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MICROFLORA AND GREASE FLEECE OF NORMAL AND FELTED WOOL SHEEP OF UKRAINIAN CARPATHIAN MOUNTAIN BREED

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Background. Sheep wool possesses felting properties, which form the basis of felt production. However, felting may also occur directly on the animal, resulting in wool defects. This phenomenon is particularly common in semi-coarse wool sheep of the Ukrainian Carpathian Mountain breed. Therefore, the aim of this study was to investigate the role of microflora and grease in the process of felt formation in ewes of this breed.

Materials and Methods. The microflora of the fleece was examined using culture-based methods on dense nutrient media. The amount of grease was determined by aqueous extraction of its salts. Wax was extracted using a Soxhlet apparatus and analyzed by thin-layer chromatography; the fatty acid composition was determined using gas–liquid chromatography after conversion to methyl esters via direct transesterification of fatty acids.

Results. Fleece with felted wool, in comparison with normal fleece, is characterized by a significantly higher sweat content ($P < 0.01$) and greater alkalinity ($P < 0.05$). This creates favorable conditions for the development of bacteria ($P < 0.01$) and mold, which, utilizing wax in their metabolic processes, reduce its amount ($P < 0.001$). A decrease in esterified cholesterol ($P < 0.05$) and an increase in polar lipid fractions ($P < 0.01$) and non-esterified fatty acids ($P < 0.05$) indicate ongoing hydrolytic processes affecting individual wax components. Changes in fatty acid composition result in an increase



in erucic acid ((13Z)-docos-13-enoic acid, C22:1 ω 9) ($P < 0.05$) and a decrease in one unidentified fatty acid ($P < 0.01$) and cerotic acid (hexacosanoic acid, C26:0) ($P < 0.01$). The latter may act within the fleece as a natural disinfectant. The total amount of saturated fatty acids in the wax of felted wool is lower, accounting for 50.46 %, compared to 58.81 % in normal wool; conversely, the proportion of unsaturated fatty acids is higher in felted wool (28.07 %) than in normal wool (16.20 %). An increased proportion of unsaturated fatty acids contributes to greater molecular susceptibility to peroxide oxidation.

Conclusion. Thus, changes occurring in the fleece environment, particularly in its microflora and grease composition, have a direct impact on the felting processes in semi-coarse wool sheep of the Ukrainian Carpathian Mountain breed.

Keywords: ewes, fleece, felted wool, microorganisms, wax, sweat, fatty acids

INTRODUCTION

Despite the fact that the modern chemical industry produces a wide range of synthetic fibers, sheep wool, owing to its unique combination of physicochemical properties, remains an extremely valuable and, in many cases, irreplaceable raw material for the production of high-quality fabrics, knitted goods, and other products, with no other fiber able to fully compete with it (Dawood, 2025). Wool uniquely combines properties such as softness (Yu *et al.*, 2021), elasticity, plasticity, effective sound and thermal insulation (Zhang *et al.*, 2025a), the ability to transmit ultraviolet radiation (Hossain *et al.*, 2024), reduction of vibrational impact, moisture absorption (maximum hygroscopicity up to 35–40 %) (Ahmed & Qayoum, 2021), the ability to be dyed in a wide range of shades (Xue *et al.*, 2025), lightness, strength (Ghasemian *et al.*, 2025), as well as high spinnability and felting capacity (El-Sayed, 2022).

The felting ability of wool is defined as its capacity to form a dense and compact mass – felt – as a result of irreversible fiber interlocking and convergence (Boostani *et al.*, 2023). This property underlies the felting industry, enabling the manufacture of cloth, felt, boots, and other felt products (Santos *et al.*, 2020; Silva *et al.*, 2022). However, wool may also undergo felting directly on the animal body, thereby becoming defective.

According to H. Tufekci & V. Sejian (2023), under conditions of high ambient temperatures, metabolic activity intensifies, accompanied by increased sweating. Animals, seeking refuge from direct sunlight, huddle together, forming shaded clusters or crawling beneath one another. As a result, conditions highly favorable for wool felting are created: elevated temperature, sufficient moisture, and increased grease accumulation on the skin surface and within the fleece. Friction between fibers within braids occurs, which, as reported by R. Li *et al.* (2025), plays a decisive role in felt formation. Consequently, fibers that detach from the skin during initial molting, as well as during animal movement, become entangled and form a felt-like layer. As noted by N. M. H. Mascarenhas *et al.* (2023), the simultaneous intensive secretion of grease at high temperatures leads to skin maceration, thereby promoting exfoliation of the superficial epidermal layer. As a result, wool from such animals is often heavily contaminated with dandruff scales, which are extremely difficult to remove during primary processing and subsequent technological use.

Fleece containing felted mats is of low economic value, since during processing it is prone only to tearing, as effective combing is virtually impossible. In addition, the wool

fibers themselves are less durable, resulting in a significant reduction in market value as high-quality textile products can be obtained only from high-grade wool raw materials (Li *et al.*, 2024; Zenda *et al.*, 2025).

This phenomenon occurs most frequently in coarse- and semi-coarse-wool sheep, particularly in sheep of the Ukrainian Carpathian Mountain breed, raised in the mountainous regions of the Ukrainian Carpathians (Suprun *et al.*, 2021).

As noted by V. Tyrunskiy *et al.* (2023), all processes within the fleece occur in a grease-rich environment that serves a protective function. The quality of wool fibers largely depends on both the quantity and quality of grease. By forming a thin coating on the fibers, wax promotes their cohesion, resulting in the formation of staples and braids, which in turn develop into a dense fleece structure.

Complex chemical processes (oxidation, saponification, hydrolysis, etc.) as well as microbiological processes continuously occur within the grease environment. An increase in bacterial contamination of the fleece leads to deterioration of both quantitative and qualitative grease parameters (Colditz *et al.*, 2022). The nature and direction of these processes are largely governed by the grease composition. In particular, alkaline conditions intensify reactions, leading to the formation of fatty acid salts (Bhavsar *et al.*, 2023).

The protective function is primarily associated with wool fat (wax) and is determined primarily by its specific composition, whose qualitative characteristics depend on an optimal balance among individual lipid classes (Jenkins & Belsito, 2023). These factors may directly influence wool felting processes in sheep.

In view of this, the present study aimed to investigate the role of fleece microflora in shaping the protective properties of grease, as well as the significance of surface lipids and their fatty acid composition in the development of wool defects, specifically felting (mat formation), in semi-coarse-wool ewes of the Ukrainian Carpathian Mountain breed.

MATERIALS AND METHODS

Experimental animals and design. Samples of normal and felted wool were taken for research from sheared fleece from the area behind the shoulder blade, during spring shearing, from 12 adult ewes of the Ukrainian Carpathian Mountain breed (6 animals with normal wool and 6 with felted wool), which belonged to the farm “Prometey” (Kolomyia, Ivano-Frankivsk region).

Research on fleece microflora. Desorption of microorganisms was carried out by grinding 1 g of wool for 5 min in a sterile porcelain mortar, with the addition of 1 mL of a 0.3 % solution of Tween-80. After that, using 99 mL of sterile water for multiple washes, wool samples were quantitatively transferred into sterile flasks, which were shaken on a schüttel apparatus for 30 min. Next, the necessary dilutions of the bacterial suspension were prepared from 10^{-1} to 10^{-9} . Plating was performed from dilutions of 10^{-6} – 10^{-9} for bacteria, 10^{-3} – 10^{-5} for actinomycetes and fungi, 10^{-2} – 10^{-4} for mold fungi, and 10^{-1} – 10^{-3} for Neurospora. The number of viable microorganisms was determined by culturing the appropriate dilutions on dense nutrient media. For bacteria, it was meat-peptone agar; for actinomycetes, Czapek; for fungi, Neurospora, and for mold fungi, Sabouraud. After 4–6 days of incubation in Petri dishes at a temperature of 30 °C, the number of colony-forming units was counted.

Determination of the total amount of wool grease (wax), sweat, and its pH.

Wool grease (wax) was extracted for 5 h in a Soxhlet apparatus with tetrachloromethane (Daly & Carter, 1954). After cooling and settling, the resulting extract was dried by evaporation. The resulting precipitate was dissolved in 10 mL of a chloroform–methanol mixture (2 : 1), 3 mL of 7.5 % potassium chloride was added, then shaken and left for 24 h to separate the liquids. The water-methanol (upper) layer with impurities was sucked off with a water-jet pump, and for further studies, the chloroform (lower) layer was used, in which lipids were dissolved.

After removing the fat grease, the wool was washed, dried, foreign impurities were separated, brought to a constant dry mass, and weighed. The mass fraction of wax was determined by the gravimetric method and expressed as a percentage in terms of pure and dry fiber.

The amount of sweat was measured by the method of aqueous extraction of its salts, and the pH (concentration of hydrogen ions) in the extract was determined using a universal ionometer EV-74 (Vlizlo *et al.*, 2012).

Research on the lipid composition of wax by TLC (thin-layer chromatography).

After determining the amount of total lipids, the obtained extract was dissolved in a chloroform–methanol mixture (2 : 1), and its composition was studied by the TLC method by applying it to Sorbfil plates. The size of the plates was 100×100 mm, and the particle size of the working layer of silica gel was 90–120 μm. The mobile phase for the separation of wax lipids was a mixture of petroleum ether and diethyl ether (4 : 1, v/v). The dried chromatograms were sprayed with 50 % sulfuric acid and carbonized at 105 °C.

The identification of individual lipid classes was carried out by comparing the chromatograms of the studied samples with the chromatogram on the surface of which controls, namely lanosterol, stearic acid, and cholesterol, were applied (Sigma Chemical Co., USA) and comparing the r_f -values of lipid classes with literature data.

Quantitative determination of individual lipid classes. Lipids from the chromatograms were transferred to centrifuge tubes, 5 mL of concentrated sulfuric acid was added, mixed, and placed in a bath with boiling water for 20 min. After cooling, centrifugation was performed for 20 min at 3000 rpm. The color intensity was measured on a spectrophotometer, in a 10 mm path-length cuvette at a wavelength of 400 nm. The content of individual lipid fractions was calculated mathematically and expressed as a percentage.

Studies of the fatty acid composition of wool fat. The fatty acid composition of surface lipids of wool was determined by converting them into methyl esters by direct transesterification of fatty acids (Stoffel *et al.*, 1959). On a gas-liquid chromatograph “Chrom-4” (Czech Republic), methyl esters of fatty acids were separated using a metal column 240 cm long and 0.3 cm in diameter, which was filled with Chromosorb 60–80 mesh, coated with 15 % polyethylene glycol succinate. The thermostat temperature was 190 °C, and the evaporator 240 °C. In this case, the air flow rate was 400 mL/min, and the carrier gas (nitrogen) – 25 mL/min.

The content of individual fatty acids was calculated using the formula:

$$X = \frac{S}{\sum S} \cdot 100 \%,$$

where X is the amount of fatty acid (%), S is the peak area of the acid, and ΣS is the sum of the areas of all fatty acid peaks.

Fatty acids were identified using standard mixtures (Supelco Inc, USA) and measuring t_r-I_r . The content of individual fatty acids was calculated mathematically according to the formulas.

Animal protection. All manipulations with ewes were carried out in accordance with the Law of Ukraine No 3447-IV "On the Protection of Animals from Cruelty" as amended on November 15, 2024, and the international principles of the Council of Europe Convention "For the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes". According to Protocol No 2 dated 25 March 2025, ethical approval for this study was granted by the Bioethical Commission of Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv.

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 \pm standard deviation ($M \pm SD$). Due to the small sample size, 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

As a result of the conducted studies of the fleece microflora, it was first established (**Table 1**) that the felted wool contains a significantly higher number of bacteria ($P < 0.01$) and mold fungi ($P < 0.001$). As for fungi, neurospora and actinomycetes, their number is practically the same in normal and defective wool. Such data clearly indicate that the fleece microflora, namely bacteria and mold fungi, plays a certain role in the processes of felting.

Table 1. Microorganism content in normal and felted wool, CFU/g ($M \pm SD$, $n = 6$)

Microorganisms	Wool	
	normal	felted
Bacteria $\times 10^9$	5.33 \pm 1.03	7.67 \pm 0.82**
Actinomycetes $\times 10^5$	1.82 \pm 0.75	1.50 \pm 0.55
Fungi $\times 10^5$	3.67 \pm 0.82	4.32 \pm 1.03
Mold fungi $\times 10^4$	2.16 \pm 1.17	4.83 \pm 0.41***
Neurospora $\times 10^3$	5.65 \pm 0.81	6.17 \pm 0.75

Note: here and below: * – statistically significant differences between normal and felted wool (* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$)

When studying the quantitative indicators of grease, we found (**Table 2**) that the increase in microorganisms in the felted wool is accompanied by a significant decrease in wax ($P < 0.001$). As for sweat, its content was significantly higher in the felted wool, with higher pH values ($P < 0.05$). These changes affect the ratio of wax to sweat. Thus, in normal wool this ratio is 1:2.35, and in felted wool it increases to 1:3.47.

Table 2. Quantitative indicators of the grease of normal and felted wool, ($M \pm SD$, $n = 6$)

Indicator	Wool	
	normal	felted
Wax, %	8.47 ± 0.32	$6.48 \pm 0.49^{***}$
Sweat, %	19.94 ± 1.30	$22.49 \pm 1.25^{**}$
Sweat pH	9.29 ± 0.59	$10.32 \pm 0.28^*$
Ratio wax to sweat	1:2.35	1:3.47

The lipid composition of the wax of the felted wool also undergoes noticeable changes. In particular, **Fig. 1** shows that against the background of practically the same amount of unesterified cholesterol, lanosterol, dehydrocholesterol, and squalene, in such wool the content of polar lipids ($P < 0.01$) and unesterified fatty acids ($P < 0.05$) significantly increases, while the content of esterified cholesterol ($P < 0.05$) decreases.

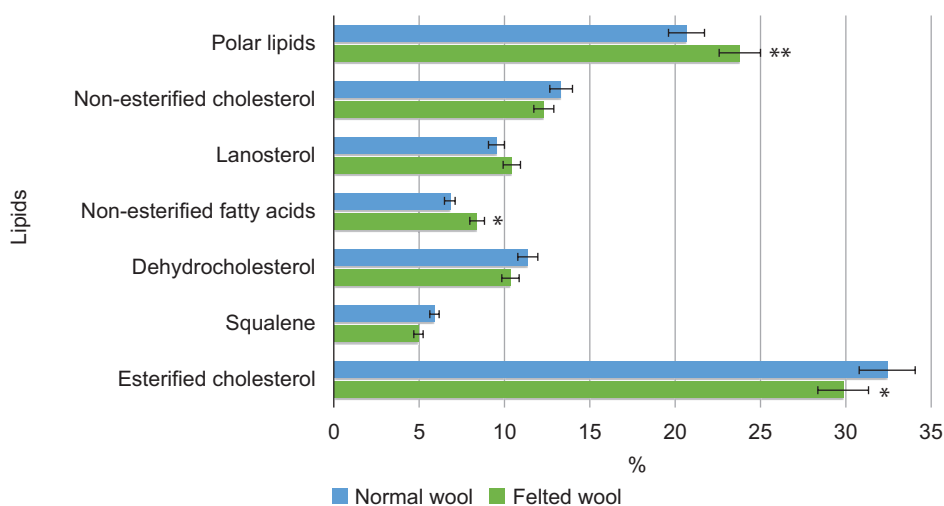


Fig. 1. Lipid composition of wax of normal and felted wool %, ($M \pm SD$, $n = 6$)

Our studies have shown (**Fig. 2**) that the fatty acid composition of wax from ewes of the Ukrainian Carpathian Mountain breed contains 23 acids, which are represented by both saturated and unsaturated acids, as well as iso acids, five of which, have not been identified yet.

The fatty acid composition of the wax of the felted wool differs from that of the normal wool in terms of the content of individual acids. In particular, this concerns a significantly higher content of erucic fatty acid ((13Z)-docos-13-enoic lauric, C22:1 ω 9) ($P < 0.05$), as well as a lower content of cerotic (hexacosanoic C26:0) acid ($P < 0.01$) and one of the unidentified acids, which we have tentatively designated as Unidentified-5 ($P < 0.01$).

In total, in the normal wool, the surface lipids contain 59.19 % of saturated fatty acids, 16.20 % of unsaturated, and 24.61 % of unidentified acids. In contrast, their proportion in the felted wool is as follows: saturated acids – 50.86 %, unsaturated – 28.07 %, unidentified – 21.07 %.

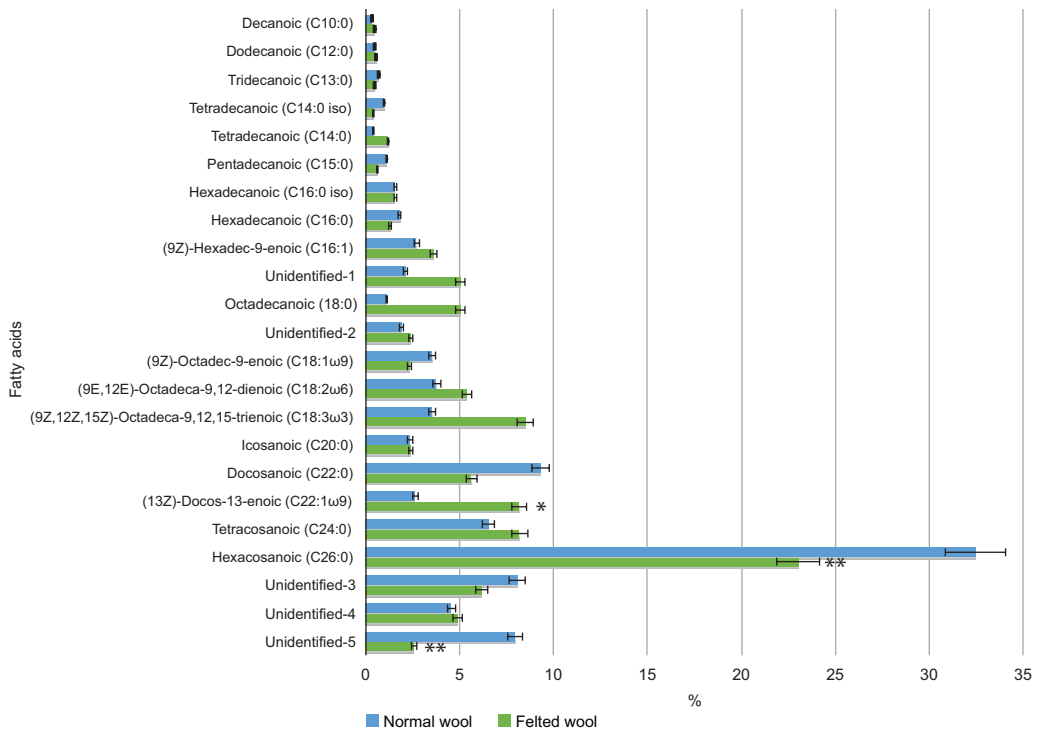


Fig. 2. Fatty acid composition of wax of normal and felted wool %, (M ± SD, n = 3)

Thus, the conducted studies showed that normal and felted wool differ in the amount of available microflora, qualitative characteristics of grease, as well as the lipid and fatty acid composition of wax.

DISCUSSION

Sheep wool possesses a wide range of unique properties inherent exclusively to this natural fiber. Even at the current level of development of synthetic fibers, it remains difficult to produce materials with thermal insulation and moisture-absorbing properties comparable to those of wool. Under modern conditions, the textile industry requires high-quality wool raw materials. However, a substantial proportion of the wool produced is defective and does not meet established quality standards. In particular, among semi-coarse-wool sheep of the Ukrainian Carpathian Mountain breed, which are raised in the mountainous regions of the Ukrainian Carpathians, a frequent defect is the felting of wool directly on the animal.

Our studies have shown that fleece microflora plays an active role in felting processes, particularly bacteria and mold fungi, whose abundance is significantly higher in felted wool compared to normal fleece. As reported by B. Petek *et al.* (2024), V. L. Vikash *et al.* (2025), and V. Thadiyan *et al.* (2025), certain bacterial species secrete enzymes – keratinases – with keratinolytic activity, enabling the hydrolysis of keratin in wool fibers. Mold fungi also exhibit keratinase-producing capacity (Bhari & Kaur, 2023); moreover, they can mechanically damage the cuticular layer of the hair through mycelial hyphal

growth (Rom *et al.*, 2025). As a result, cuticle cells of damaged fibers become bent and irregularly torn, individual scales are detached, and the fiber surface is deformed, leading to fiber entanglement and the formation of a dense felted mat, as also demonstrated by J. Zhang *et al.* (2025b).

Since wool fat consists predominantly of stearic components, it is relatively resistant to saponification (Allafi *et al.*, 2022). At the same time, A. F. El-Fiky *et al.* (2022) and S. Ali *et al.* (2023), describing the action of microbial lipases, indicate their ability to hydrolyze wool wax. Comparable results were obtained in the present study. Thus, in felted wool, the content of polar lipids and non-esterified fatty acids increases significantly, while the proportion of esterified cholesterol decreases, indicating ongoing hydrolytic processes within the grease environment.

In this context, a reference can be made to our previous findings, which demonstrated that a sharp increase in bacterial colonization of the fleece leads to deterioration of grease protective properties (Tkachuk *et al.*, 2024). The present study confirms this observation, as an increase in bacterial and mold fungal abundance in felted wool is accompanied by a significant reduction in wax content and an increase in sweat, thereby altering their ratio. Specifically, in normal wool this ratio is 1:2.35, whereas in felted wool it increases to 1:3.47. As is well established (Sarma *et al.*, 2024), the protective properties of grease are primarily determined by the wax-to-sweat ratio, with optimal protection observed when the proportion of sweat per unit of wax is lower.

In felted wool, higher sweat pH values were observed compared to normal wool. As noted by Q.-Y. Liu *et al.* (2025), microbial growth is more active under alkaline conditions. In addition, high-pH sweat may alter fiber structure, thereby negatively affecting wool quality. Conversely, as reported by G. Gelaye *et al.* (2021), wax protects wool fibers from adverse exogenous factors, a function that largely depends on its lipid composition, particularly its fatty acid profile.

Our studies have shown that the fatty acid composition of surface lipids in ewes of the Ukrainian Carpathian Mountain breed includes 23 fatty acids, comprising saturated, unsaturated, and iso acids. Five fatty acids have not been identified yet. Differences were observed between the fatty acid composition of wax in normal and felted wool. In particular, felted wool exhibited a significantly higher content of erucic acid ((13Z)-docos-13-enoic acid, C22:1 ω 9), as well as a lower content of one unidentified fatty acid and, notably, cerotic acid (hexacosanoic acid, C26:0). The latter, according to B. Singh and S. Singh (2003), M. Rehan *et al.* (2020), and F. Rwegoshora *et al.* (2023), exhibits antimicrobial properties and is therefore of particular importance, as it may function as a natural disinfectant within the fleece. A reduced content of this fatty acid was observed specifically in wool with higher microbial contamination.

The total proportion of saturated fatty acids in the wax of felted wool is lower, amounting to 50.46 %, compared to 58.81 % in normal wool, whereas the proportion of unsaturated fatty acids is higher in felted wool (28.07 %) than in normal wool (16.20 %). In this context, it should be noted that an increase in unsaturated fatty acids enhances molecular susceptibility to peroxide oxidation (Cao *et al.*, 2024).

Thus, the present study established the characteristic features of the microflora composition, as well as quantitative and qualitative parameters of fleece grease, in felted wool of sheep of the Ukrainian Carpathian Mountain breed.

CONCLUSIONS

The fleece of ewes of the Ukrainian Carpathian Mountain breed with felted wool, in comparison with normal fleece, is characterized by a higher abundance of bacteria and mold fungi. Favorable conditions for the development of these microorganisms in felted wool arise from an increased sweat content and elevated alkalinity. Microbial utilization of wax during metabolic activity results in a reduction of its content, which occurs through the hydrolysis of individual components, as evidenced by an increase in polar lipid fractions and non-esterified fatty acids, accompanied by a decrease in esterified cholesterol.

The fatty acid composition of wax is also altered. In particular, felted wool contains a significantly higher proportion of erucic acid ((13Z)-docos-13-enoic acid, C22:1 ω9), as well as a reduced content of one unidentified fatty acid and cerotic acid (hexacosanoic acid, C26:0). The latter, owing to its antimicrobial properties, may function as a natural disinfectant within the fleece. These compositional changes result in a decline in the protective properties of wax.

As a consequence, conditions become favorable for the adverse effects of microflora on wool fiber structure, particularly on the cuticular layer, which ultimately may lead to fiber damage and promote the development of felting processes. However, the present study does not fully elucidate the underlying mechanisms, thereby highlighting the need for further research into the origin and progression of this defect in sheep wool.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors received no specific funding for this work and declare no conflict of interest.

Human Rights: the article does not contain any experiments with humans.

Animal Rights: all international, national and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [V.T.; N.M.; N.O.]; methodology, [V.T.; N.M.]; investigation, [V.T.; N.O.]; resources, [V.T.; N.O.]; data curation, [V.T.; B.K.; B.A.]; writing – original draft preparation, [V.T.; N.M.; B.A.]; writing – review and editing, [V.T.; N.O.]; visualization, [N.M.; B.A.]; supervision, [V.T.; B.K.]; project administration, [V.T.; B.A.]; funding acquisition, [-].

All authors have read and agreed to the published version of the manuscript.

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МІКРОФЛОРА І ЖИРОПІТ РУНА НОРМАЛЬНОЇ ТА ЗВАЛЯНОЇ ВОВНИ ОВЕЦЬ УКРАЇНСЬКОЇ ГІРСЬКОКАРПАТСЬКОЇ ПОРОДИ

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Обґрунтування. Овечій вовні властива валкоздатність, яка покладена в основу валяльного виробництва. Однак вовна може звалюватись і на тілі вівці, стаючи, таким чином, дефектною. Це явище поширене і у напівгрубововнових овець української гірськокарпатської породи. З огляду на це, метою роботи було вивчити роль мікрофлори та жиропоту руна у процесах виникнення звалку у вівцематок цієї породи.

Матеріали та методи. Мікрофлору руна досліджували за допомогою висівання на щільні живильні середовища. Кількість поту визначали водною витяжкою його солей. Віск екстрагували в апараті Сокслета і вивчали методом тонкошарової хроматографії, а жирнокислотний склад – на газорідному хроматографі, методом переведення у метилові ефіри завдяки прямій переетерифікації жирних кислот.

Результати. Руно зі зваляною вовною, порівняно з нормальною, характеризується вірогідно вищим вмістом поту ($P < 0,01$) вищої лужності ($P < 0,05$). Це сприяє розвитку бактерій ($P < 0,01$) і пліснявих грибів ($P < 0,001$), котрі, використовуючи у процесах своєї життєдіяльності віск, знижують його кількість ($P < 0,001$). Зменшення естерифікованого холестеролу ($P < 0,05$) та зростання фракцій полярних ліпідів ($P < 0,01$) і неестерифікованих жирних кислот ($P < 0,05$) вказують на процеси гідролізу окремих компонентів воску. А зміни у жирнокислотному складі призводять до зростання ерукової кислоти ((13Z)-докоз-13-еноєнової, C22:1ω9) ($P < 0,05$) та зниження

однієї з неідентифікованих кислот ($P < 0,01$) і церотинової (гексакозанової C26:0) жирних кислот ($P < 0,01$). Остання може виступати в руні як природний дезінфектант. Загальна кількість насичених кислот у воску зваляної вовни є нижчою і становить 50,46 %, порівняно з нормальною вовною (58,81 %); а ненасичених, навпаки, у зваляній вовні більше (28,07 %), порівняно з нормальною (16,20 %). А збільшення кількості ненасичених жирних кислот сприяє зростанню вразливості молекул до пероксидного окиснення.

Висновки. Отже, зміни, які відбуваються у середовищі руна і, зокрема, його мікрофлори та жиропоту, мають безпосередній вплив на процеси звалювання напівгрубої вовни овець української гірськокарпатської породи.

Ключові слова: вівцематки, жиропіт, зваляна вовна, мікроорганізми, віск, піт, жирні кислоти