatty acid emulsions in the diet of sheep, which affect the internal lipids of wool, and in particular, their fatty acid composition, can be used to improve the physical, and therefore technological characteristics of wool raw materials.

**Keywords:** ewes; lambs; internal lipids; hexadecanoic acid; octadecanoic acid; nutritional factors; diets.

### Introduction

Increasing wool productivity of sheep and improving the quality of wool largely depends on their nutrition (Musati et al., 2024) since, after genetic factors, nutritional factors have the greatest influence on the processes of wool fiber formation (Chishti et al., 2021; Gelaye et al., 2021). Fats are of great importance in sheep feeding (Ponnampalam et al., 2024), which is due to their high energy value, as well as their multifaceted biological effect on the animals' body (Gaffield et al., 2022). In particular, fats are a source of fatty acids, including essential ones, which are not synthesized in the body of sheep, or are synthesized in small quantities. These fatty acids have a significant impact on the functioning of the hair follicle (Welle, 2023).

Wool fiber is a product of the functional and metabolic activity of the hair follicle (Yue et al., 2023; Zhang & Chen, 2023), where processes are genetically regulated (Qiu et al., 2025). Depending on the location, the following morphological parts of the wool fiber are distinguished: the root, which is located deep in the skin, in the so-called hair sac or sheath of the hair bulb, at the base of which there is a hair papilla, rich in blood vessels and nerves, and consists of living cells capable of reproduction by mitotic division; the shaft, which covers the surface of the skin and protects the body from cooling and the skin from mechanical damage (Lv et al., 2020; Matos et al., 2022).

The hair shaft, or simply hair, is made up of three layers: the cuticle, cortex, and medulla, which are interconnected by cell-membrane complexes. The outer scale-like layer, or cuticle, is formed of dense keratinized cells (Kicinska-Jakubowska et al., 2024), which overlap each other in a tiled manner, thus protecting the hair from the effects of negative environmental factors (McMullen & Zhang, 2020). The cuticle forms a protective barrier reinforced by a lipid network, which accounts for only 1–9% of the fiber mass, but plays a crucial role in maintaining its hydrophilicity and protection (Tkachuk et al., 2014). The cortex, or cortical layer, is located under the cuticle

and accounts for up to 90% of the total mass of the hair and mainly determines its physical-mechanical and chemical properties (Csuka et al., 2023). It is built of several layers of spindle-shaped cells pressed against each other. The cells of the cortex consist of densely arranged cylindrical filamentous macrofibrils, which in turn are built with microfibrils, which are formed by intermediate filaments immersed in an amorphous medium (matrix) (Zoccola et al., 2023). Finally, the medullary layer, or medulla, is located in the center of the fiber and is characterized only by the awn and dead hair, and in some cases of the transitional hair (Fellows et al., 2021).

Wool fiber consists of 95% keratin, while the remaining components include pigments, mineral elements, water, and lipids. The latter account for up to 3% of wool structure (Tkachuk et al., 2024), and they are the main components of the plasma membranes of hair cells. Internal lipids are localized both in the cuticle and cortex, and in cell membrane complexes (CMCs), which connect the cells of the cuticle and cortex (Ghermezgoli et al., 2020). Recent studies have also confirmed the presence of lipids in the medulla (Kaneta et al., 2021). In particular, Sandt & Borondics (2021) showed that the concentration of lipids in this layer of the hair is 3–20 times higher than in the surrounding cortex. Structural lipids, due to their specific fatty acid composition, play no less a role in hair than in the stratum corneum of the epidermis, influencing its appearance and properties (Szalay & Wertz, 2023; Mijaljica et al., 2024). In particular, they determine its surface properties and protect against negative environmental factors, and also affect physical properties, such as strength, elasticity, hygroscopicity, etc. (El-Fiku et al., 2021).

The main amount of fatty acids in the composition of free internal lipids includes hexadecanoic (C16:0), octadecanoic (C18:0) and (9Z)-octadec-9-enoic (C18:1 $\omega$ 9), and in bound lipids – 18-methyleicosanoic (18-MEA). The latter, whose thickness is approximately 1.1 nm (Song et al., 2024), connects internal lipids through thioether bonds with hair proteins on the outer surface of  $\beta$ -cuticular cells (Wang

et al., 2024). This lipid F layer is considered to be fundamental in determining the surface properties of the hair by forming a hydrophobic barrier (Cozzolino et al., 2024), giving the fiber smoothness and shine, enhancing its resistance to aging, and protecting against environmental stress factors (Sanders et al., 2023).

Thus, the aim of this work was to expand our knowledge of the fatty acid composition of the internal lipids of sheep wool, and to investigate the influence of nutritional factors on them and, in particular, the inclusion of a water-soluble complex of fatty acids in the diets.

## Materials and methods

*Experimental animals and design.* The studies were carried out in the educational and scientific production center "Komarnivske" of the Lviv National University of Veterinary Medicine and Biotechnology named after S. Z. Gzhytsky, on adult ewes of the Prekos breed, during the winter-stalling period of housing. According to the principle of pair analogues (breed of sheep, their age and live weight), two groups of ewes (10 head each) were selected, of which one was the control, and the other was the experimental. At the beginning of the experiment, the ewes were in the last period of pregnancy, and from the middle of the experiment – in the first period of lactation.

During the equalization period, which lasted 10 days, all ewes received a basic ration balanced according to feeding standards. During the experimental period, which lasted 95 days, the animals of the control group received the basic diet, and the ewes of the experimental group received a 3% water-soluble complex of fatty acids "Essential lipid complex" (ELC) produced by LLC EcoProFeed (Ukraine) as part of their feed.

This complex is an aqueous emulsion of fatty acids, which includes 54.5% – (9E,12E)-octadeca-9,12-dienoic (C18:2 $\omega$ 6), 24% – (9Z)-octadec-9-enoic (C18:1 $\omega$ 9), 10% – hexadecanoic (C16:0), 6% – (5Z,8Z,11Z,14Z)-icos-5,8,11,14-tetraenoic (C20:4 $\omega$ 6), 4% – octadecanoic (C18:0) and 1.5% – (9Z,12Z,15Z)-octadeca-9,12,15-trienoic (C18:3 $\omega$ 3). The energy value of the complex is 880 kcal per 100 g. As a result, the crude fat content in the diet of pregnant ewes increased from 46.41 to 58.41 g, i.e. by 12 g, and in lactating ewes – from 62.81 to 80.81 g, by 18 g. The ewes were fed in groups, with daily weighing of the feed consumed and free access to water.

All manipulations with sheep were carried out in compliance with the international principles of the Council of Europe Convention "On the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Meulen-Frank et al., 2017) and the resolutions of the National Congress of Ukraine on Bioethics (2010), and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" as amended on 15.11.2024.

*Isolation of internal lipids of wool.* Wool samples for biochemical studies were taken at the end of the experiment from the experimental ewes and their offspring, in the area behind the shoulder blade. The samples were washed in a neutral detergent and extracted in a Soxhlet apparatus with tetrachloromethane (Sigma Aldrich, USA) for 5 hours to remove surface fats.

To isolate free internal lipids, the studied wool samples were re-extracted for 5 hours with a mixture of chloroform/methanol (2:1) in a Soxhlet apparatus. The lipid extract obtained in this way was dried under vacuum, and used further for studies of the fatty acid composition.

Bound internal lipids were obtained by the alkaline hydrolysis method according to Wertz & Downing (1988). For this purpose, wool samples, after extracting of free internal lipids, were hydrolyzed at 60 °C by two-hour treatment in 100 mL of 1 M sodium hydroxide solution (Tianjin Dingxin Chemical Co Ltd, China) in 90% methanol (SRP Ltd, Ukraine). After cooling, the samples were transferred to separating funnels, 100 mL of chloroform (Chemico Group, UK) and 25 mL of distilled water were added to each. The lower chloroform layer was collected after 12 hours, and the upper phase was acidified with 6 M hydrochloric acid solution (PPC Group, Poland) and re-extracted by mixing with 100 mL of chloroform. After settling, the lower chloroform layer was collected and added to the previously obtained extract, and dried by evaporation. The resulting precipitate was dissolved in 10 mL of a chloroform-methanol mixture (2 : 1) and

3 mL of 7.5% potassium chloride (Luxion, China) was added. After 24 h, the upper phase was removed using a water jet pump, and the lower one, which contained lipids, was used for further studies of the fatty acid composition.

*Determination of the fatty acid composition of internal wool lipids.* To determine the fatty acid composition of internal wool lipids, they were converted into methyl esters by direct transesterification of fatty acids (Stoffel et al., 1959). The separation of fatty acid methyl esters was carried out using a gas-liquid chromatograph Chrom-4 (Czech Republic) under the following conditions: a metal column 2,400 mm long and 3 mm in diameter was filled with Chromosorb 60–80 mesh (Chromatographic Specialties Inc, Canada), coated with 15% polyethylene glycol succinate (TRC Canada), with the thermostat temperature of the columns at 190 °C, evaporator at 240 °C, with an air flow rate of 400 mL/min and a carrier gas (nitrogen) flow rate of 25 mL/min.

The identification of fatty acids was performed using standard mixtures (Supelco USA), and retention indices (tr-Ir) of all components were measured. The content of individual fatty acids was calculated using formulas.

*Statistical analysis.* The analysis of the obtained experimental data was carried out using the Statistica 7.0 program (StatSoft Inc., USA). Data are presented as mean  $\pm$  standard deviation. ANOVA analysis of variance was used to compare the control and experimental groups, followed by analysis of the significance of the differences. The significance of the differences was considered statistically significant at  $P < 0.05$  and less.

## Results

The results of the conducted studies, first of all, showed that the composition of free internal lipids contains 19 fatty acids, three of which have not yet been identified by us. These acids include both saturated and unsaturated acids, as well as iso and antiiso acids.

It has been established that feeding ewes as part of the main diet a water-soluble complex of fatty acids affects the fatty acid composition of free and bound internal lipids of wool, and this applies to both the wool of ewes and the wool of lambs obtained from them.

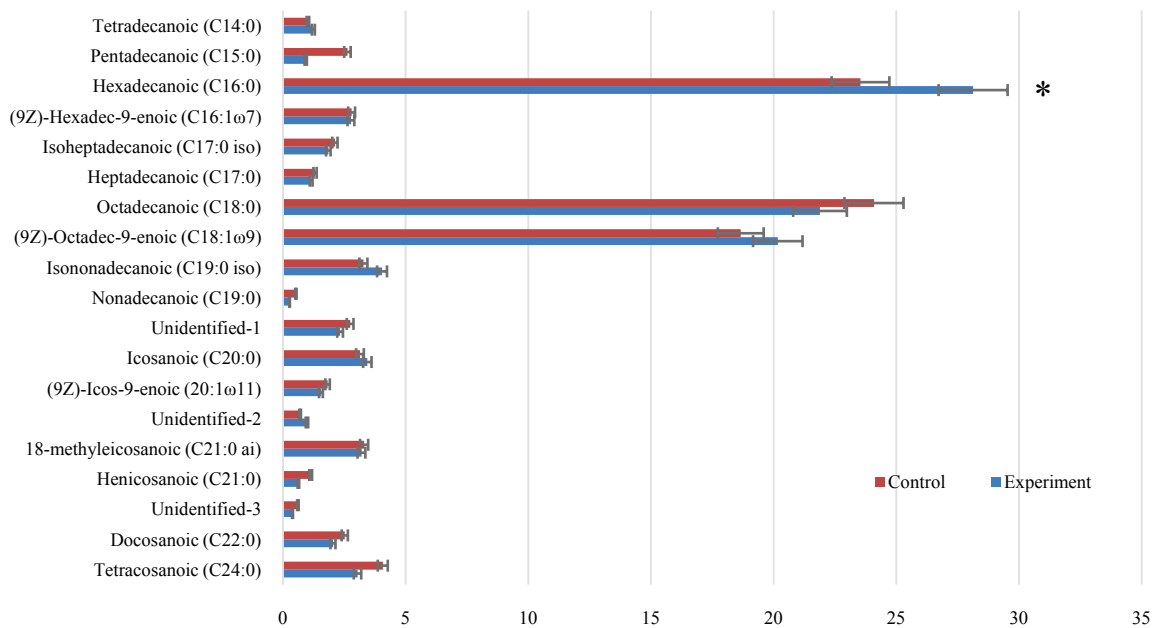
In particular, from the data of Figures 1 and 2 it is clear that in the composition of free internal lipids the content of hexadecanoic (C16:0) fatty acid significantly increased, in the wool of ewes from 23.54% to 28.13% ( $P < 0.05$ ) and in lambs obtained from them from 21.71% to 25.61% ( $P < 0.01$ ). These changes are quite natural given that the fatty acid emulsion, which was included in the ewes' diets, contains 10% of this acid.

As for other fatty acids, we did not observe any significant changes in their content in the composition of free internal lipids. Moreover, the saturation index, i.e. the ratio of the sum of saturated to unsaturated fatty acids, also practically did not change, and was 3.12 and 2.93 in the control group of ewes and 2.77 and 3.21 in the experimental group, respectively.

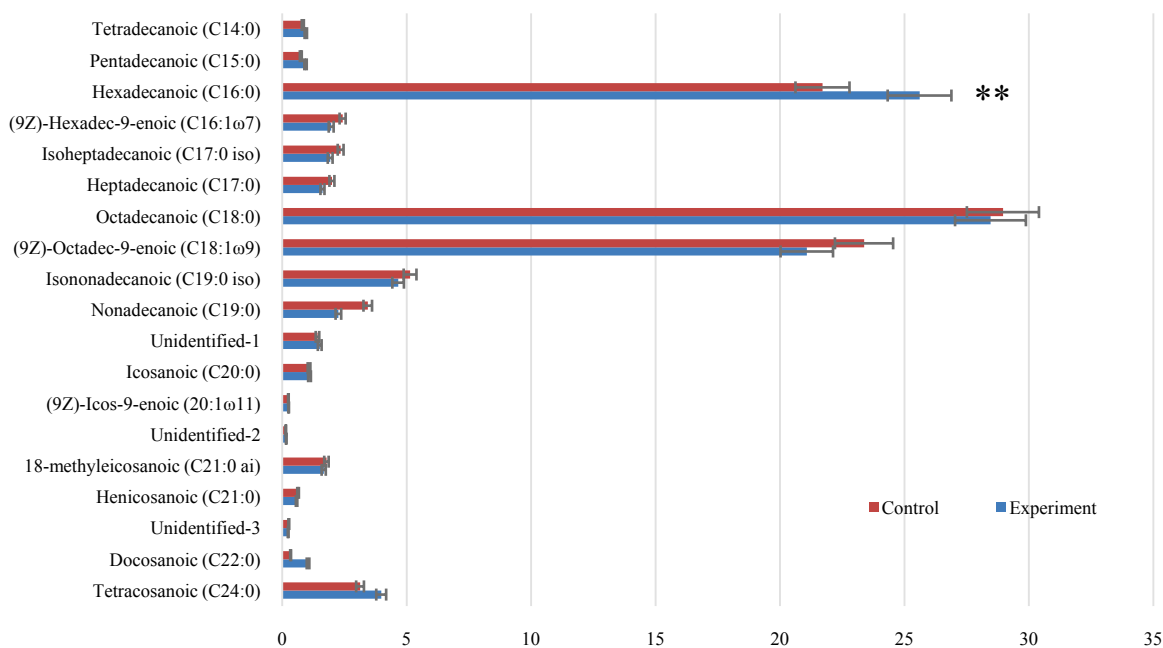
The composition of bound internal lipids is represented by 12 fatty acids, of which one is unidentified, and the largest amount is 18-MEA. However, the nutritional factors we have considered did not lead to significant changes in the content of this acid. Instead, there were changes in the content of fatty acids that were part of the complex that was included in the diet of experimental animals, namely: (9Z)-octadec-9-enoic (C18:1 $\omega$ 9), hexadecanoic (C16:0) and octadecanoic (C18:0) ones. The content of these acids in bound internal lipids of wool increased.

From the data in Figure 3 it is clear that in the wool of ewes the increase in (9Z)-octadec-9-enoic (C18:1 $\omega$ 9) acid is insignificant. Instead, the amount of hexadecanoic (C16:0) ( $P < 0.05$ ) and octadecanoic (C18:0) ( $P < 0.05$ ) fatty acids increased significantly. These changes led to an increase in the total amount of saturated acids from 84.49% in the control group to 88.37% in the experimental group.

As for lambs, only octadecanoic (C18:0) ( $P < 0.05$ ) fatty acid significantly increased (Fig. 4), while hexadecanoic (C16:0) and (9Z)-octadec-9-enoic (C18:1 $\omega$ 9) acids only tended to increase, but this is not significant.



**Fig. 1.** Fatty acid composition of free lipids in ewes' wool (% ,  $x \pm SD$ ,  $n = 4$ )



**Fig. 2.** Fatty acid composition of free lipids of lambs' wool (% ,  $x \pm SD$ ,  $n = 4$ )

The data we obtained indicate that the inclusion of a water-soluble complex of fatty acids in the basic diet of ewes has a direct effect on the fatty acid composition of both free and bound internal lipids of wool, and therefore affects its physical, and therefore, ultimately, technological properties, both in the ewes themselves and in the lambs obtained from them.

## Discussion

Energy is an important factor in sheep feeding, the deficiency of which negatively affects their growth and productivity (Wang et al., 2020; Kaitholil et al., 2024), while increasing the energy level in the diet leads to increased gains and promotes their development (Dong et al., 2020; Kang et al., 2024). Considerable attention is paid to fatty acids in animal feeding (Andrade et al., 2024; Duan et al., 2025), and especially in sheep, the lack of which leads to a number of disorders in the organs and systems of their body (Tajonar et al., 2023; Zhou

et al., 2024). Also, the literature contains practically no data on the effect of feed additives containing  $\omega$ -3,  $\omega$ -6 and  $\omega$ -9 fatty acids on the fatty acid composition of internal lipids of sheep's wool.

In this regard, we conducted a study of the effect of feeding ewes with an emulsion of fatty acids as part of their basic diet on their content in sheep wool. The emulsion included saturated hexadecanoic (C16:0), octadecanoic (C18:0) and unsaturated (9E,12E)-octadeca-9,12-dienoic (C18:2 $\omega$ 6), (9Z)-octadec-9-enoic (C18:1 $\omega$ 9), (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic (C20:4 $\omega$ 6), (9Z,12Z,15Z)-octadeca-9,12,15-trienoic (C18:3 $\omega$ 3) acids.

It is known that an important role in the formation of the physico-chemical characteristics of hair belongs to lipids, which are responsible for its hydrophobic properties (Coderch et al., 2022; Vikash et al., 2025). Previously, Havryliak et al. (2012) showed that the fatty acid composition of free internal lipids is represented by 19 acids, and the composition of covalently bound is represented by 10 respectively. The data obtained by us now coincide with these results for free inter-

nal lipids, and differ somewhat for covalently bound. Thus, in the composition of free internal lipids we found 19 fatty acids, and in the composition of bound we found 12.

According to Inostroza et al. (2024) the main fatty acids of free lipids are hexadecanoic (C16:0), octadecanoic (C18:0) and (9Z)-octadec-9-enoic (C18:1 $\omega$ 9), the total content of which is about 60% of

their total amount, which is consistent with our results. According to our data, these three fatty acids constitute the largest amount of the entire fatty acid composition of free internal lipids, and their total amount is from 66% to 75%, depending on the age, sex and nature of feeding of sheep.

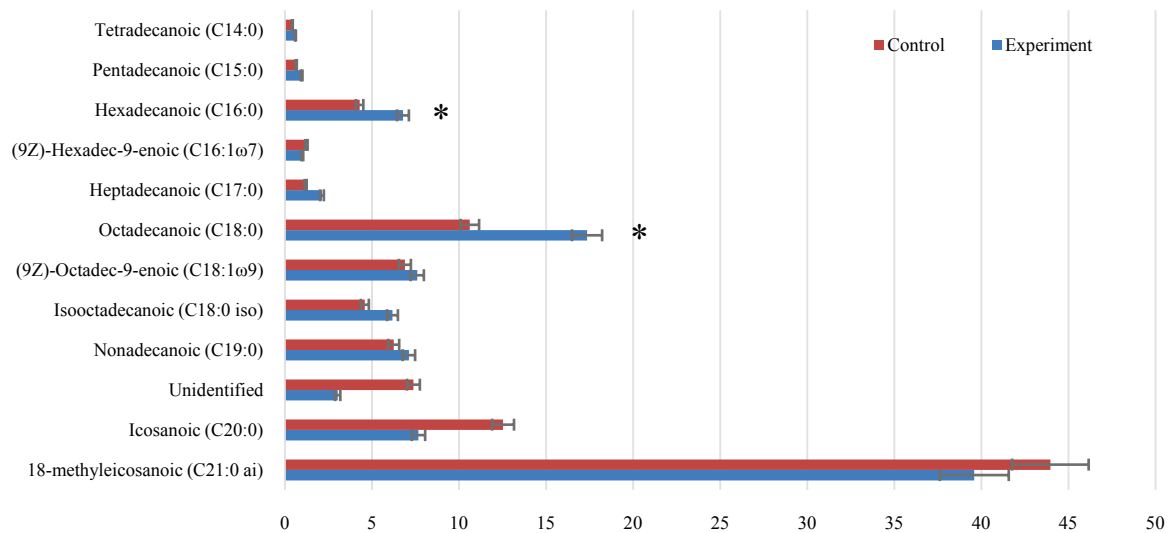


Fig. 3. Fatty acid composition of bound lipids of ewes' wool (% ,  $x \pm SD$ ,  $n = 4$ )

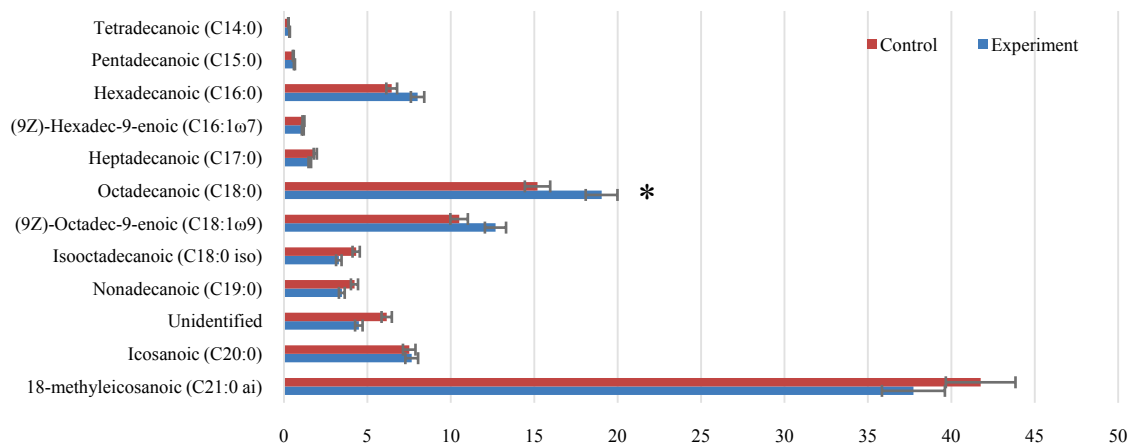


Fig. 4. Fatty acid composition of bound lipids of lambs' wool (% ,  $x \pm SD$ ,  $n = 4$ )

Coderch et al. (2023) indicate that the main amount of fatty acids of hair is located in the cell-membrane complexes of the cuticle, which are built from two  $\beta$ -layers: upper and lower. The upper layer contains the most covalently bound 18-MEA, which forms a monolayer intercalated with other fatty acids stabilized by van der Waals and electrostatic interactions. The lower layer is formed mainly by (9Z)-octadec-9-enoic (C18:1 $\omega$ 9), octadecanoic (C18:0) and hexadecanoic (C16:0) acids. We recorded the largest changes in the latter. In particular, when ewes were fed a water-soluble complex of fatty acids, the amount of hexadecanoic (C16:0) acid in the wool of animals of the experimental group significantly increased, and these changes apply to both ewes ( $P < 0.05$ ) and lambs obtained from them ( $P < 0.01$ ).

Instead, in bound structural lipids, the main share is 18-MEA, the amount of which is almost 40% according to the literature data (Braz et al., 2023; Foggia et al., 2024). The results obtained by us range from 37.7–43.9%. No significant changes in this acid under the influence of the nutritional factors we considered were observed. Instead, changes in other acids were recorded. In particular, in the wool of ewes of the experimental group, the amount of hexadecanoic (C16:0) ( $P < 0.05$ ) and octadecanoic (C18:0) ( $P < 0.05$ ) acids significantly increased, and in the wool of lambs obtained from them, the amount of octadecanoic (C18:0) ( $P < 0.05$ ), which is logical, since these acids

were part of the complex that was included in the diet of ewes. Inostroza et al. (2022) note that the amount of saturated fatty acids in sheep wool is 65.82%, and for unsaturated it was 34.18%. According to the data we obtained, in the composition of free internal lipids, the total amount of saturated acids exceeds 70%, and 80% in bound, and unsaturated varies within 25% and 10%, respectively. However, we have not yet identified a certain number of acids. In particular, in free internal lipids there are 3 acids, and in bound lipids there is 1.

Nutritional factors do not have a significant effect on the total number of saturated and unsaturated acids, only in lambs of the experimental group in bound lipids did the share of saturated acids increase to 88.37%, compared to the control group, in which their share was 84.49%. So, from the data obtained it clearly follows that the nutritional factors we are concerned with affect the fatty acid composition of internal lipids of wool, both in ewes and in lambs obtained from them.

## Conclusion

Inclusion of a water-soluble complex of fatty acids in the basic diet of ewes led to a significant increase in free internal lipids of hexadecanoic (C16:0) acid from 23.54% to 28.13% in the wool of ewes ( $P < 0.05$ ), and from 21.71% to 25.61% in the wool of lambs obtained

from them ( $P < 0.01$ ). The fatty acid composition of bound internal lipids is characterized by a significant increase in hexadecanoic (C16:0) ( $P < 0.05$ ) and octadecanoic (C18:0) ( $P < 0.05$ ) acids in ewes of the experimental group, and in lambs obtained from them – octadecanoic (C18:0) ( $P < 0.05$ ). No significant changes in the total amount of saturated and unsaturated acids under the influence of the nutritional factors we have considered were recorded, only in lambs of the experimental group in bound lipids did the amount of saturated acids increase to 88.37%, compared to the control group, in which their amount was 84.49%. The data obtained as a result of the studies clearly indicate the influence of the feed ingredients of the studied complex on the fatty acid composition of the internal lipids of sheep wool, which directly affects the physical, and therefore the technological properties of wool. However, our studies do not fully reveal the mechanisms and nature of this influence, which opens up prospects for further research in this direction.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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