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Research Article

Cholesterol Esters in Sheep Wool Upon Inclusion of a Fatty Acid Emulsion in Their Diet

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Abstract | Nutritional factors are decisive in the formation of wool productivity of sheep. In this regard, the aim of our work was to investigate the effect of fermented emulsion of fatty acids on the quantitative and qualitative composition of cholesterol esters of surface and internal lipids of wool of Prekos breed ewes and their lambs. Surface lipids (wax) were isolated by extraction in a Soxhlet apparatus with tetrachloromethane, free internal lipids by repeated extraction with a mixture of chloroform methanol (2:1), bound internal lipids after alkaline hydrolysis. Cholesterol esters were separated in the n-heptane toluene system (8:2). Studies have shown that feeding an emulsion of fatty acids slows down oxidation processes in the grease, as indicated by a probable increase in the composition of surface lipids of esterified cholesterol and, in particular, saturated esters and a decrease in monounsaturated acids. In the composition of free internal lipids of wool of ewes and lambs of the experimental group, the content of saturated acid esters ($P< 0.001$) increases and decreases monounsaturated ($P < 0.05$). In addition, the content of di-, tri- and tetraenoic acid esters ($P < 0.05$) also decreased in the experimental group of ewes. In cholesterol esters of bound internal lipids, the number of saturated acid esters significantly increases only in the experimental group of lambs ($P < 0.001$). Given the importance of cholesterol esters of internal lipids in the formation of physicochemical, and therefore technological characteristics of hair, the results obtained can be used in the production of wool as a raw material for the textile industry.

Keywords | Ewes, Lambs, Wool, Wax, Internal lipids, Fatty acid esters

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INTRODUCTION

Wool is one of the main products of sheep farming, and is widely used in the textile industry. It is characterized by environmental friendliness and by a number of positive sanitary and hygienic properties, including combined heat- and sound-insulating capacity, lightness, softness, and high hygroscopicity (Allafi *et al.*, 2022; Patrucco *et al.*, 2024). In terms of chemical composition, wool fiber is 94-95% proteins (with approximately 80-85% of which is keratin) and only 2-3% is lipids (Mattiello *et al.*, 2023; Vikash *et al.*, 2025).

However, the importance of the latter for the formation of physicochemical parameters of the hair is difficult to overestimate, since lipids are crucial in protecting wool fibers from the negative effects of environmental factors. In particular, they prevent fiber fragility and thinning, serve as a barrier against moisture loss, and also positively affect the main technological characteristics of wool by improving its shine, elasticity and fiber tensile strength (Sanders *et al*., 2023).

Wool lipids are divided into two groups: Surface and internal. As shown by Raghav *et al*. (2022), surface lipids are a product of the secretory activity of the sebaceous glands, which is secreted onto the skin surface directly through the hair follicle canal. These lipids are called wax, since they do not contain acylglycerols, which are the main components of any type of fat, or phospholipids, as confirmed by the studies of Duzelbayeva *et al*. (2023). In contrast, Ruttler *et al*. (2022) reported that their main components are complex esters of lanosterol and cholesterol, and they also contain non-esterified fatty acids and squalene.

Csuka *et al*. (2023) showed that internal wool lipids are formed as a result of biosynthesis in the matrix cells of the hair follicle, and they are conventionally divided into two groups: free and protein-bound. Coderch *et al*. (2023) indicate that free lipids are stabilized in the hair by van der Waals and electrostatic interactions, and sometimes by hydrogen or even ionic bonds, and are released from the fibers by extraction with polar solvents. According to Tkachuk *et al*. (2024). Free internal lipids are mainly represented by ceramides, glucosylceramides, cholesterol sulfate, as well as esterified and unesterified cholesterol.

Instead, Wang *et al*. (2024) note that bound lipids can only be isolated after preliminary alkaline hydrolysis, since they are attached to proteins on the outer surface of epicuticular cells via 18-methyleicosanoic acid (18-MEA) through thioether bonds. Weiand *et al*. (2024) indicate that in this way a lipid monolayer is formed, which determines the surface properties of the hair and makes it hydrophobic. On the fiber surface, 18-MEA is expressed together with shorter, unbranched, straight fatty acids, forming a protective layer, and also, thanks to the methyl group, creates a disordered surface that can give the hair useful tribological properties (Bergendal *et al*., 2025).

However, as Ross *et al*. (2022) point out, due to the constant exposure of wool fiber to various factors that negatively affect its structure and physicochemical characteristics, such as ultraviolet radiation, weather factors and conditions of animal production, the lipid barrier is damaged and loses its inherent functions. In particular, Fernandes *et al*. (2023) emphasize that the loss of 18-MEA reduces hydrophobicity and increases surface friction, leading to dryness, tangling, and hair damage, which in turn, according to Qi *et al*. (2021), can lead to reduced tensile strength and brittleness.

The lipid composition of wool is influenced by a number of factors, including nutritional factors, as mentioned by Habeeb (2025). According to Tkachuk *et al*. (2025), the use of a fat supplement in sheep diets slows oxidation processes in both internal and surface lipids of wool, which in turn leads to an improvement in the protective properties of the wool grease and the physicochemical characteristics of wool fibers.

Thus, the aim of this work was to examine the content of cholesterol esters in surface and internal wool lipids and to investigate the effect of including a fermented water-soluble fatty acid complex in the diet of sheep.

MATERIALS AND METHODS

Experimental animals and design

The study was conducted on full-aged ewes of the Prekos breed, in the winter-stall period of keeping, in the conditions of the educational and scientific production center «Komarnivske» Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv, Ukraine. According to the principle of pair analogues, taking into account breed, age, and live weight, two groups of animals (control and experimental) of 10 heads each were selected. In the first half 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. The experiment lasted 95 days.

All ewes during the 10-day equalization period received a basic diet balanced according to feeding standards; and during the experimental period, the animals of the experimental group had their compound feed supplemented with 3% of the fatty acid emulsion (Essential Lipid Complex ELC) produced by LLC EcoProFeed, Ukraine.

The emulsion is obtained by enzymatic treatment of oils in the presence of glycolipids and polysaccharides using a lipolytic enzyme complex of microorganisms of the genus *Bacillus pseudomonas* and *Bacillus subtilis*. The complex includes: linoleic (9E,12E)-octadeca-9,12-dienoic $C_{18:2\omega6}$) 54.5%, oleic (9Z)-octadec-9-enoic $C_{18:1\omega9}$) 24%, palmitic (hexadecanoic $C_{16:0}$) 10%, arachidonic (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic $C_{20:4\omega6}$) 6%, stearic (octadecanoic $C_{18:0}$) 4% and linolenic (9Z,12Z,15Z)-octadeca-9,12,15-trienoic $C_{18:3\omega3}$) 1.5% fatty acids. As a result, the crude fat content in the diet of pregnant ewes increases from 46.4 to 58.4 g (by 25.9%), and in lactating ewes from 62.8 to 80.8 g (by 28.7%) 100 g of emulsion contains 880 kcal.

All animals were kept under the same conditions, and feeding was group, with free access to water and daily weighing of the consumed feed. For the studies, wool samples taken at the end of the experiment from the area behind the shoulder blade were used.

Isolation of surface and internal lipids

To obtain surface lipids (wax), wool was extracted in a Soxhlet apparatus with tetrachloromethane (Sigma Aldrich, USA) for 5 hours. After that, a test was conducted with Sudan Black B for the absence of residual lipids. To obtain free internal lipids, wool samples were re-extracted with a chloroform–methanol (2:1) mixture in a Soxhlet apparatus for 5 hours. Bound internal lipids were obtained after preliminary alkaline hydrolysis [20]. For this purpose, wool samples, after removal of free internal lipids, were hydrolyzed by two-hour treatment in 100 ml of 1 M NaOH solution (Tianjin Dingxin Chemical Co Ltd, China) in 90% methanol (SRP Ltd, Ukraine) at 60 °C. Then 100 ml of chloroform (Chemico Group, UK) and 25 ml of distilled water were added to each sample. After 12 hours the lower chloroform layer was collected, and the upper phase was acidified with 6 M HCl solution (PPC Group, Poland) and re-extracted by mixing with 100 ml of chloroform. After settling, the lower chloroform layer was collected and combined with the previous extract, which was then evaporated. The resulting residue was dissolved in 10 ml of a chloroform–methanol mixture (2:1) and 3 ml of 7.5% KCl solution (Luxion, China) was added. After 24 hours the upper phase was collected with a water-jet pump, and the lower phase containing lipids was used for further research.

The lipids obtained in this way were studied by thin-layer chromatography in the hexane–diethyl ether system (4:1) with preliminary saturation of the chamber with solvent vapors. Separation was carried out on (Sorbfil) plates.

Study of cholesterol esters

After identification of esterified cholesterol, silica gel with this lipid fraction was scraped from the plates into test tubes. 5 milliliters of a chloroform–methanol mixture (2:1) was added to the test tubes and left for extraction for 24 hours. The extraction mixture was then filtered into clean tubes. The obtained cholesterol esters were applied to silica gel plates, and separation was carried out in the n-heptane–toluene (8:2) system without preliminary saturation of the chamber with solvent vapors. Separation in this solvent system was repeated three times. After each separation, the chromatograms were dried at room temperature and developed in iodine vapors. Identification of individual lipid classes was performed by comparing the chromatogram of the studied samples with that of the controls and by comparing r_f values with literature data. The amount of esterified cholesterol was calculated mathematically per 1 g of clean wool, and the content of its individual classes was expressed as a percentage.

For the research, reagents of qualification not lower than chemically purewere were used.

Statistical analysis

The data obtained as a result of the experiment were analysed using Statistica 12.0 software (StatSoft Inc., USA). The results are presented as mean ± standard deviation (M ± SD). Due to the small sample size n = 4 per group (content of esterified cholesterol and content of wax) and n = 5 per group (content of free and bound internal lipids wool), both parametric (Student`s t-test) and non-parametric (Mann-Whitney U test) methods were used to compare two independent groups. Differences were considered statistically significant at $p < 0.05$ and less.

RESULTS

The results of the conducted studies showed the effect of feeding ewes an emulsion of fatty acids as part of their main diet on the content of esterified cholesterol in the surface and internal lipids of wool. Changes were observed both in ewes and in the lambs obtained from them.

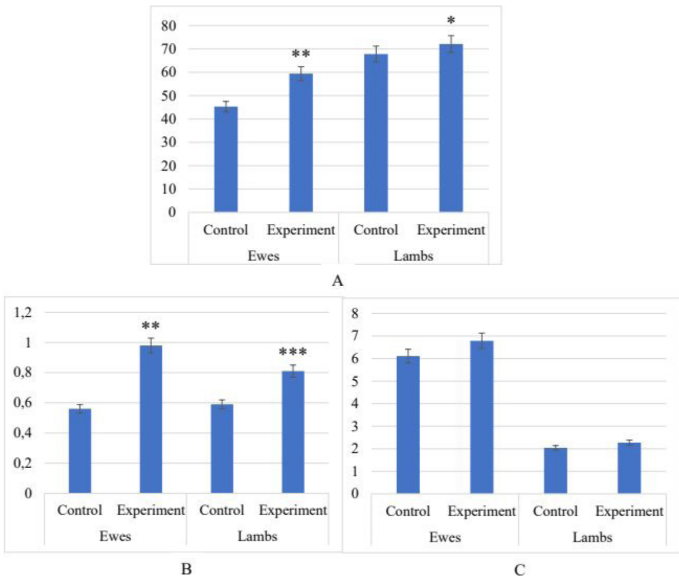

Figure 1: Content of esterified cholesterol, mg per 1 g of wool (mean ± SD, n = 4).
A: Surface lipids (wax), B: internal free lipids, C: internal bound lipids. The differences are significant between the control and experimental groups (* – P <0.05; ** – P <0.01; *** – P <0.001).

In particular, according to the data in Figure 1, in animals of the experimental group, compared with the control group, the content of esterified cholesterol in surface lipids significantly increased from 45.27 to 59.43 mg per 1 g of wool in ewes (P < 0.01) and from 67.81 to 72.09 mg per 1 g of wool in lambs (P < 0.05). Similar changes were also observed in free internal lipids. Thus, in ewes the content of esterified cholesterol increased from 0.56 to 0.98 (P < 0.01), and in lambs from 0.59 to 0.81 (P < 0.01) mg per 1 g of wool. However, in bound internal lipids, under the influence of the applied factors, no statistically significant changes in esterified cholesterol were recorded.

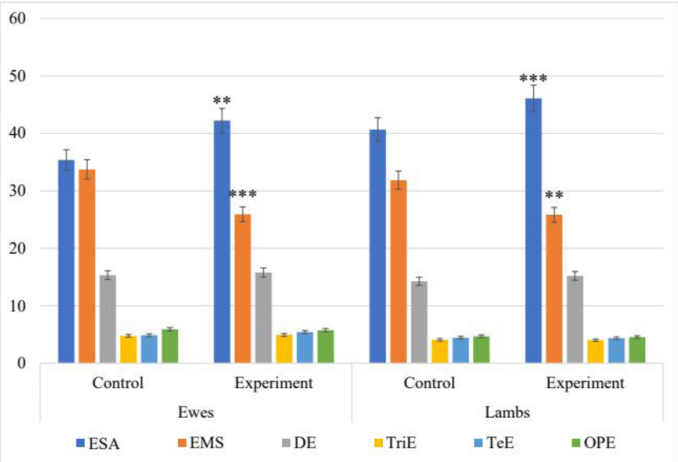

Figure 2: Content and composition of wax cholesterol esters, % (mean ± SD, n = 4).
ESA: esters of saturated acids, EMS: esters of monounsaturated acids, DE: diene esters, TriE: triene esters, TeE: tetraene esters, OPE: other polyene esters. The differences are significant between the control and experimental groups (* – P <0.05; ** – P <0.01; *** – P <0.001).

The cholesterol esters of surface lipids (wax) were divided into six main classes (Figure 2), the largest proportion of which consisted of esters of saturated and monounsaturated acids. It was precisely these esters that showed statistically significant changes in the experimental animals whose diet included a fatty acid emulsion. In the experimental group, a significant increase in saturated esters and a decrease in monounsaturated esters were observed. These changes were characteristic of both the ewes and the lambs obtained from them. In particular, the amount of saturated acid esters in ewes increased from 35.39 to 42.23% (P < 0.01), and in lambs from 40.69 to 46.09% (P < 0.001). Conversely, the content of monounsaturated acid esters in ewes decreased from 33.74 to 25.93% (P < 0.001), and in lambs from 31.87 to 25.83% (P < 0.01).

The cholesterol esters of free internal lipids, under the conditions of our study, were divided into four classes (Figure 3). Among them, the largest proportion, as in wax cholesterol esters, falls on esters of saturated and monounsaturated fatty acids, and it is precisely these that undergo the greatest changes under the influence of the applied factors. In the animals of the experimental group, the content of saturated acid esters significantly increased from 47.72 to 53.53% in ewes (P < 0.001) and from 48.57 to 50.97% in lambs (P < 0.01), while the content of monounsaturated acid esters decreased from 38.32 to 35.06% in ewes (P < 0.05) and from 37.36 to 35.48% in lambs (P < 0.05). In addition, in the experimental group of ewes, the content of di-, tri-, and tetraenoic acid esters decreased from 10.63 to 7.85% (P < 0.05).

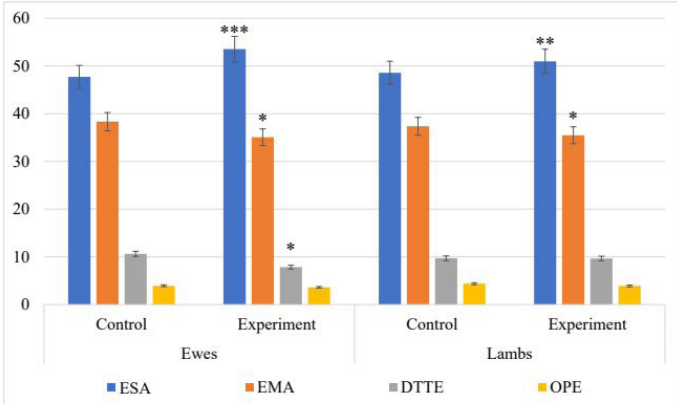

Figure 3: Content and composition of cholesterol esters of free internal lipids wool, % (mean ± SD, n = 5).
ESA: esters of saturated acids, EMS: esters of monounsaturated acids, DTTE: di-, tri- and tetraene esters, OPE: other polyene esters. The differences are significant between the control and experimental groups (* – P <0.05; ** – P <0.01; *** – P <0.001).

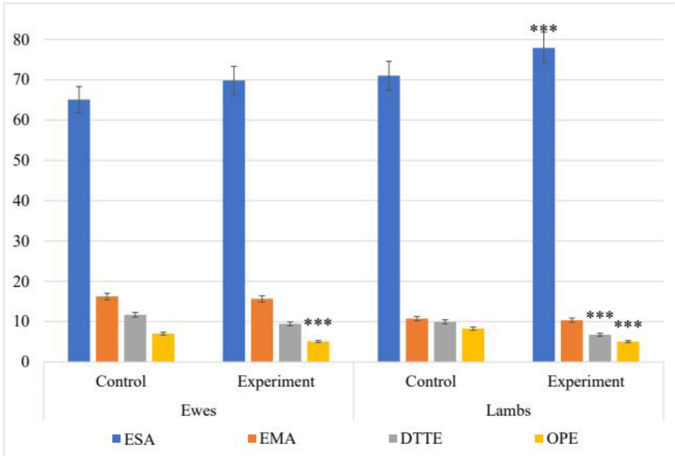

Figure 4: Content and composition of cholesterol esters of bound internal lipids wool, % (mean ± SD, n = 5).
ESA: esters of saturated acids, EMS: esters of monounsaturated acids, DTTE: di-, tri- and tetraene esters, OPE: other polyene esters. The differences are significant between the control and experimental groups (* – P <0.05; ** – P <0.01; *** – P <0.001).

According to the data presented in Figure 4, the cholesterol esters of bound (as well as free) internal lipids were divided into four fractions, of which more than 65% corresponded to esters of saturated fatty acids. However, their amount significantly increased only in the experimental group of lambs (P < 0.001). This increase occurred due to a decrease in the esters of di-, tri-, and tetraenoic acids (P < 0.001) and in other polyene esters (P < 0.001). In contrast, although in the experimental group of ewes, compared with the control, the amount of saturated acid esters increased from 65.08 to 69.85%, this increase was not statistically significant. It occurred due to a decrease in the esters of di-, tri-, and tetraenoic acids (from 7.00 to 5.06%) and, especially, other polyene acids (P < 0.001).

Thus, the inclusion of a water-soluble fatty acid complex in the main diet of ewes led to significant changes in the quantitative and qualitative composition of cholesterol esters of surface and internal wool lipids, both in the ewes themselves and in the lambs obtained from them.

DISCUSSION

The diverse nature of sheep productivity requires that their diets be provided with complete, high-energy feeds (Ge *et al*., 2023; Wang *et al*., 2024), and, as indicated by Tajonar *et al*. (2023) and Andrade *et al*. (2024), with essential fatty acids. Unfortunately, the literature hardly discusses their role in the formation of the qualitative characteristics of wool. Therefore, we conducted a study on feeding ewes an emulsion of fatty acids obtained through enzymatic treatment of oils using a lipolytic enzyme complex of microorganisms of the genera Pseudomonas and Bacillus subtilis, in order to determine their effect on the content of cholesterol esters in the surface and internal lipids of sheep wool.

The results obtained in the studies of many authors, in particular Hervas *et al*. (2021) and Telle-Hansen *et al*. (2022), indicate a positive effect of including fatty acids in the animals' diets. Our research showed that feeding fatty acids positively affects the protective properties of wool grease, as evidenced by an increase in the content of esterified cholesterol, and in particular, in the esters of saturated acids. As reported by Jenkins and Belsito (2023), and Fuster *et al*. (2025), the qualitative characteristics of wool wax are determined primarily by its specific lipid composition.

The changes recorded in our study, under the influence of the applied nutritional factors, indicate a slowing of oxidation processes in the wool grease. As is known, lipid oxidation is a continuous chain reaction that occurs when unsaturated fatty acids react with oxygen to form lipid hydroperoxides. These compounds are unstable and decompose into secondary oxidation products, which, in turn, can generate free radicals, as noted by many researchers, including Ponnampalam *et al*. (2022) and Zhou *et al*. (2024). As a result, these changes may negatively affect the protective properties of the wool grease and be reflected in the technological characteristics of wool raw material.

Wool is the most popular natural protein fiber used in the textile industry because it possesses a range of unique properties (Taleb *et al*., 2022). During its annual growth, wool is constantly exposed to various adverse external factors that cause damage and degradation of its structure and chemical composition (Starkova *et al*., 2022), leading to a deterioration of the physical properties of the fibers (Coderch *et al*., 2022). An important role in forming the physicochemical characteristics of the fiber belongs to internal lipids. According to Ishihara *et al*. (2021) and El–Fiky *et al*. (2021), they form a surface lipid barrier that regulates the water balance in the fiber. Our research showed that in free internal lipids of the wool of ewes from the experimental group that is, those fed with the fatty acid emulsion there was a statistically significant increase in esterified cholesterol content due to an increase in the esters of saturated acids. In contrast, the amount of esters of monounsaturated acids decreased in the experimental group. These changes were characteristic of both the wool of ewes and the wool of their lambs.

Regarding the bound internal lipids, the inclusion of a fatty acid emulsion in the diet of ewes did not cause statistically significant changes in the total amount of esterified cholesterol. However, changes in the qualitative composition of cholesterol esters were recorded. In the wool of lambs, due to a decrease in di-, tri-, tetraenoic (P < 0.001) and other polyene esters (P < 0.001), the content of saturated acid esters significantly increased (P < 0.001). As we showed earlier, feeding ewes with a fatty acid emulsion led to an increase in the total amount of saturated bound acids in lamb wool (Motko *et al*., 2025). A statistically significant decrease in other polyene cholesterol esters was also observed in the experimental ewes (P < 0.001). In the context of the above, it should be emphasized that Sandt and Borondics (2021) indicated the importance of cholesterol esters of internal lipids in forming the physicochemical characteristics of the fiber. In particular, cholesterol directly affects the physical properties of wool fibers, especially their strength (Kartal *et al*., 2021; Atav *et al*., 2025).

Thus, the data obtained as a result of the conducted research clearly indicate the effect of the water-soluble fatty acid complex under study on the quantitative and qualitative composition of cholesterol esters in the surface and internal lipids of sheep wool, which has a direct influence on the protective properties of wool grease, as well as on the physicochemical and, consequently, technological parameters of wool as a raw material for the light and textile industries. However, the studies conducted do not fully explain these mechanisms, which opens prospects for further research in this direction.

CONCLUSION

Fermented fatty acid emulsion, introduced into the basic diet of ewes, promotes an increase in esterified cholesterol in the surface and free internal lipids of the wool of both ewes and lambs obtained from them. The increase in saturated acid esters in the wax of ewes from 35.39 to 42.23% ($P < 0.01$), and in lambs from 40.69 to 46.09% ($P < 0.001$), and the decrease in monounsaturated acids in ewes from 33.74 to 25.93% ($P < 0.001$), and in lambs from 31.87 to 25.83% ($P < 0.01$) indicate a slowdown in the oxidation processes in the wool grease, which ultimately may have a positive effect on its protective properties. In the experimental group, in free internal lipids of wool, the content of esters of saturated acids significantly increases in ewes ($P < 0.001$) and lambs ($P < 0.01$), and the content of monounsaturated acids decreases in ewes ($P < 0.05$) and lambs ($P < 0.05$). In addition, the content of di-, tri-, and tetraenoic acid esters also decreased in the experimental group of ewes ($P < 0.05$). In cholesterol esters of bound internal lipids, more than 65% are esters of saturated acids. However, their number significantly increases only in the experimental group of lambs ($P < 0.001$), due to a decrease in di-, tri-, and tetraenoic ($P < 0.001$) and other polyene esters ($P < 0.001$). The results obtained indicate a positive effect of the relevant nutritional factors on the composition of cholesterol esters of surface and internal lipids of wool, which may affect its technological qualities. However, these studies do not fully reveal the mechanisms and nature of this process and require further clarification.

NOVELTY STATEMENT

This study is the first to test the effect of feeding fermented emulsion of fatty acids on the quantitative and qualitative composition of cholesterol esters of surface and internal lipids of wool of ewes and their lambs. It is recommended to add 3% fatty acid emulsion to ewes' feed.

AUTHOR'S CONTRIBUTION

VT: Conceptualization. Formal analysis. Supervision. Writing review and editing.
NM: Investigation. Resources. Methodology. Writing original draft.
BK: Data Curation. Funding Acquisition. NO: Validation. Project Administration.
AB: Visualization. Software.

Ethical approval

All procedures with sheep were carried out in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 2005), Council of Europe Directive No. 2010/63/EU, and the Law of Ukraine No. 3447-IV On the Protection of Animals from Cruelty as amended on 15.01.2024. According to protocol No 2 (25.03.2025) from the bioethical commission of the Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv have obtained ethical approval for the study.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI-assisted technologies were used in the writing, editing, data analysis, and preparation of manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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