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Influence of microorganisms of fleece on the structure, physical properties, amino acid and mineral composition of sheep wool

V. M. Tkachuk*, P. V. Stapay**, N. Z. Ohorodnyk*, N. R. Motko*, A. O. Boyko*

*Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv, Lviv, Ukraine
**Institute of Animal Biology, National Academy of Agrarian Sciences, Lviv, Ukraine

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Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv, Pekarska st., 50, Lviv, 79010, Ukraine. Tel.: +380-32-239-26-43. E-mail: admin@lvet.edu.ua

Institute of Animal Biology, National Academy of Agrarian Sciences, Vasylia Stusa st., 38, Lviv, 79034, Ukraine. Tel.: +380-32-260-07-95. E-mail: inenbiol@mail.lviv.ua

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Wool is an irreplaceable raw material for the textile industry. However, a significant amount of woolen raw material is of low quality. In particular, microbiological destruction of fibers is the most widespread type of damage to wool. In view of this, the aim of our work was to clarify the role of the microflora of sheep fleece in the processes of degradation of the fiber structure and to establish the degree of their chemical and physical changes. The study focused on the wool of ewes from the Askanian fine-wool breed. Microbial analysis was performed by culturing samples on solid nutrient media. The structure was studied using transmission electron microscopy. The keratoses content was determined through the oxidation of wool with superacids. The amino acid composition was analyzed via ion chromatography using an AAA-400 amino acid analyzer. The mineral composition was assessed with an atomic absorption spectrophotometer, model S-115 PC. Fineness was measured using a micrometer, and the tensile strength of the wool was evaluated using a DSh-3M apparatus. It is shown that as a result of the vital activity of the fleece microflora, damage to the structure of wool fibers occurs, which is accompanied by changes in the surface of the cuticular layer, which is indicated by a probable increase in beta-keratoses. In such wool, the total amount of amino acids decreases (by 25.6 g/kg, or 2.7%) due decreases in arginine, valine, histidine, lysine and cystine. The reduction of histidine and lysine may be related to the highest content of these amino acids in the cuticular layer, which undergoes significant changes. Wool damaged by microflora is characterized by a reduced level of such mineral elements as copper and sulfur. A decrease in the latter with a decrease in cystine may indicate the destruction of disulfide bonds in the fiber by proteolytic enzymes of microorganisms. This ultimately leads to the deterioration of the physical properties of such wool, in particular, a decrease in strength by 20.5% (8.3 versus 6.6 cN/tex) and fineness by 7.9% (23.9 versus 22.0 μm). These data provide the opportunity to improve the quality of wool raw materials, but do not reveal the enzymatic mechanisms of the effect of microorganisms on wool fiber, which requires further research.

Keywords: microflora of fleece; keratin; amino acids composition; mineral elements; wool strength; fiber fineness.

Introduction

The total population of sheep in the whole world is about 1.32 billion head, from which 1.746 million tons of wool are sheared annually (FAO, 2023). Today, the share of wool for the production of textile products in the world is only 3%. However, the cost of finished products made from it is 50 or more times higher than the cost of these raw materials and ensures the functioning of light industry, engineering, trade, transport, etc. (El Sabry et al., 2023; Popescu & Stanescu, 2024).

Despite the wide use of artificial and chemical fibers, the advantages of natural wool are undeniable. High hygroscopicity, elasticity and strength, good thermal insulation properties make wool an irreplaceable raw material for the textile industry now and in the future (Yang et al., 2022). However, a significant amount of woolen raw material is currently still of low quality. In particular, microbiological destruction of fibers is the most widespread type of damage to woolen raw materials, both directly on the sheep and during its storage.

Sheep wool belongs to the group of keratins, the characteristic feature of which is a complete set of amino acids and, above all, sulfur-containing ones, which can be used by microorganisms for the synthesis of their own proteins. (Giteru et al., 2022; Tegegne, 2023). However, they can be used only by microorganisms that possess proteolytic enzymes, namely exoproteases capable of hydrolyzing keratin to individual amino acids (Liu et al., 2023). Several microorganisms including bacteria, actinomycetes and fungi have been reported to produce keratinases. These keratinases are predominantly extracellular serine proteases or metallo-endoproteases, and have a protease and disulphide reductase dual nature (Ibrahim et al., 2022). Currently, keratinolytic enzymes are known, which belong to at least 14 different families of proteases, among which disulfide reductases are distinguished, which catalyze the degradation of keratin by breaking disulfide bonds (Qiu et al., 2020).

As Chilakamarry et al. (2021) point out in their studies, degradation of keratin occurs mainly by proteolysis, sulfitolysis and deamination. Proteolysis and sulfitolysis, under the action of microbial enzymes, cause breaks in disulfide bonds, and deamination leads to the release of -NH_2 groups.

In the process of keratin damage, microorganisms first damage the cuticular layer, and then penetrate into the cortex. As a result of a dislocation of the fiber structure of the scales and the cells of the cortical layer, they are no longer connected to each other and the fiber disintegrates (Rom et al., 2024; Vikash et al., 2025). Some microorganisms, in addition to disrupting the fiber structure, reduce its quality by coloring the wool in red, blue, dirty green, and sometimes yellow (Denman et al., 2022). It was shown that the increase of microorganisms in sheep fleece leads to yellowing of fibers which significantly affects its lipid composition (Tkachuk et al., 2014).

Long-term action of microorganisms leads to a decrease in the amount of all amino acids that are part of the fiber. This is especially true for coarse wool, where the total amount of amino acids decreases by 10–12%, and slightly less for fine wool (4–5%). It should be noted that the number of amino acids that form disulfide bonds, namely cystine and methionine, as well as polar (hydrophilic) amino acids that provide hydrogen bonds, such as serine, glycine, threonine, tyrosine, is significantly reduced (up to 25–33%). In the primary structure of keratin, the N-terminal group is serine, and the C-terminal group is tyrosine, and therefore, a decrease in the number of these amino acids indicates a dislocation of the primary structure of the protein (Zhang & Fan 2021; Feroz et al., 2020).

The high stability and resistance to dissolution in various solvents and the three-dimensional network structure of keratin are due to the presence of disulfide bonds (Mi et al., 2025). Changes in the amino acid composition of proteins of wool fibers under the action of spontaneous microflora indicate the destruction by microorganisms of peptide and disulfide bonds, which ensure the stability of the primary structure of keratin, as well as hydrogen bonds, which play the main role in stabilizing the spatial structure of proteins (secondary, tertiary and quaternary) (Mattiello et al., 2023).

A number of authors, and in particular (Collie et al., 2025; Vikash et al., 2025) believe that thus, under the action of proteolytic enzymes of microorganisms, keratin is decomposed into amino acids, as a result of which their number is significantly reduced, especially cystine, methionine, serine, glycine, threonine and tyrosine. This leads to breaks in covalent and hydrogen bonds, fibers lose their strength, elasticity, and shine. However, the issue of microbiological damage to wool has not been fully clarified. In view of this, the aim of our work was to clarify the role of the microflora of sheep fleece in the processes of degradation of the fiber structure and to establish the degree of their chemical and physical changes.

Materials and methods

Ethics. 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" and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" as amended on 15.11.2024.

The design of animal experiments. The object of the research was wool samples, which were collected after the spring shearing from the shoulder blade area of twenty ewes of the Askanian fine-wool breed, which belonged to the Institute of Animal Husbandry of the Steppe Regions named after M.F. Ivanov "Askania Nova". All animals were under the same conditions of keeping and feeding. The obtained samples were divided into wool that was in normal condition, and wool which had visual signs of damage and defects. The presence or absence of damage was confirmed by scanning electron microscopy as previously reported (Tkachuk et al., 2024). For further research, five samples of damaged wool, which had the greatest microbiological insemination, were selected (a natural process of colonization was observed). Five samples of undamaged wool with a low level of microbial contamination served as controls.

Microbiological studies. After that, microorganisms were isolated from all the studied samples. For this purpose, 1 g of wool was ground for 5 min in a sterile porcelain mortar, adding 1 mL of a 0.3% Tween-80 solution. Then the wool sample was quantitatively transferred into a sterile flask, using 99 mL of sterile water for repeated washings. Flasks with samples were shaken on a Schuttel apparatus for 30 min, after which the necessary dilutions of the bacterial suspension were prepared by a commonly used method. The number of viable microorganisms was determined by sowing appropriate dilutions on dense nutrient media: meat-peptone agar was used for bacteria, Sabouraud was used for fungi, neurospores and molds, Czapek one used for actinomycetes. Colonies were counted after 4–5 days of incubation in Petri dishes at a temperature of 30 ºC.

Damaged wool with the highest microbiological insemination was selected for further research: the average content of bacteria was 7.2 x 10^9, fungi content was 6.6 x 10^5, molds content was 5.4x10^4, neurospores content was 4.6x10^3, actinomycetes content was 4.4 x 10^5 CFU/g. Samples of undamaged wool with a low level of microbial contamination served as controls: the average content of bacteria was 2.6 x 10^9, fungi content was 4.2 x 10^5, molds content was 4.8 x 10^4, neurospores content was 3.6 x 10^3, actinomycetes content was 2.2 x 10^5 CFU/g.

After that, the wool samples were washed with a neutral washing solution, thoroughly rinsed and dried. Wool fat (wax) was removed by extraction in a Soxhlet apparatus with tetrachlormethane for 5 hours.

Study of fiber structure (transmission electron microscopy). The structure of the wool was studied using a transmission electron microscope PEM-100. Bundles of 10–20 pieces of parallel-placed hairs 8–10 mm long were selected for the research. To obtain images, the samples were fixed using an osmium fixative (a 1.5% solution of osmium tetroxide in a 0.2 N cacodylate buffer (pH 7.2) for 24 h at room temperature, then washed and dehydrated according to the generally accepted scheme). To obtain images, dehydrated preparations were poured into epoxy resin EPON 812 and MNA. Before filling, the sample was saturated with a filling medium diluted with propylene oxide in a ratio of 1:1, and then in a clean filling medium for 12 h at room temperature.

The blocks were polymerized in a thermostat at 60 ºC for 48 hours. From the obtained blocks, the samples were prepared on the UMTP 6M ultratome using a diamond knife from the company "Diatome" (Switzerland). Ultrathin sections were counterstained with lead citrate and uranyl acetate and used for viewing and photography. In the research, osmium oxide from the Sigma company was used, and the rest of the reagents were from the Fluka company. Photographs in the transmission electron microscope were obtained using a digital camera "Sony H9".

Study of keratoses. The quantitative ratio of keratoses was determined according to the method of Asquith & Parkinson (1966). According to this technique, superacids oxidize the disulfide bonds in the keratin molecule. The final splitting of the fibers is achieved with the help of alkali. For this, samples of clean, degreased and dry wool weighing 1 g were completely immersed in a 1.6% solution of peracetic acid in an Erlenmeyer flask for 48 hours, at a temperature of 25 ºC, with constant shaking on a Schuttel apparatus. Then the wool was washed with distilled water and dried at room temperature.

The oxidized wool was immersed in a solution of sodium hydroxide (CAS, Germany) (10 mL of 0.02 N NaOH per 100 mg of wool) and left for 24 hours with constant shaking. Then the dissolved fraction was filtered on Buchner funnels through pre-weighed paper filters (blue tape); the insoluble part that remained on them (beta-keratosis) was washed with distilled water, then with alcohol, alcohol-ether and ether. The sediment was dried and weighed together with the filter. The content of beta-keratin was determined gravimetrically.

Alpha-keratosis was isolated from the filtrate by adding concentrated acetic acid to pH 4.0 (a white precipitate formed) and obtained as a precipitate on the filter (blue band). The filtrate that remained corresponded to gamma-keratosis. First, it was heated to 40 ºC and a syrup was obtained, which turned into a powder after the addition of absolute alcohol. The resulting precipitates were dried, together with the filter, and weighed. The content of the fractions was calculated gravimetrically and shown in percentage.

Study of amino acid composition. The amino acid composition of wool keratin was determined using an AAA-400 amino acid analyzer (Ingos, Czech Republic) by the ion chromatography method (ISO 13903: 2005) after protein hydrolysis with a 6 N hydrochloric acid solution for 24 hours at a temperature of 110 ºC. The principle of operation of the analyzer is based on the chromatographic chemisorption of amino acids on a cation exchange resin followed by elution and determination of the type of amino acid by color reaction with ninhydrin. The separation of amino acids was carried out under the following conditions: the chromatographic column was filled with Ostion Ingos sorbent (Czech Republic), the mobile phase was a combination of 4 citrate buffer solutions with pH 2.7–8.0, regeneration of the column with pH 14.0, post-column derivatization of amino acids with ninhydrin in solution to form chromophoric complex compounds, two-channel photometric detection at 570 nm, automatic control of multistage chromatographic analysis was carried out using Chromulan 0.82 Ingos software.

Determination of mineral composition. To determine the mineral composition, wool samples were previously mineralized by the method of wet ashing followed by acid extraction. In the prepared samples, macro- and microelements were determined on an atomic absorption spectrophotometer S-115 PC using an acetylene-air mixture. The element concentration in the sample was calculated using a computer program according to the calibration curve.

Physical indicators. From the physical parameters, the fineness was determined using a micrometer and the tensile strength of wool was determined using the DSh-3M apparatus.

Statistical analysis. The obtained experimental data were analyzed using Statistica 12.0 software (StatSoft Inc., USA). The data were statistically analyzed using ANOVA. The results are expressed as mean and standard error (x ± SE). Differences between the data were considered significant at $P < 0.05$.

Results

Figure 1 shows cross-sections of wool fiber. In the picture, two morphologically different areas are clearly distinguished, namely the cuticle and the cortex. Electron microscopic studies indicate a complex structure of the cuticular layer Figure 1.

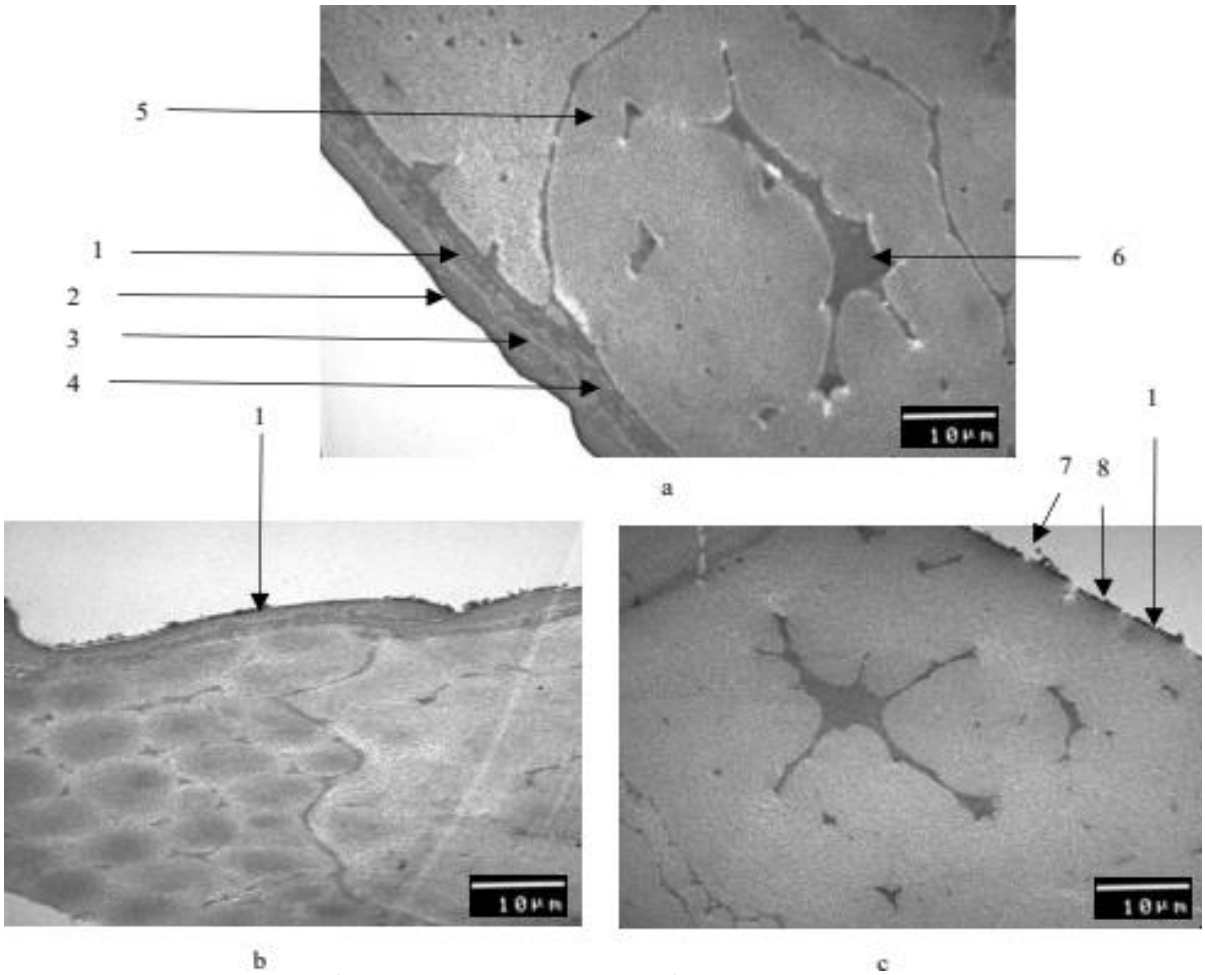

Fig. 1. Cross-sections of normal (*a*) and damaged (*b, c*) wool fiber: *1* – cuticle, *2* – epicuticle, *3* – exocuticle, *4* – endocuticle, *5* – cortex, *6* – remains of the nucleus; *7* – ruptured cuticle, *8* – thinning cuticle

When studying the macrostructure of wool, i.e. determining individual fractions of keratoses in it (Table 1), it was established that the ratio between alpha, beta and gamma keratoses is disturbed in fibers that have undergone destructive changes. From the data in Table 2, it can also be seen that in wool damaged by microorganisms, the content of alpha-keratosis probably increased ($P < 0.05$) and beta-keratosis decreased ($P < 0.01$).

Table 1
Macrostructure of normal and damaged sheep wool (%, x ± SE, n = 5)

Keratosis	Normal wool	Damaged wool
Alpha	59.72 ± 1.07	65.44 ± 1.25*
Beta	12.64 ± 0.47	10.01 ± 0.13**
Gamma	27.63 ± 0.62	24.56 ± 1.36

When studying the amino acid composition (Table 2), it was found that the amount of amino acids in damaged wool decreased by a total of 25.6 g/kg, or 2.7%, compared to wool in normal condition. The reduction of amino acids in damaged wool occurs due to the depletion of arginine ($P < 0.05$), valine ($P < 0.05$), histidine ($P < 0.05$), lysine ($P < 0.05$) and cystine ($P < 0.05$).

It is shown (Table 3) that in comparison with normal wool, damaged wool probably contains less sulfur ($P < 0.01$). The data in this table also indicate significant changes in copper levels in damaged wool. Compared to normal wool, the amount of this element decreased by 19.1% ($P < 0.05$).

Consequently, the microflora of sheep fleece causes significant changes in the structure and chemical composition of wool fibers, impacting their strength.

Table 2
Amino acid composition of normal and damaged sheep wool (g/kg, x ± SE, n = 5)

Amino acid	Normal wool	Damaged wool
Cystine	119.90 ± 2.44	100.84 ± 4.23*
Methionine	5.02 ± 0.14	4.78 ± 0.06
Proline	51.60 ± 1.23	53.08 ± 0.80
Glycine	57.44 ± 2.22	54.66 ± 1.33
Alanine	39.74 ± 0.85	38.82 ± 1.21
Serin	93.96 ± 1.70	108.58 ± 5.74
Tyrosine	36.60 ± 0.72	35.14 ± 0.99
Aspartic acid	77.76 ± 1.75	76.20 ± 0.47
Glutamic acid	93.22 ± 4.02	105.02 ± 3.09
Leucine	76.08 ± 1.47	73.22 ± 0.84
Valin	51.70 ± 0.51	48.60 ± 0.89*
Isoleucine	38.02 ± 2.28	37.34 ± 2.12
Phenylalanine	26.10 ± 1.15	25.22 ± 1.44
Lysine	27.52 ± 0.51	23.14 ± 1.02*
Threonine	63.14 ± 1.54	58.94 ± 2.72

Arginine	62.10 ± 2.90	53.78 ± 0.96*
Histidine	9.40 ± 0.39	7.28 ± 0.61*
Tryptophan	15.32 ± 0.38	14.34 ± 0.60
Total amino acids	944.62	918.98

It was established (Table 4) that the tensile strength of fibers in damaged wool decreased by 20.5% (8.3 versus 6.6 cN/tex, $P < 0.01$). As for the fineness of wool, in damaged hair it decreased by 7.9% (23.9 versus 22.0 μm), although this was insignificant.

Table 3

Mineral composition of normal and damaged sheep wool (x±SE, n = 5)

Element	Normal wool	Damaged wool
Sulphur, g/kg	37.91 ± 0.23	35.92 ± 0.33**
Calcium, g/kg	2.11 ± 0.08	1.97 ± 0.06
Phosphorus, g/kg	0.30 ± 0.01	0.28 ± 0.02
Potassium, g/kg	0.89 ± 0.04	0.86 ± 0.04
Magnesium, g/kg	0.41 ± 0.03	0.39 ± 0.02
Sodium, g/kg	0.49 ± 0.02	0.46 ± 0.02
Zinc, mg/kg	147.02 ± 2.97	144.70 ± 2.85
Ferrum, mg/kg	105.38 ± 1.79	110.26 ± 3.32
Copper, mg/kg	8.06 ± 0.36	6.52 ± 0.27*

Table 4

Physical properties of normal and damaged sheep wool (x ± SE, n = 5)

Indicator	Normal wool	Damaged wool
Strength, cN/tex	8.31 ± 0.21	6.56 ± 0.25***
Fineness, μm	23.85 ± 0.42	21.96 ± 0.55

Discussion

As a result of conducted electron microscopic studies, it was established that the cuticular layer is significantly damaged in wool that has undergone destructive changes by fleece microorganisms. In particular, its significant thinning is observed, and in some cases, complete destruction. We will remind you that the wool cuticle is made up of several layers, namely epicuticles, exocuticles, and endocuticles (Caven et al., 2022). According to Bergendal et al. (2025), Motko et al. (2025) the epicuticle, or the outer layer of the cuticle, is a thin hydrophobic layer that contains a significant amount of 18-methyleicosanoic acid, which is bound to the proteolipid membrane and is characterized by a high cystine content. Under the epicuticle there are the A-layer and the actual exocuticle, which are characterized by a high cystine content and hydrophobic properties (Zhang et al., 2022). These layers ensure the mechanical integrity and chemical resistance of a hair. The endocuticle is of non-keratin origin and is built from remnants of cytoplasmic structures. The line connecting these layers has an irregular shape. Coderch et al. (2023) indicate that all structural components of the cuticle are connected to each other by CMC (cell-membrane complexes). The latter, as well as the endocuticle, are the main ways of diffusion of various substances inside a hair. CMCs are built mainly from lipids and polysaccharides (Coderch et al., 2025). The main function of the CMC is to ensure intercellular contacts between different structural components of the hair, such as cuticle-cuticle, cuticle-cortex, cortex-core. Our studies have shown that the cortex occupies the main part of the fiber and is of the greatest importance in the formation of the physical and mechanical properties of a hair. Cortical cells are densely placed next to each other and are oriented along the axis of the hair. Studies of cross-sections of wool show that almost all these cells are round in shape. Instead, cells that are directly adjacent to the cuticle are mostly more flattened in the same direction as the cuticle. Remains of nuclei are observed in cortical cells.

The data obtained by us during the study of the ultrastructure of the fiber are also confirmed during the study of the macrostructure, that is, the determination of individual fractions of keratoses in it. In particular, it was established that the ratio between alpha, beta and gamma keratosis is disturbed in fibers that have undergone destructive changes. We emphasise that wool keratoses correspond to different structural components of the fiber. In particular, alpha-keratosis corresponds to the proteins of the macro- and microfibrils of cells of the cortex, beta-keratosis to the cuticle and cell membranes, gamma-keratosis to the interfibrous substance, the cementing substance, that is, the fiber matrix (Petek et al., 2024; He et al., 2025). In wool damaged by microorganisms, the content of alpha-keratosis probably increases ($P < 0.05$) and beta-keratosis decreases ($P < 0.01$). The decrease of the latter is obviously related to the negative effect of the fleece microflora on the cuticular layer of the hair, which leads to its damage. Therefore, the consequences of destructive changes in damaged fibers are clearly visible from the data of the macrostructure of wool, and biochemical studies of the amino acid and mineral composition reveal their nature and depth.

When studying the amino acid composition, it was found that the amount of amino acids in damaged wool decreases occurs through depletion of arginine, valine, histidine, lysine and cystine, which is associated with the degradation of the chemical structure of these amino acids under the influence of microorganisms present in the sheep fleece. Regarding the reduction of histidine and lysine, it is possible that this is connected with the greatest content of these amino acids in the cuticular layer, which, as mentioned above, undergoes significant changes in damaged wool. As for the probable ($P < 0.05$) reduction of cystine in damaged wool, it should be recalled that the wool fiber is a network of polypeptide chains interconnected by covalent and non-covalent bonds (Wang et al., 2024). The most important among them are disulfide bridges formed by the sulfur-containing amino acid cystine (Csuka et al., 2023). They are formed in the process of fiber formation, namely, at the last stage of keratinization. Thanks to these bonds, keratin fibers are insoluble in water and more resistant to chemical and physical factors compared to other proteins (Oussadi et al., 2025).

Damaged wool probably contains less sulfur ($P<0.01$). These data clearly reflect changes in the amino acid composition, since any alteration in the total sulfur content in wool primarily depends on the cystine content, which is closely associated with its physical and mechanical properties (Sun et al., 2022; Branisa et al., 2024). The data also indicate significant changes in copper levels in damaged wool. It is worth noting that copper, as a component of certain enzymes, plays a role in numerous processes, including the pigmentation and keratinization of wool fibers. It is believed (Ghimis et al., 2023; Hossain et al., 2024; Kalahroodi et al., 2024) that copper, as a bacteriostatic agent, can be excreted through the skin and thus suppress the growth and reproduction of the bacteria of the fleece.

Previous studies by Brandelli et al. (2010) described the effect of microbial keratinases on the structure and composition of wool. Unfortunately, we did not conduct such studies. Instead, our data provide additional information on the influence of sheep fleece microflora on the physical properties of wool, in particular its strength and fineness. In particular, it was established that the tensile strength of fibers in damaged wool decreases. This is consistent with the disruption of disulfide bonds in damaged wool, which play a direct role in shaping such a physical property of wool as its strength (Li et al., 2025; Wang et al., 2025). As for the fineness of wool, in damaged hair it decreases. These data are quite logical considering the significant damage to the cuticular layer by microorganisms of wool fibers (Yue et al., 2024). Consequently, in the processes of damage to the wool fiber by microorganisms, its structure is damaged, chemical composition and physical characteristics.

Our experiment is only a separate fragment of the whole problem, and it clearly indicates that fiber damage begins during the annual growth of wool. In addition, such studies clearly indicate that in different sheep of the same type, which are in the same conditions, the processes of fiber damage proceed differently, since out of 20 head there are 5 samples with clearly expressed damage and 5 samples that are completely normal. Therefore, the importance of our studies is that they indicate the existence of different causes for the development of fleece microflora. This, in particular, is the quantity and quality of wool grease, as evidenced by our previous studies (Tkachuk et al., 2024).

Damage to fibers directly on the animal is only the initial stage. The next stage is damage to fibers during wool storage after shearing. Therefore, all of the above said clearly indicates the importance of the problem and, in general, the novelty of such studies. Revealing the mechanisms and causes of microbiological damage to wool fibers

will contribute to the development of methods for its prevention and reduction. This will make it possible to influence the industry, and wool processing enterprises will receive high-quality raw materials.

Conclusion

In line with the study's objectives, the influence of sheep fleece microflora on merino wool damage was examined. It was found that an increase of total number microorganisms creates favorable conditions for their impact on the structure of wool fibers. The result of the vital activity of the sheep fleece microflora is damage to wool fibers, which leads to changes in the structure, in particular in the surface cuticular layer. These changes lead to a deterioration in the physical characteristics of the fibers, notably a decrease in fineness and strength. This leads to a deterioration in the technological characteristics of wool. The obtained data can be used to improve the quality of wool raw materials, however, they do not reveal the enzymatic mechanisms of the influence of microorganisms on wool fiber, which requires further research.

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