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EVALUATION OF SURFACE LIPIDS OF SHEEP WOOL FOLLOWING DIETARY INCLUSION OF EMULSIFIED FATTY ACID COMPLEX

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ABSTRACT: Fatty acids, particularly ω-3, ω-6, and ω-9 play vital roles in sheep nutrition, but their influence on the protective properties of wool grease remained unclear. This study assessed the effects of dietary supplementation with an emulsified fatty acid complex on both the qualitative and quantitative characteristics of wool surface lipids in adult Prekos ewes and their lambs. The experimental group received a water-soluble emulsion containing linoleic, oleic, palmitic, arachidonic, stearic, and α-linolenic acids incorporated into the basal diet. Wax content was determined via Soxhlet extraction, and sweat salts were measured by aqueous extraction. Lipid classes were separated by thin-layer chromatography, and fatty acid profiles were quantified using gas-liquid chromatography. Results indicate a significant increase in wax secretion in ewes ($P < 0.01$) and lambs ($P < 0.05$), along with a decrease in sweat pH among lambs ($P < 0.05$). In ewe wax, levels of lanosterol ($P < 0.01$) and esterified cholesterol ($P < 0.05$) were elevated; lamb wax exhibited increases in lanosterol ($P < 0.05$) and dehydrocholesterol ($P < 0.05$). Both ewes and lambs showed a reduction in polar lipid content ($P < 0.05$), suggesting diminished accumulation of oxidative products. Analysis of fatty acid composition in the ewe group revealed significant increases in cerotic (C26:0; $P < 0.001$), lauric (C12:0; $P < 0.01$), and oleic (C18:1; $P < 0.01$) acids. Therefore, dietary inclusion of an emulsified fatty acid complex enhances the protective properties of wool grease by modulating wax and fatty acid composition, with potential benefits for fiber integrity and resilience.

Keywords: Emulsified fatty acids; Fleece lipids; Hexacosanoic acid; Sweat secretion; Wool wax.

INTRODUCTION

Sheep nutrition is one of the most important factors affecting their productivity (Burezq and Khalil, 2022). Diet is the most accessible means by which meat (Costa et al., 2023), milk (Vargas-Bello-Pérez et al., 2021), and wool yield (Chishti et al., 2021) can be systematically enhanced, as well as the physicochemical and consequently technological properties of wool (Kitaeva et al., 2023). As Li et al. (2024) report, lipids play a crucial role in sheep nutrition; a deficiency leads to growth retardation, impaired reproductive performance, reduced productivity, and deterioration of product quality. Alba et al. (2021) emphasize that fat digestibility in sheep is high and depends on fat's physicochemical properties, fatty acid composition, and dietary balance. Furthermore, Gelaye et al. (2021) report a significant influence of dietary factors on both the quantitative and qualitative parameters of sheep fleece grease.

Wool grease is the product of the secretory activity in the sebaceous and sweat glands (Aissani et al., 2022). Wool grease is the product of secretory activity in the sebaceous and sweat glands (Aissani et al., 2022). Although the precise function of sebaceous secretion remains under investigation, it is generally believed to prevent skin dryness, impart softness and elasticity to the epidermis, and provide water repellency. The presence of active hydrolytic enzymes particularly arylsulfatase in sebaceous secretion suggests additional roles in detoxifying endogenous and exogenous compounds, disinfecting the hair-follicle cavity, and potentially participating in the desquamation of cells within the follicular sheath (Raghav et al., 2022).

Wool grease or its purified form, lanolin is widely used in pharmacology and cosmetology (Abou Taleb and El-Sayed, 2021). Owing to its content of unsaturated fatty acids such as oleic, linoleic, and α-linolenic acids (Cholewinska and Michalak, 2018), lanolin exerts a beneficial effect on the skin by forming a protective film on epidermal surface, maintaining moisture levels by reducing transepidermal water loss by 20–30% (Souto et al., 2021), thereby preventing cracking (Kang et al., 2022).

Albanell et al. (2018) and Duzelbayeva et al. (2023) describe sheep sebaceous secretions as a complex mixture of esters of primary and secondary alcohols alongside free long- and medium-chain fatty acids. The principal constituents are esters of cholesterol, lanosterol, and three additional C_{30} alcohols analogous to lanosterol; minor components include cerebrosterol and 25-oxycholesterol, the latter arising via autoxidation (Ruttler et al., 2022). Sweat predominantly contains alkali metal salts—chiefly potassium, with lesser sodium—and, under high humidity, the presence of potassium

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soaps and organic acids renders wool grease a natural detergent (Molik et al., 2023). Wool wax is the sole grease component that positively influences wool's physicochemical properties (Dominguez et al., 2003). By coating fibers with a thin layer, wax promotes inter-fiber adhesion forming staples and braids that contribute to a compact fleece. This protective coating shields wool from mechanical and botanical contaminants, as well as environmental stressors (e.g., solar radiation, precipitation) during growth, storage, and initial processing. Lanolin's protective efficacy stems primarily from its specific lipid composition and the optimal balance among lipid classes (Jenkins and Belsito, 2023).

Wool quality depends heavily on both the quantity and quality of wax, which vary with breed, individual characteristics, husbandry practices, seasonal and climatic conditions, and diet (El-Sayed et al., 2018). Given these influences, the present study aimed to investigate the effects of dietary supplementation with an emulsified fatty acid complex on the qualitative and quantitative characteristics of sheep wool grease.

MATERIALS AND METHODS

Experimental animals and design

The study was conducted during the winter stall-housing period on adult Prekos ewes maintained at the Educational and Scientific Production Center "Komarnivske," Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Lviv, Ukraine. Using paired-analogue matching for breed, age, and live weight, two groups of ten ewes each were formed: a control group and an experimental group. At the start of the trial, all ewes were in the late gestation period; from mid-trial onward, they entered early lactation.

Following a 10-day adaptation period during which all animals received a basal diet balanced according to established feeding standards, a 95-day experimental period commenced. Control ewes continued on the basal diet, whereas experimental ewes received, in addition, 3 % (w/w) of a water-soluble fatty acid complex ("Essential Lipid Complex," LLC EcoProFeed, Ukraine; 880 kcal per 100 g). This emulsion comprised linoleic acid (C18:2 ω-6; 54.5 %), oleic acid (C18:1 ω-9; 24 %), palmitic acid (C16:0; 10 %), arachidonic acid (C20:4 ω-6; 6 %), stearic acid (C18:0; 4 %), and α-linolenic acid (C18:3 ω-3; 1.5 %). It was produced by enzymatic treatment of oils using a lipolytic enzyme complex from *Bacillus pseudomonas* and *Bacillus subtilis* in the presence of glycolipids and polysaccharides. This supplementation increased dietary crude-fat intake by 12 g (from 46.41 g to 58.41 g) in pregnant ewes and by 18 g (from 62.81 g to 80.81 g) in lactating ewes.

All animals were group-fed, with daily weighing of the provided feed, and had *ad libitum* access to water. Feed and water intakes were recorded throughout the experimental period.

Sampling

At the end of the experiment, wool samples including surface grease were collected from the experimental ewes and their lambs in the region posterior to the scapula for subsequent biochemical analyses.

Determination of the total amount of wax, sweat, and pH of sweat

Wool fat (wax) was extracted from fleece samples with tetrachloromethane (Sigma-Aldrich, USA) using a Soxhlet apparatus for 5 h (Daly and Carter, 1954). After cooling and phase separation, the extract was evaporated to dryness. The residue was dissolved in 10 mL of chloroform–methanol (2:1; Chemico Group, UK/SRP Ltd, Ukraine), and 3 mL of 7.5 % potassium chloride solution (Luxion Co., China) was added. Samples were shaken and allowed to separate for 24 h; the upper aqueous–methanol layer was removed by suction, and the lower chloroform layer containing lipids was retained for analysis. The defatted wool was washed, dried to constant mass, cleared of debris, and weighed. Wax content was determined gravimetrically and expressed as a percentage of clean, dry fiber. Sweat salts were extracted aqueously, and the pH of the extract was measured using a universal ion-selective meter.

Lipid-class separation by thin-layer chromatography (TLC)

The lipid extract was re-dissolved in chloroform–methanol (2:1) and applied to 100 × 100 mm silica-gel TLC plates (Sorbfil; particle size 90–120 μm). Lipid classes were separated using a mobile phase of petroleum ether–diethyl ether (4:1, v/v; Carlo Erba, Italy). After development and drying, plates were sprayed with 50 % sulfuric acid (Alhim, Ukraine) and charred at 105 °C. Individual lipid classes were identified by comparison with reference standards (cholesterol, stearic acid, lanosterol; Sigma-Aldrich, USA) and published R_f values.

Quantitative determination of individual lipid classes

Lipid bands were scraped from the silica, transferred to centrifuge tubes, and treated with 5 mL concentrated sulfuric acid. Tubes were mixed thoroughly, heated in a boiling water bath for 20 min, then cooled and centrifuged at 3,000 rpm for 20 min. The absorbance of the supernatant was measured at 400 nm in a 10 mm path-length cuvette. Concentrations of each lipid class were calculated against calibration curves and expressed as percentages of total wax.

Determination of the fatty acid composition of wax

Surface lipids were converted to fatty acid methyl esters via direct transesterification (Stoffel et al., 1959). Separation was performed on a "Chrom-4" GLC (Czech Republic) equipped with a 2,400 mm × 3 mm metal column packed with Chromosorb (60–80 mesh) coated with 15 % polyethylene glycol succinate. Operational conditions were: column thermostat, 190 °C; injector, 240 °C; airflow, 400 mL/min; carrier gas (N_2), 25 mL/min. Fatty acids were identified by comparison to Supelco standard mixtures, and retention times (t_R) were recorded. Individual fatty acid percentages were calculated using standard quantitative formulas.

Statistical analysis

All data were processed using Statistica 12.0 (StatSoft Inc., USA). Results are presented as mean ± SD. Group comparisons were made by one-way ANOVA, followed by post-hoc tests for pairwise significance. Differences were considered significant at $P < 0.05$.

RESULTS

The conducted studies (Table 1) first revealed that feeding ewes, a water-soluble complex of fatty acids, leads to a significant increase in wax secretion in the experimental animals from 12.21 to 14.89% ($P < 0.01$). Consequently, the wax-to-sweat ratio in grease significantly improved from 1:1.15 in controls to 1:0.96 in treated ewes. No significant differences were observed in total sweat content or sweat pH between control and experimental ewes under the conditions of this study.

Table 1 - Indicators of grease content of ewe's fleece of ewes, (x ± SD, n = 4)

Indicator	Control	Experiment
Amount of wax, %	12.21 ± 1.08	14.89 ± 0.37**
Amount of sweat, %	14.04 ± 0.91	14.26 ± 1.22
pH of sweat	8.61 ± 0.21	8.43 ± 0.23
Wax: sweat ratio	1: 1.15	1: 0.96

SD= Standard deviation; **=P < 0.01.

The lipid composition of the wax in ewes of the experimental group was altered (Figure 1). Specifically, the nutritional factors applied led to a significant increase in esterified cholesterol, from 37.08% to 39.91% ($P < 0.05$), and lanosterol, from 7.36% to 10.65% ($P < 0.01$), as well as a decrease in polar lipids, from 21.97% to 18.39% ($P < 0.05$). In contrast, the fractions of esterified cholesterol, non-esterified fatty acids, dehydrocholesterol, and squalene did not exhibit significant changes. Similar changes observed in the grease of ewes were also found in the grease of their lambs (Table 2). Specifically, in the experimental group, the wax content significantly increased from 13.50% to 14.99% ($P < 0.05$). This increase, in turn, led to alterations in the wax-to-sweat ratio. In the control group, the ratio was 1:1, while in the experimental group, it was 1:0.81. Unlike ewes, in the lambs from the control group, the pH of sweat significantly decreased from 7.78 to 7.04 ($P < 0.05$), while the amount of sweat also showed a tendency to decrease. In the lipid composition of the wax in lambs (Figure 2), as observed in ewes, the experimental group exhibited an increase in lanosterol, from 7.60% to 9.47% ($P < 0.05$), and a decrease in polar lipids, from 9.35% to 7.64% ($P < 0.05$). However, unlike ewes, the fraction of dehydrocholesterol in the wax of lambs significantly increased from 11.43% to 13.48% ($P < 0.05$). No significant changes were observed in esterified and non-esterified cholesterol, non-esterified fatty acids, or squalene in the lambs of the experimental group.

The fatty acid composition of ewe wax consists of 23 acids, including both saturated and unsaturated, as well as iso-acids (Figure 3). It is noteworthy that five of these acids have not yet been identified. The fatty acid composition of the wax in ewes from the experimental group differs from that of the control group in terms of the content of individual acids. Specifically, the experimental group exhibits a significantly higher content of lauric acid (dodecanoic C12:0) ($P < 0.01$), oleic acid (9Z)-octadec-9-enoic C18:1ω9) ($P < 0.01$), cerotic acid (hexacosanoic C26:0) ($P < 0.001$), and one unidentified acid ($P < 0.05$), as well as a lower content of linoleic acid (9E,12E)-octadeca-9,12-dienoic C18:2ω6) ($P < 0.05$). The total amount of unsaturated fatty acids in the wax of the control group is 27.92%, while in the experimental group, it is 13.21%.

These findings demonstrate that inclusion of a water-soluble fatty-acid complex in the diet of ewes modifies both the lipid-class composition and fatty-acid profile of wool grease, with implications for its protective functions and, by extension, the physicochemical properties of wool fibers.

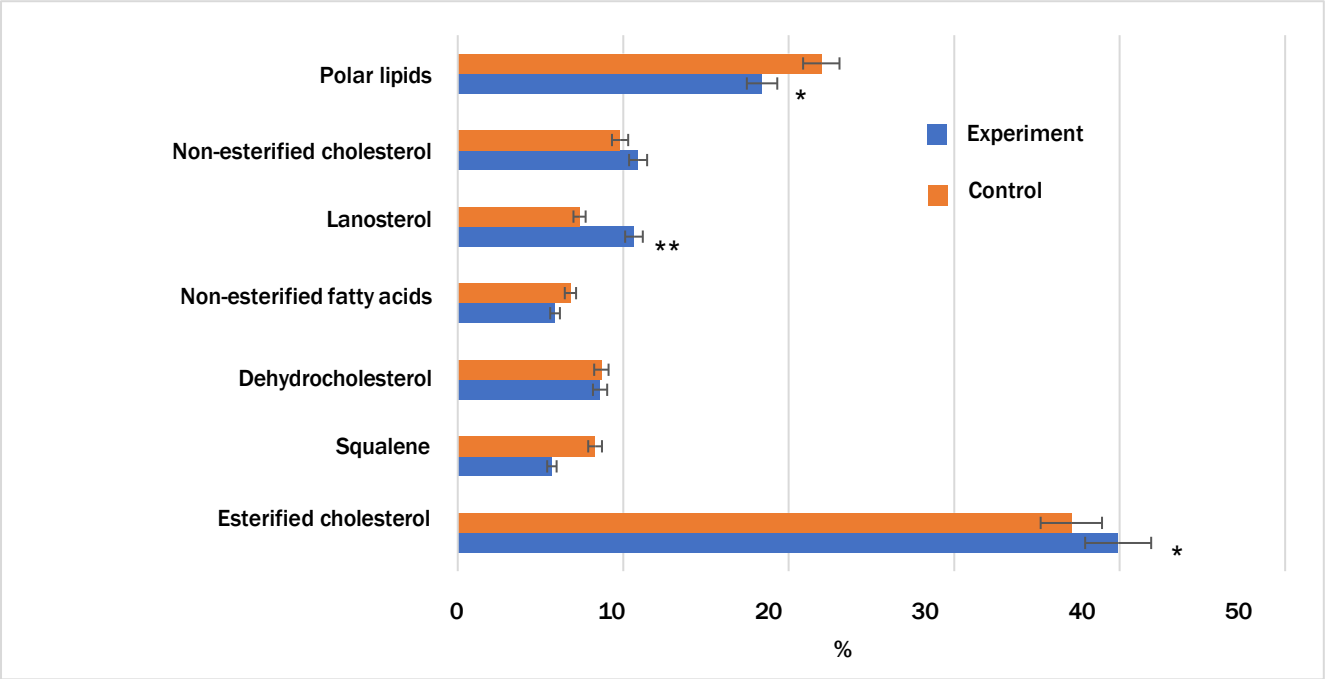

Figure 1 - Lipid composition of the grease of the ewe's fleece (M ± SD, n = 4). SD= Standard deviation; *=P < 0.05, **=P < 0.01

Table 2 - Indicators of fleece grease of lambs, (x ± SD, n = 4)

Indicator	Control	Experiment
Amount of wax, %	13.50 ± 0.77	14.99 ± 0.91*
Amount of sweat, %	13.48 ± 0.96	12.10 ± 1.49
pH of sweat	7.78 ± 0.23	7.04 ± 0.50*
Wax : sweat ratio	1: 1	1: 0.81

SD= Standard deviation; *=P < 0.05

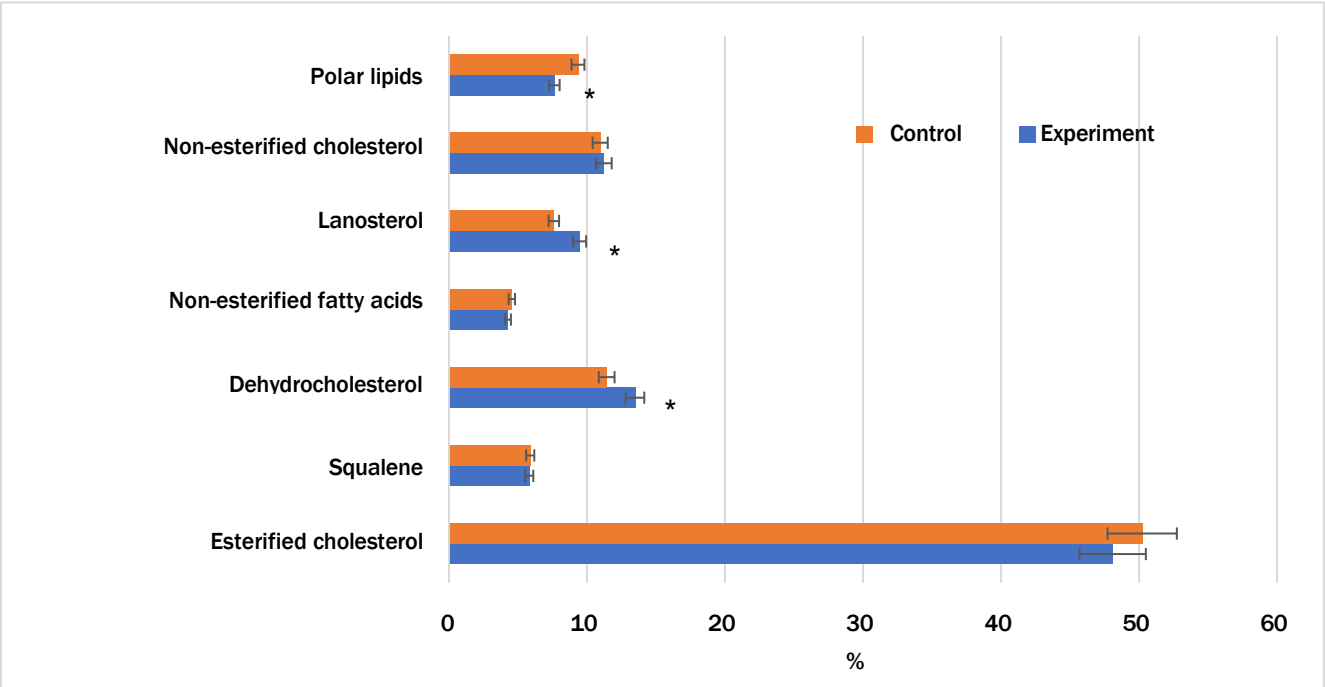

Figure 2 - Lipid composition of the wool grease wax of lambs, (M ± SD, n = 4). SD= Standard deviation; *=P < 0.05.

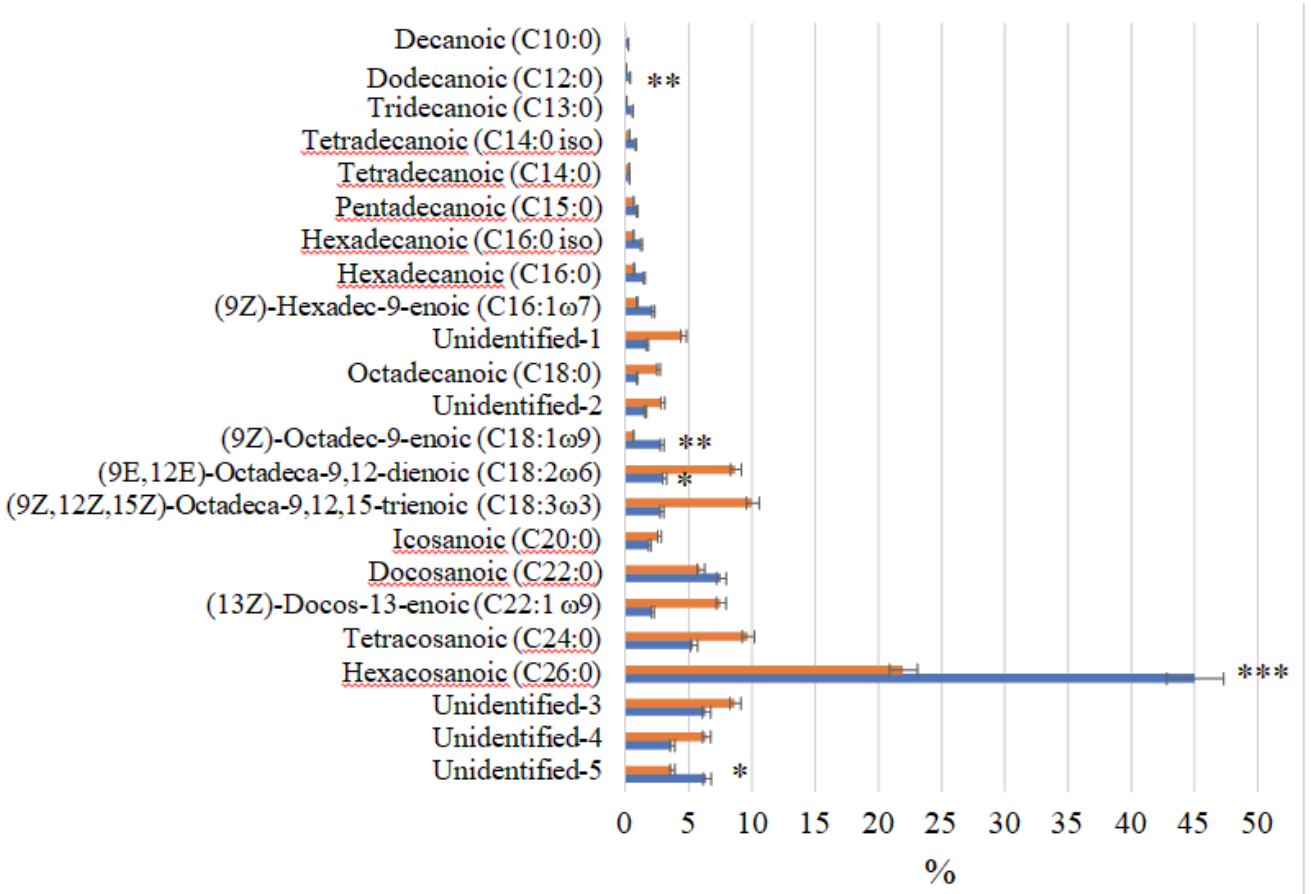

Figure 3 - Fatty acid composition of the grease wax of the ewe's fleece, (M ± SD, n = 3). SD= Standard deviation; *=P < 0.05, **=P < 0.01, ***=P < 0.001.

DISCUSSION

Enhancing the productivity and quality of sheep products depends largely on balanced nutrition, particularly the provision of optimal energy levels, which profoundly influence metabolic processes (Hervás et al., 2021; Oualyou et al., 2024). In recent years, researchers in small-ruminant nutrition have increasingly focused on improving feeding efficiency (Dijkstra et al., 2025). Alba et al. (2021) highlight that, given the diverse demands of sheep for meat, milk, and wool production, high-energy feeds are critical. Tajonar et al. (2023) emphasize the essential role of unsaturated fatty acids, noting that their deficiency disrupts phospholipid metabolism, impairs membrane binding capacity and fluidity, and compromises lipoprotein formation and lipid transport.

Despite extensive research on energy-dense supplements, data remain scarce regarding the effects of ω-3, ω-6, and ω-9 fatty acids on the protective properties of sheep fleece grease. To address this gap, we supplemented the basal diet of ewes with a water-soluble fatty acid complex containing both saturated (palmitic C16:0; stearic C18:0) and unsaturated acids linoleic (C18:2 ω-6), oleic (C18:1 ω-9), arachidonic (C20:4 ω-6), and α-linolenic (C18:3 ω-3). Rumen fermentation hydrogenates these unsaturated acids to saturated forms, reducing methane production and mitigating the risk of acute tympany (Yang et al., 2022).

Wool grease secreted by sebaceous glands as lanolin plays a vital role in maintaining fiber integrity by preventing moisture ingress (Molik and Potocka, 2019; Lis, 2024; Meng et al., 2025). Present findings demonstrate that dietary inclusion of the lipid complex significantly increased wax content in both ewes ($P < 0.01$) and their lambs ($P < 0.05$), improving the wax-to-sweat ratio. A wax-to-sweat ratio of less than 1: 1 corresponds to superior protective properties. Additionally, lambs in the experimental group exhibited a trend toward reduced sweat volume accompanied by a significant decrease in sweat pH ($P < 0.05$). As we reported previously, elevated sweat concentrations, particularly under alkaline conditions accelerate wax degradation (Tkachuk et al., 2024).

The findings of Johnson et al. (2023) indicate that lanolin's qualitative characteristics depend primarily on its specific lipid composition. Present results corroborate this assertion: experimental ewes exhibited significant increases in lanosterol ($P < 0.01$) and esterified cholesterol ($P < 0.05$), while their lambs showed elevated levels of lanosterol ($P < 0.05$) and dehydrocholesterol ($P < 0.05$), accompanied by a decrease in polar lipids ($P < 0.05$). The reduction in polar lipids may reflect diminished accumulation of oxidative products.

Regarding the fatty acid profile of the surface lipids of wool, the amount of oleic acid (9Z)-octadec-9-enoic C18:1ω9) in the ewes of the experimental group significantly increased ($P < 0.01$). This is consistent with the fact that the water-soluble lipid complex contains 24% of this acid. However, the observed decrease in linoleic acid (9E,12E)-octadeca-9,12-dienoic C18:2ω6) ($P < 0.05$), despite its 54.5% presence in the complex, remains somewhat unclear. In the wax of the animals in the experimental group, an increase in lauric acid (dodecanoic C12:0) ($P < 0.01$) and, particularly, cerotic acid (hexacosanoic C26:0) ($P < 0.001$) was observed, with cerotic acid being the most abundant of all fatty acids. Notably cerotic acid plays a crucial role due to its antimicrobial properties (Singh and Singh, 2003; Rehan et al., 2020), functioning as a natural disinfectant within the fleece. Although five of the twenty-three identified fatty acids remain uncharacterized, one of these unknowns increased significantly under dietary treatment ($P < 0.05$). Changes in the fatty-acid composition reduced the proportion of unsaturated acids to 13.21 % in the experimental group versus 27.92 % in controls. This shift likely enhances oxidative stability, as higher unsaturated-acid content correlates with increased susceptibility to peroxide oxidation (Cao et al., 2024).

Therefore, dietary inclusion of a fatty acid emulsion enhances the protective properties of sheep wool grease by increasing total wax content and optimizing its lipid and fatty acid composition.

CONCLUSION

Dietary supplementation of ewes with a water-soluble fatty acid complex increased the secretion of wool surface lipids, resulting in an improved wax-to-sweat ratio; in lambs, sweat pH also decreased. The lipid composition of wool wax shifted, with a reduction in polar lipids in both supplemented ewes and their offspring—likely reflecting lower accumulation of oxidation products. Enhanced protective properties of wool grease in the experimental group were evidenced by elevated levels of lanosterol, esterified cholesterol, and dehydrocholesterol. The fatty acid profile of experimental ewes' wax was characterized by higher concentrations of oleic acid (C18:1 ω-9), lauric acid (C12:0), and notably cerotic acid (C26:0); the latter, owing to its antimicrobial activity, may serve as a natural disinfectant within the fleece. Overall, these findings demonstrate that dietary inclusion of a fatty acid emulsion enhances the protective functions of wool grease, with positive implications for fleece quality. However, the current study does not fully address the impact of these nutritional interventions on the physicochemical and technological properties of wool fibers, highlighting avenues for future research.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethical approval

All manipulations with animals were carried out by the international principles of the Council of Europe Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» and the resolutions of the National Congress of Ukraine on Bioethics (2010), which comply with the current legislation of Ukraine, in particular the Law of Ukraine No. 3447-IV «On the Protection of Animals from Cruelty» as amended on 15.11.2024. According to protocol No 93 (03.06.2021) from the bioethical commission of the Institute of Animal Biology NAAS have obtained ethical approval for the study.

Authors' contribution

T.Vitalii: Writing – original draft, Validation, Supervision, Methodology, Conceptualization. K.Bogdan: Writing – original draft, Resources, Funding acquisition, Conceptualization, O.Nataliia: Writing – original draft, Project administration, Methodology. M.Nataliia: Writing – original draft, Methodology.

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Competing interests

The authors declare no competing interests in this research and publication.

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