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INTERNAL LIPIDS OF SHEEP WOOL WHEN A WATER-SOLUBLE COMPLEX OF FATTY ACIDS IS INCLUDED INTO THEIR DIET

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Background. An effective way to increase the productivity of sheep is through the use of fats in their feeding. With this in mind, the effect of a water-soluble complex of fatty acids on the internal lipids of wool sheep has been studied.

Materials and Methods. The diet of ewes of the Prekos breed of the research group included 3 % of a water-soluble complex of fatty acids – the essential lipid complex (ELC): linoleic – 54.5 %, oleic – 24 %, palmitic – 10 %, arachidonic – 6 %, stearic – 4 %, and linolenic – 1.5 %. Wool productivity was calculated over a skin area of 36 cm^2, and the live weight gain of lambs was measured at birth and again in 21 days. Free internal lipids were released by extraction in a Soxhlet apparatus, and bound lipids were released after preliminary alkaline hydrolysis. Wool strength was determined using the DSh-3M apparatus.

Results. The emulsion of fatty acids contributes to the intensification of wool and milk production processes, as evidenced by an increase in wool growth in ewes by 28.8%, and an increase in the live weight of lambs by 9.7 %.

The total amount of both free (P <0.05) and bound internal lipids (P <0.05) increased in the wool of experimental ewes. In the composition of free lipids, the content of non-esterified fatty acids (P <0.05) and non-esterified cholesterol (P <0.05) significantly decreases, while its esterified fraction increases (P <0.01). The same trend was noted in covalently bound lipids, but only a decrease in non-esterified cholesterol in lambs was observed. These changes indicate a slowdown in lipid oxidation processes under the influence of the dietary factors applied.

In the composition of both free and bound internal lipids, the content of ceramides significantly increased (*P* <0.05), applying to both the wool of ewes and the lambs obtained from them. In bound lipids, in ewes, a significant increase in glucosyl ceramides was noted (*P* <0.05), and in lambs, in cholesterol sulfate (*P* <0.001).

Changes in the quantitative and qualitative composition of internal lipids lead to an increase in wool strength from 7.38 to 8.03 cN/tex, i.e. by 8.8 % (*P* <0.05).

Conclusion. Feeding emulsion of fatty acids to ewes leads to an increase in productivity and positively influences both the quantitative and qualitative composition of internal lipids in the wool of ewes and their lambs.

Keywords: wool, emulsion, gains, internal lipids, ceramides, strength

INTRODUCTION

There is an intensive search and study of various dietary additives to reduce the price of feed, increase animal productivity, and improve animal husbandry products (der Poel *et al.*, 2020; Akintan *et al.*, 2024; Buchko *et al.*, 2024). The largest portion of expenses for keeping agricultural animals, including sheep, is spent on feed (Salami *et al.*, 2019; Burezq & Khalil, 2022).

An effective way to increase the productivity of farm animals is the use of fats in their feed. Adding fats to diets contributes to a more rapid growth of animals, reduces feed costs per unit of growth, increases the feed protein utilization ratio, shortens the fattening period, and improves the quality of animal products (Costa *et al.*, 2023).

The use of fat additives in animal feed has a positive effect associated with their high energy value, as well as wide-ranging biological effects on the body (Gaffield *et al.*, 2022). The digestibility of fats by sheep is high and depends on their physical and chemical properties, fatty acid composition, as well as the balance of rations (Li *et al.,* 2024). A deficiency of fats leads to a disruption of metabolic processes, damage to the skin, a decrease in the body's resistance to infectious diseases (Jia *et al.*, 2021), and an increase in feed costs per unit of production (Alba *et al.*, 2021).

Fats are a source of essential fatty acids: linoleic, linolenic, and arachidonic, which are not synthesized in the body of animals, or are synthesized in small amounts (Meale *et al.*, 2015). The addition of fatty acids, particularly eicosapentaenoic acid and docosahexaenoic acid, to ewes in the last period of pregnancy changes the profile of fatty acids in blood plasma, colostrum, and milk, and can increase lipogenesis (Coleman *et al.*, 2018).

The inclusion of fats in the diet affects the consumption of feed by animals. In particular, studies have shown that unsaturated fatty acids activate the receptors in the hypothalamic satiety center, stimulating food consumption (Allen *et al.,* 2020). The use of fats improves the palatability of feed and the energy value of diets (de Lima *et al.*, 2023).

Lipids are of great importance in the nutrition of sheep, especially ewes during periods of physiological stress (pregnancy and lactation). Thus, a lack of nutrients and energy value in feed can lead to a decrease in live weight of lambs at birth and survival of offspring (Ahmadzadeh-Gavahan *et al.*, 2023), as well as a decrease in wool production in ewes (Doyle *et al.*, 2021).

Wool fiber is 95 % made of keratin, and the remaining 5 % consists of lipids, pigments, mineral elements, and water (Jose *et al.*, 2023). The hair structure contains up to 3 % of lipids, which are both free and bound to proteins (Csuka *et al.*, 2023). The latter can be released only after preliminary alkaline hydrolysis, because they are attached to proteins through 18-methyleicosanoic acid (18-MEA) via thioester bonds. However, a certain amount of them can be released by organic solvents without preliminary alkaline hydrolysis; these are the so-called free lipids (Tokunaga *et al.*, 2019).

Internal lipids are localized both in the cuticle and cortex of the hair, and in cell-membrane complexes (CMCs), which connect the cells of the cuticle and cortex (Ghermezgoli *et al.*, 2020). The CMC of the cuticle consists of the upper and lower β-layers. The upper one is composed of covalently bound 18-MEA, forming a monolayer intercalated with other fatty acids, which are stabilized by van der Waals and electrostatic interactions. The lower layer does not contain 18-MEA, but is formed mainly by straight-chain fatty acids, namely palmitic (C16:0) and oleic (C18:0). These layers are separated from each other by a delta layer, which is a glycoprotein or globular protein (Robbins, 2009). In contrast, the CMC of cortical cells consists of non-esterified fatty acids, cholesterol, or cholesterol sulfate, and ceramides. The outer edge of each cortical cell is surrounded by a two-layer structure, which is also separated by a delta layer. The surface between the cuticle and the cortex is a complex CMC, which is formed on the cuticle side by a mixed monolayer of covalently and non-covalently bound fatty acids (lower β-layer), and on the cortical side by a double layer of non-esterified fatty acids, cholesterol, and ceramides. The cuticular monolayer and the cortical bilayer are also separated by a delta layer (Coderch *et al.*, 2023).

Wool lipids play an important role in protecting the fiber from damage. It was established that under the influence of external negative environmental factors, particularly the microflora of the fleece, ultraviolet radiation (Habe *et al.,* 2011; Ross *et al.*, 2022), weathering, and various mechanical factors (Fernandes *et al.*, 2023), the hair is damaged, leading to a decrease in the amount of structural lipids (including 18-MEA). This reduces its hydrophobicity, strength, and shine, making the fiber dry and brittle (Nagase, 2019).

MATERIALS AND METHODS

Experimental animals and design. The study was conducted on ewes of the Prekos breed under the conditions of the educational and scientific production center "Komarnivske" of the Lviv National University of Veterinary Medicine and Biotechnology named after S. Z. Gzhitskyi (Ukraine). According to the principle of matched pairs (based on breeding of animals, their age, and live weight), two groups of ewes (10 heads each) were formed: one control and one experimental. The experiment was carried out during the winter-stall period. At the beginning of the experiment, the ewes were in the late stage of pregnancy, and starting from the middle of the experiment, they entered the first period of lactation.

All interventions were performed in accordance with the international principles of the Council of Europe Convention "On the Protection of Vertebrate Animals Used for Experimental or Other Scientific Purposes" (Simmonds, 2017), EU Directive No. 609 (1986) and the resolutions of the National Congress of Ukraine on Bioethics (2010), which comply with the current legislation of Ukraine, in particular, Article 26 of the Law of Ukraine of 16.10.2012 No 5456-VI "On the Protection of Animals from Cruelty", as amended of 04.08.2017.

During the equalization period, which lasted 10 days, all animals received the main diet, balanced according to feeding norms. During the experimental period, which lasted 95 days, animals of the control group received the basic diet from the equalization period. The ewes of the experimental group received 3 % of the water-soluble complex of fatty acids – "Essential Lipid Complex – ELC" (LLC EcoProFeed, Ukraine) as part of the compound feed, which increased the crude fat content in the diet of pregnant ewes from 46.41 to 58.41 g, and in lactating ewes from 62.81 to 80.81 g. ELC is an aqueous emulsion of the following fatty acids: linoleic – 54.5 %, oleic – 24 %, palmitic – 10 %, arachidonic – 6 %, stearic – 4 %, and linolenic – 1.5 %. The energy value of ELC is 880 kcal/100 g.

Animals were fed in groups. Actual feed intake was determined daily for two consecutive days by weighing feed and its residues. Animals had free access to water. The rations of the experimental animals are presented in the table.

Diets of experimental ewes

Indicator	Pregnant ewes		Lactating ewes	
	control	experiment	control	experiment
Meadow hay, kg	0.700	0.700	1.100	1.100
Wheat straw, kg	0.200	0.200	0.200	0.200
Haylage (vetch-oat mixture), kg	2.100	2.100	2.500	2.500
Beet pulp, kg	1.000	1.000	1.500	1.500
Total compound feed, kg: including:	0.400	0.412	0.600	0.618
– wheat, kg	0.133	0.133	0.200	0.200
– oats, kg	0.133	0.133	0.200	0.200
– barley, kg	0.133	0.133	0.150	0.150
– ELC, kg	–	0.012	–	0.018
Premix, kg	0.01	0.01	0.015	0.015
Feed units	1.57	1.57	2.15	2.15
Exchange energy, mJ	17.40	17.84	23.79	24.45
Dry substance, kg	2.22	2.22	2.94	2.94
Crude protein, g	187.87	187.87	251.16	251.16
Crude fiber, g	589.24	589.24	773.14	773.14
Crude fat, g	46.41	58.41	62.81	80.81

Sampling procedures. The control of the wool productivity of experimental ewes was carried out by recording the growth of fibers on the measured skin area of 36 cm^2, and the live weight gain of the lambs born from them by weighing them at birth and again 21 days later.

Biochemical analysis. The selected wool samples were washed in a neutral detergent, after which, to remove surface fats, they were extracted in a Soxhlet apparatus with tetrachloromethane for 5 hours.

To release free internal lipids, the wool samples were re-extracted with a chloroform/methanol mixture (2:1) in a Soxhlet apparatus for 5 hours. The lipid extract was dried under vacuum and weighed. Bound internal lipids were obtained by the alkaline saponification method described by P. W. Wertz & T. D. Downing (1988). For this, the wool samples remaining after the free internal lipids were separated and hydrolyzed by a two-hour treatment at 60 °C in 100 mL of a 1 M sodium hydroxide solution in 90 % methanol. The samples were cooled and transferred to separatory funnels. Then, 100 mL of chloroform and 25 mL of distilled water were added to each sample. After 12 hours, the lower chloroform layer was removed, and the upper phase was acidified with a 6 M hydrochloric acid solution and then re-extracted by mixing with 100 mL of chloroform. After settling, the lower chloroform layer was removed, combined with the previously obtained extract, and dried by evaporation. The resulting precipitate was dissolved in 10 mL of a chloroform-methanol mixture (2:1), and 3 mL of 7.5 % potassium chloride was added to each sample. After 24 hours, the upper phase was removed using a water jet pump, and the lower phase, which contained lipids, was used for analysis.

The total amount of internal lipids was determined by weighing, and their composition was determined by thin-layer chromatography. Sorbfil plates measuring 10×10 cm were used, with a working layer of fractionated wide-pore silica gel (particle size 90–120 micrometers); the thickness of the sorbent layer on one plate was ±5 micrometers. Lipids were separated in two solvent systems: petroleum ether/diethyl ether, 4:1, v/v (system 1), and chloroform/methanol/water, 65:25:4, v/v (system 2).

After drying, the chromatograms were sprayed with 50% sulfuric acid and charred at 105 °C. For the quantitative determination of lipids, the spots from the plates were scraped into test tubes, concentrated sulfuric acid was added, and the mixture was heated to 105 °C. The optical density of each fraction was measured on a KFC-3 spectrophotometer at a wavelength of 400 nm in a cuvette with a thickness of 1 cm. Individual classes of lipids were identified by comparing the chromatograms of the samples with reference standards for cholesterol, stearic acid, and lanosterol (Sigma Chemical Co., USA) (Marsh & Weinstein, 1966). Other lipid classes were identified by comparing their rf-values to those found in literature (Wertz & Downing, 1989). The content of individual lipid components was calculated mathematically and expressed as a percentage.

For physical measurements, the tensile strength of wool was determined using the DSh-3M apparatus (Vlizlo, 2012).

Statistical analysis. The data were analyzed using Statistica 6.0 software pack (StatSoft Inc., USA). Data are presented as mean ± standard error. Analysis of variance ANOVA was used to compare the groups, followed by an analysis of the significance of the differences. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

It is known that the body of a sheep, for the normal course of the process of wool formation, needs not only a sufficient amount of plastic, but also energy substrates (Khan *et al.,* 2012). The addition of fat supplements to the rations of ewes has a positive effect on both the growth of wool and the live weight of lambs (Haddad & Younis, 2004).

As a result of our research, it was first established (**Fig. 1**) that feeding ewes a 3 % emulsion of fatty acids (ELC) as part of the compound feed leads to an increase in both wool and milk productivity. For instance, in the control group, wool growth in ewes was 0.323 mg/cm^2/day, while in the experimental group, it was 0.416 mg/cm^2/day, representing an increase of 28.8 %. Simultaneously, the live weight gain of lambs from the control group of ewes was 248.71 g/day, whereas for the experimental group it was 272.95 g/day, indicating a 9.7 % increase in milk yield in the experimental group.

These findings clearly demonstrate that the lipid supplement promotes the intensification of both wool and milk production, leading to an increase in lamb live weight gain as a result of the latter.

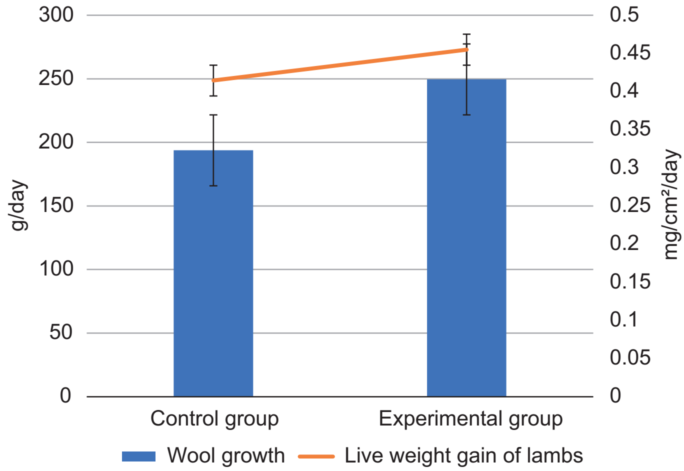

Fig. 1. Average daily growth of wool in ewes and live weight of lambs, (M ± SE, n = 5)

Two types of internal lipids can be distinguished in wool fibers: free and bound. Notably, the latter can only be obtained after preliminary alkaline hydrolysis. Their quantity depends on many exogenous and endogenous factors and can make up to 2 % of the fiber mass. The content of free lipids typically varies around 1 % (Tkachuk *et al.*, 2014).

As a result of our research on the total internal lipid content in sheep's wool (**Fig. 2**), we first identified their age-related characteristics. The amount of total lipids in lambs is significantly lower compared to that in ewes' wool. This applies to both their free (0.48 % vs. 0.66 %) and bound (0.90 % vs. 1.54 %) forms.

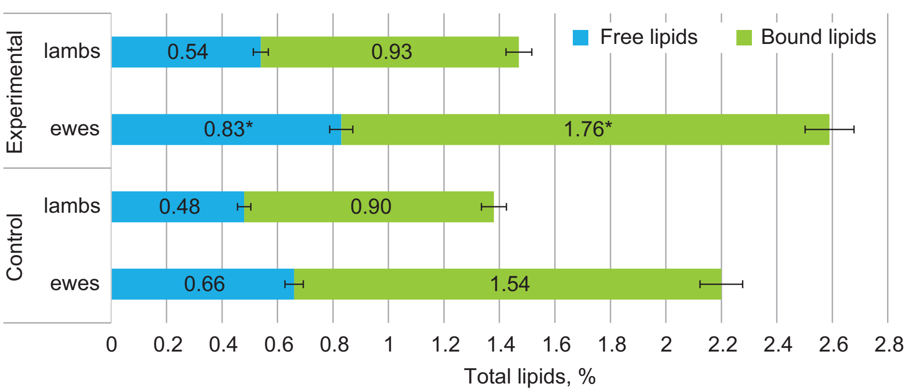

Fig. 2. The content of total internal lipids in the wool of ewes and lambs, (M ± SE, n = 5)
Note: here and in the future: * – the differences are significant between the control and experimental groups (* – P <0.05; ** – P <0.01; *** – P <0.001)

We also demonstrated that feeding ewes with the ELC lipid supplement leads to an increase in the fraction of free lipids (from 0.66 % to 0.83 %) and bound lipids (from 1.54% to 1.76 %) in their wool. Conversely, no such changes were observed in lambs, whose wool exhibited almost the same total lipid content in both the control and experimental groups.

Analysis of data on the qualitative composition of free and bound internal lipids of wool showed that there are significant differences between them (**Figs. 3** and **4**). In particular, the composition of the fraction of free lipids contains a much smaller amount of non-esterified fatty acids than in the bound fraction, and this is characteristic both of the wool of ewes and, especially, of lambs.

In addition, free lipids are quantitatively dominated by the non-esterified cholesterol fraction. In particular, its share accounts for more than 60 percent, and the esterified fraction accounts for only 8–14 %. In bound lipids, the fraction of esterified cholesterol in the wool of ewes accounts for about 40 percent, and non-esterified cholesterol accounts for about 25 percent. At the same time, in the wool of lambs, the ratio between cholesterol fractions is very close, but still the non-esterified cholesterol fraction predominates quantitatively. It is obvious that a significant amount of non-esterified cholesterol can indicate a higher level of oxidation processes, as a result of which the content of all forms of fatty acids is significantly reduced.

Under the conditions of our research, we were not able to clearly establish which classes increase the total lipid content in the wool of ewes and lambs of the research group. Only significant changes in the ratio between their separate fractions were recorded. In particular, from the data in **Figure 3**, it can be seen that in the wool of animals that were fed a water-soluble complex of fatty acids as part of the main diet, the content of non-esterified cholesterol in the composition of free internal lipids separated in system 1 probably decreases and its esterified fraction increases. At the same time, the amount of non-esterified fatty acids probably decreases in the wool of ewes of the experimental group. In covalently bound lipids, the tendency to the same changes as in free lipids is followed, but only a decrease in non-esterified cholesterol in lambs was significant. The nature of such changes indicates the slowing down of lipid oxidation processes under the influence of the dietary factors we have applied.

It is shown (**Fig. 4**) that in the wool of ewes and lambs, almost half of the free and bound lipids separated in system 2 are ceramides, and a quarter are sulfolipids. However, in the wool of lambs, the content of the latter is somewhat lower, compared to ewes, and does not exceed 20 percent.

Regarding the effect of ELC on the internal lipids of wool separated in system 2, the content of their main component – ceramides – significantly increases in the experimental group, and this applies to free and covalently bound lipids of both ewes and lambs obtained from them. And in the wool of ewes of the research group, in bound lipids, a significant increase in glucosyl ceramides was also noted. In this regard, it should be recalled that in the structural composition of hair, ceramides play an important role, taking part in the formation of intracellular lamellae, forming a paired bilayer (Coderch *et al.*, 2014).

There is some uncertainty regarding the significant increase in bound lipids, particularly cholesterol sulfate, in the wool of lambs from the experimental group. However, it should be emphasized that cholesterol sulfate plays a crucial role in the formation of hair monolithicity. It acts as an intercellular “cement” and serves as an amphipathic component, essential for constructing the double lipid layer (Strott & Higashi, 2003; Fandrei *et al.*, 2022).

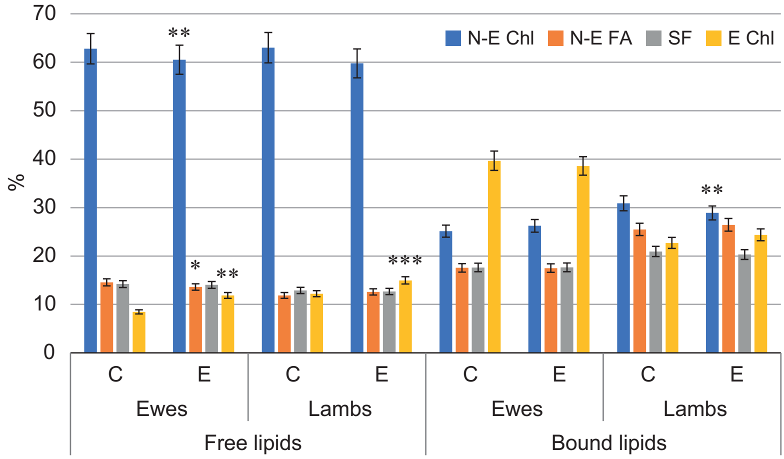

Fig. 3. The content of internal lipids in the wool of ewes and lambs, (M ± SE, n = 5)
Note: C – control, E – experiment. Separated in system 1 (N-E Chl – non-esterified cholesterol, N-E FA – non-esterified fatty acids, SF – sterol fraction, E Chl – esterified cholesterol)

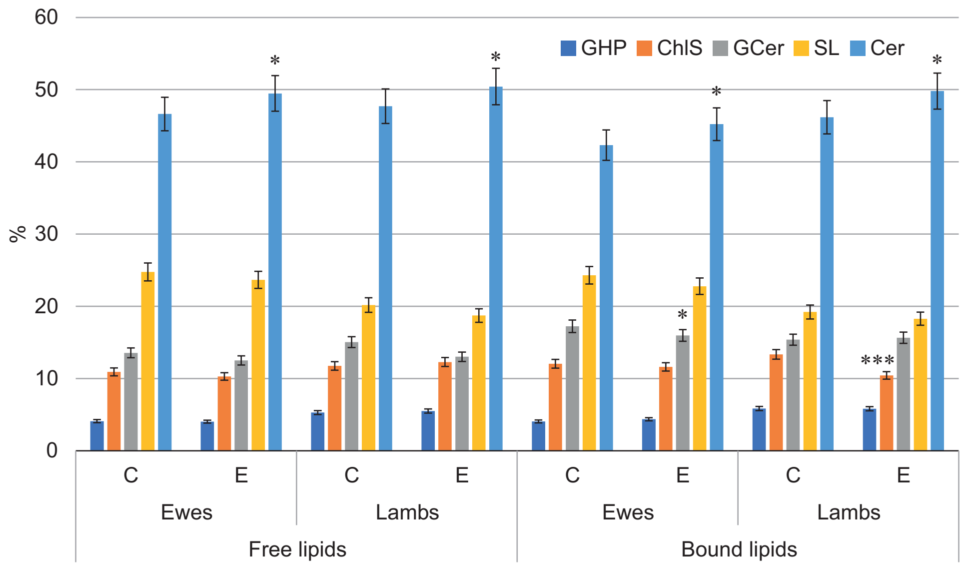

Fig. 4. The content of internal lipids in the wool of ewes and lambs, (M ± SE, n = 5)
Note: C – control, E – experiment. Separated in system 2 (GHP – Glycolipids of the highest polarity, ChlS – cholesterol sulfate, GCer – glucosylceramides, SL – sulfolipids, Cer – ceramides)

It is known that indicators of its strength depend on the content of internal lipids in the hair (Coderch *et al.*, 2017). Similar data were obtained under the conditions of our research. In particular, the increase in the amount of total internal lipids in the wool of ewes of the research group, and the changes that occur in their qualitative composition, ultimately lead to an increase in fiber strength from 7.38 to 8.03 cN/tex, which is 8.81 % higher compared to the control group (**Fig. 5**).

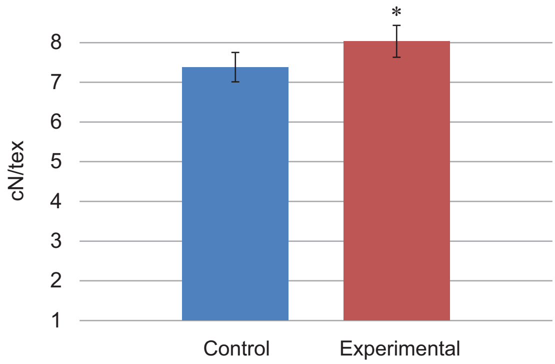

Fig. 5. The strength of the wool of ewes, (M ± SE, n = 5)

It is evident from the data obtained that the nutritional factors applied positively influence both the quantitative and qualitative composition of internal lipids in the wool of ewes and their lambs. This enhancement results in improved physical parameters of the wool, particularly an increase in strength, thereby elevating the overall quality and technological characteristics of the wool raw materials.

CONCLUSIONS

The introduction of a water-soluble complex of fatty acids into the diet of ewes contributes to an increase in their wool productivity by 28.8 %, and increases in the live weight of lambs obtained from them by 9.7 %. Feeding ELC lipid supplement to ewes leads to an increase in the content of bound (P <0.05) and free (P <0.05) lipids in their wool; and slowing of their oxidation processes, which is indicated by a significant decrease in the content of non-esterified fatty acids (P <0.05), non-esterified cholesterol (P <0.01) and an increase in its esterified fraction (P <0.01). In the composition of both free and bound internal lipids, the content of ceramides significantly increases (P <0.05), and this applies to both the wool of ewes and the lambs obtained from them. Changes in the quantitative and qualitative composition of internal lipids lead to an increase in wool strength by 8.8 % (P <0.05).

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

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

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ВНУТРІШНІ ЛІПІДИ ВОВНИ ОВЕЦЬ ЗА ВКЛЮЧЕННЯ ДО ЇХНЬОГО РАЦІОНУ ВОДОРОЗЧИННОГО КОМПЛЕКСУ ЖИРНИХ КИСЛОТ

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Обґрунтування. Ефективним способом підвищення продуктивності овець є використання в їхній годівлі жирів. З огляду на це, досліджено вплив водорозчинного ферментативного комплексу жирних кислот на внутрішні ліпіди вовни овець.

Матеріали та методи. Вівцематкам породи прекос дослідної групи до складу комбікорму включено 3 % водорозчинного ферментативного комплексу жирних кислот – essential lipid complex (ELC): лінолевої – 54,5 %, олеїнової – 24 %, пальмітинової – 10 %, арахідонової – 6 %, стеаринової – 4 % та ліноленової – 1,5 %. Вовнову продуктивність обліковували на площі шкіри 36 см^2, прирости живої маси ягнят – зважуванням під час народження та на 21-й день. Вільні внутрішні ліпіди виділяли за допомогою екстракції в апараті Сокслетта, а зв'язані – після попереднього лужного гідролізу. Міцність вовни визначали на апараті ДШ-3М.

Результати. Емульсія жирних кислот сприяє інтенсифікації процесів вовно- і молокоутворення, на що вказує зростання приростів вовни у вівцематок на 28,8 %, та живої маси ягнят – на 9,7 %.

У вовні дослідних вівцематок збільшується загальна кількість як вільних (P <0,05), так і зв'язаних внутрішніх ліпідів (P <0,05). У складі вільних ліпідів вірогідно знижується вміст неестерифікованих жирних кислот (P <0,05), неестерифікованого холестеролу (P <0,05) та зростає його естерифікована фракція (P <0,01). У ковалентно зв'язаних ліпідах відмічено таку ж тенденцію, однак вірогідним є лише зниження неестерифікованого холестеролу у ягнят. Такі зміни вказують на сповільнення процесів окиснення ліпідів під дією застосованих нами аліментарних чинників.

У складі і вільних, і зв'язаних внутрішніх ліпідів вірогідно зростає вміст церамідів (P <0,05), причому це стосується як вовни вівцематок, так і отриманих від них ягнят. У зв'язаних ліпідах вівцематок відмічено вірогідне зростання глюкозил церамідів (P <0,05), а у ягнят – холестерол сульфату (P <0,001).

Зміни у кількісному та якісному складі внутрішніх ліпідів призводять до зростання міцності вовни з 7,38 до 8,03 сН/текс, тобто на 8,8 % (P <0,05).

Висновки. Згодовування вівцематкам емульсії жирних кислот забезпечує підвищення продуктивності й позитивно впливає як на кількісний, так і на якісний склад внутрішніх ліпідів вовни вівцематок і їхніх ягнят.

Ключові слова: вовна, емульсія, прирости, внутрішні ліпіди, цераміди, міцність

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