

Original Research Paper

Effect of Protein Feed Additives on Skin and Fur Quality of American Mink

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Abstract: The purpose of the research is to study the effect of hydrolysates on the skin and fur quality. To assess morphological parameters of skin, as well as the commercial characteristics of the raw materials obtained, we formed three groups (one control and two experimental) of 3-month-old males (25 animals in each group) and females (50 animals in each group). Control group minks received the main conditioning diet. For four months, the minks from Experimental Group I received 2 mL/day of hydrolysate in addition to the main diet and the minks from Experimental Group II received 4 mL/day of the additive, right up to the moment of the scheduled on-farm euthanasia (after 7 months). All the experimental animals received protein hydrolysate in addition to the main diet according to the feeding standards adopted on the farm and in accordance with the feeding recommendations, taking into account the amino acid composition of the diet. It was established that the use of the protein feed additive-hydrolysate (an effective dose is 4 mL/day) has a positive effect on the commercial characteristics of mink pelts. Hydrolysate increases pelt size and area, skin thickness due to its papillary layer, increases fur length, density and strength; enlarges bundles of more numerous underfur hairs and makes hairs longer but not coarse. The impact of the feed additive on the skin glandular apparatus, as well as on the epidermis thickness has not been proven. The performed tests demonstrated that all the size and quality characteristics of air-dried pelts from the experimental animals are better than those from the control ones. The largest average pelt area was observed in Experimental Group I and the highest percentage of extra-large pelts was observed in Experimental Group II. It was found that Group II had the highest percentage of both extra-large and defect-free pelts, although, in practice (without feed additives) these two indicators are inversely related. Processing and analysis of the data allowed us to evaluate the combined indicator of pelt quality-in males from Experimental Groups I and II the quality score significantly exceeds that one in the control group by 11 and 26%, respectively; and in females - by 22 and 24%, respectively.

Keywords: American Mink, Feed Additive, Hydrolysate, Skin and Fur, Histology, SEM

Introduction

Nowadays many people oppose fur farming, however, it should be noted that this factor does not affect a dynamic growth of the fur animal population. This industry is one of the most effective sectors of animal husbandry, this industry is closely related with the other

ones, processing included. The mink is one of the most farmed fur-bearing animals. This fact is explained by the high demand for its furs (Ludwiczak and Stanisz, 2019). Denmark is one of the leading producers of mink pelts. In the Russian Federation production of mink pelts decreased by 40% (Balakirev, 2021; Khusainova and Vorozheykina, 2019). The increase in the number of

minks and the number of produced pelts resulted in overproduction and, subsequently, led to the industry crisis caused by the drop in fur prices (FCUSA, 2018). The American cage-bred mink is used as a major source of raw furs in modern fur farming (Pereverzeva, 1982) and global fur trade (Thirstrup *et al.*, 2017).

Fur quality and pelt size are the main productivity criteria of fur-bearing animals. The fur price depends on these criteria and in its turn, the price accounts for the efficiency of the whole production sector (Jensen *et al.*, 2017).

Feed composition and its quality are the most important factors having an impact on the fur quality, as well as the growth, development and reproductive characteristics of minks (Lyhs *et al.*, 2019; Kvarnikova, 2017).

In comparison with other areas of animal husbandry, fur farming in cages is the youngest sector, though technologically advanced (Lyhs *et al.*, 2019).

Fur farming in cages has some specific features associated with the peculiarities of the species. Regardless of domestication, the animals kept in cages for fur farming purposes have not lost their predatory instincts. One of the key and urgent tasks of fur farming and veterinary medicine is to improve the diet of minks so that they could use their full genetic potential for production of high-quality fur, as well as to modernize the ways of correcting metabolic processes in these animals in case they get an unbalanced diet (Kvarnikova, 2017; Kvarnikova and Kharlamov, 2013).

In 2020, a mutation of the SARS-CoV-2 coronavirus was detected in minks in Denmark, the Netherlands, Spain, Sweden, Italy and the United States. The mutant is transmitted to humans and a decision was made to cull all the minks in these countries (Konishi, 2021; Mossburg and Ries, 2020). In the future, it will be necessary to take effective measures (including improved feeding) to restore the breeding stock of these valuable fur-bearing animals. In Russia, there are 22 mink farms in 14 regions with a breeding stock of about 300 thousand animals and all the fur farms are operating now behind the closed doors.

The feed additives based on hydrolysates of animal origin are known to be highly effective (Bachinskaya, 2019). In particular, such animal products as protein hydrolysates are of great practical interest, because they have good functional properties; are produced from cost-saving raw materials and are used in veterinary medicine as a source of free amino acids (Yongqing *et al.*, 2017; Etemadian *et al.*, 2021).

At the same time, the effect of hydrolysates on the skin and fur quality has not been sufficiently studied, especially with regard to skin and fur microstructures, including one of the American minks. The goal of our research is to fill this gap.

Materials and Methods

To assess morphological parameters of skin, as well as the commercial characteristics of the raw materials obtained, we formed three groups (one control and two experimental) of 3-month-old males (25 animals in each group) and females (50 animals in each group). Control minks received the main conditioning diet. For four months, the experimental minks from Group I received 2 mL/day of hydrolysate in addition to the main diet and minks from Group II received 4 mL/day of the additive, right up to the moment of the scheduled on-farm euthanasia (after 8 months). All the experimental animals received protein hydrolysate according to the feeding standards adopted on the farm and in accordance with the feeding recommendations, taking into account the amino acid composition of the diet (Balakirev and Kladovshnikov, 2007; GOST, 2013). In order to determine commercial characteristics of the raw materials obtained, the pelts from control and experimental minks were sorted into corresponding consignments according to the GOST requirements (GOST, 2013).

We used a standard histological method to obtain paraffin sections with subsequent hematoxylin-eosin staining (Merkulov, 1969; Kiernan, 1999) in order to study chest skin samples from six minks in the compared groups (fixed in 10% neutral formalin). The obtained preparations were analyzed in a Zeiss Axio Vert A1 light microscope. Micrographs were taken using high-resolution AxiocamICc 5 color cameras in the Axiocam Zen 2 lite software at magnifications of $\times 100$ and $\times 400$.

We performed Scanning Electron Microscopy (SEM) of the chest and abdomen skin hairs from ten control and experimental animals in the compared groups using microscopes JSM 840 A (Japan) and TESCAN (Czech Republic) according to the standard method (Sokolov *et al.*, 1988; Dyulger *et al.*, 2020). The hairs were sorted into categories and orders under a binocular magnifier and after their thickness was measured in light-optical microscope Amplival (VEB Carl Zeiss Jena), as well as in Leica DMLS (Germany) with a digital video camera using a $\times 10$ eyepiece and $\times 10$, $\times 40$ and $\times 63$ objectives. Micrographs and electronograms were edited using software Axio Vision v. 3.0 (Carl Zeiss), Photoshop CS6, Adobe Photo shop Elements 11, improving only contrast and brightness of the image and the shot composition. Morphometry was performed in the TescanAtlas software Statistica 10 (USA) was used for statistical analysis of morphometric indicators. The following indicators were taken: $M \pm m$ -arithmetic mean with the error of the arithmetic mean; σ - standard deviation; p - Student's t-test. The following values were taken as

relative indicators: H/D-the ratio of the cuticle scale height to the hair shaft thickness at its location, in the narrowed base and in the maximally expanded part (granna) of the shaft; d/D-the ratio of smaller and larger diameters of the shaft cross sections; Me/D×100% - the ratio of the medulla thickness to the hair thickness. The number of measurements is 10 for each sample.

Results of the Research Discussion

Commercial Quality of Pelts

Commercial quality of the produced. Pelts is the main practical result of any research into the fur farming; commercial quality refers to those useful characteristics of pelts that contribute to their quality and value. Commercial quality is a combination of skin and fur characteristics, as well as of general pelt characteristics and includes such indicators as the pelt area, its mass, thermal conductivity and strength (Ludwiczak and Stanisz, 2019; Mossburg and Ries, 2020). According to the data received, all the size and quality characteristics of air-dried pelts from the experimental animals are better than from the control ones (Table 1).

The largest average pelt area was observed in Experimental Group I and the highest percentage of extra-large pelts was observed in Experimental Group II. It suggests that the pelts from males in Group II were longer than in Group I and in the Control Group, which is a significant advantage when fur sewing patterns are cut out. It is noteworthy that Group II has the highest percentage of both extra-large and defect-free pelts, although, in practice (without feed additives) these two indicators are inversely related.

Processing and analysis of the data allowed us to evaluate the combined indicator of pelt quality- in males from Experimental Groups I and II, the quality score significantly exceeds that one in the control group, (by 11 and 26%, respectively); and in females - by 22 and 24%, respectively.

Mink Hairs

Our data on the mink hairs are quite consistent with those published. The fur consists of guide hairs, several categories of guard hairs, intermediate hairs and underfur hairs growing in bunches (Fig. 1a, 6). Thickness of the guide hairs of the studied animals slightly exceeds the limits known for this species-133 μM for the winter mink and 101.9±14.46 μM for the winter belly fur (Table 2). Thus, thickness of guide hairs ranges from 113 to 136 μM in control animals and from 113 to 158 μM in experimental animals, i.e., there is a slight trend towards thicker hair shafts in experimental animals, although we did not find statistically significant differences ($p = 0.582$). The same is observed in medulla development. In control animals, the medulla

thickness accounts for 46-82% of the hair shaft thickness and for 42-90% ($p = 0.924$) in experimental animals.

Statistically the guide hairs are significantly longer in the experimental animals ($p = 0.04$; $n = 100$) (Table 1). In control animals, it ranges from 7 to 21 mM and in experimental animals from 8 to 22 mM.

The microstructure of all hair categories is different, especially in guide hairs and underfur. Moreover, the cuticle scale pattern of the former differs at the base end of the hair (ring-shaped and semi-ring-shaped), in the area above the base end of the shaft (spear-like), in front of the most expanded part of the shaft-granna (cone-like) and in-granna (ring-shaped and semi-ring-shaped, flattened) (Fig. 16, г, д). The number of rows of spear-like scales ranges from six to seven across the shaft. The cuticle scale pattern of underfur is also spear-like, the scales along the shaft are highly elongated and the shaft is surrounded with three of them (Fig. 16, inset).

The comparison of the cuticle index shows significant differences between different parts of the shaft, that is variations in the cuticle pattern along the shaft (Table 2). Comparison of this indicator in control and experimental minks revealed a statistically reliable fact that ($p = 0.003$) the height of the spear-like scales on the chest hairs is greater in the experimental animals (in the area above the base of the shaft) due to the elongation of the spear-like scales of the cuticle (up to 60.8 μM and 66.2 μM, respectively); and the height of the scales in granna is less ($p = 0.04$) in experimental minks (up to 8.4 μM and 7.0 μm, respectively). The other microstructural characteristics of hairs in the control and experimental groups (morphometry, the form of shaft diameter at the base and in granna, mesh medulla, cortical layer) (Fig. 1r) are quite similar in the compared animals.

Skin Microstructure

Basic characteristics of mink chest skin are quite similar to those of other Mustelinae representatives. The chest skin is thick, non-pigmented, slightly wrinkled, with a thin no pigmented epidermis and a well-developed stratum corneum. The papillary layer is the skin basis and it differs little from the reticular layer by thickness and orientation of the collagen fiber bundles, most of which meander along the surface of the skin. The border between the papillary and reticular layer goes at the level of the hair follicles. Hair-lifting muscles are absent. Sebaceous glands are sac-shaped, well developed and unpigmented. Sweat glands are sausage shaped, rare. Hair bundles consist of a large guard hair and quite numerous underfur hairs, which come out of one hair sheath in the upper parts of the skin. Fat cells are rare in the reticular layer. The comparative analysis

showed that thickness of the skin and its layers differs in control and experimental minks. Epidermis in the control animals is more flattened and smoothed than in the experimental minks (Fig. 2a, б), although significant differences in its thickness have not been found (Table 2).

It can be observed on the longitudinal sections that there is an increase in the absolute and relative skin thickness due to its papillary layer, which is significantly thicker in experimental animals, mainly because hair follicles are

embedded deeper (Table 3, Fig. 2б, r). The sebaceous glands are well developed, holocrine, have a rounded shape and each hair bundle has one or two of them. Their sizes do not significantly differ in the compared mink groups (Table 3). Experimental minks have more hair bundles, which are thicker and cover a larger area. In addition, this group has significantly more underfur hair follicles in the bundle than the control group, however, the sizes of the follicles are similar (Table 3).

Table 1: Morphometry and commercial quality of pelts from American mink males and females

Indicator	Males			Females		
	Experiment I (2 mL/day of hydrolysate)	Experiment II (4 mL/day of hydrolysate)		Control	Experiment I (2 mL/day of hydrolysate)	Experiment II (4 mL/day of hydrolysate)
Number of pelts, items.	20	20	20	20	20	20
Pelt area, dm ²	11.12	11.59	11.22	8.57	8.98	9.20
Extra large pelts, %	70.0	80.0	90.0	10.0	25.0	30.0
Defect-free pelts, %	35.0	40.0	55.0	13.0	15.0	17.0
Quality score, %	108.0±10.0	119.0±8.0	134.0±9.0	88.0±11.0	110.0±7.0	112.0±11.0

Table 2: Morphometry of the chest and belly guide hairs of the studied American mink males

Indicator	Studied animals			
	Control (N* = 5)		Experimental animals (N = 5)	
Thickness (D):	M ± m	σ	M ± m	σ
Guide hair, n** = 33, μm	135.6±3.7	21.2	133.3±2.2	14.0
Medulla				
Me/D, %, M ± m; σ	63.1±1.6	9.1	62.9±2.3	12.2
Guide hair length, n = 100, mm	12.4±0.3	3.0	13.3±0.3	3.3
Cuticle Index:				
guide hair above				
the base of the shaft, n = 10	1.05±0.04	0.09	1.2±0.2	0.35
The same before granna, n = 10	0.32±0.03	0.07	0.32±0.05	0.1
same in granna, n = 10	0.19±0.1	0.21	0.07±0.01	0.02

*N - the number of studied animals; **n - the number of measurements

Table 3: Morphometry of chest skin microstructures in American mink adult males

Indicator	Control animals		Experimental animals		t-test* p
	M ± m	σ	M ± m	σ	
Thickness:					
skin, μm	749.0±12.6	14.5	993.6±51.6	44.3	0.001
epidermis, μm	13.2±3.9	3.4	10.3± 2.7	2.6	0.20
papillary layer:					
μm	433.6±15.1	33.7	748.8±21.8	48.7	0.001
% Of skin thickness	50.8	—	76.3	—	—
Area of the sebaceous gland, μm ²	3607.5±408.0	204.0	3502.4±358.4	255.8	0.92
Depth of the sebaceous gland, μm	203.0±24.0	17.0	345.6±114.5	93.0	0.08
Area of the underfur follicle bundle, mm ²	5865.0±2262.0	1146.1	11430.7±713.3	1008.8	0.04
Number of underfur hairs in a bundle.	18.5±1.5	3.9	24.7±1.9	4.6	0.03
Underfur follicle area, mm ²	339.0±113.1	113.0	601.7±183.1	150.0	0.32

*statistically significant values are given in bold

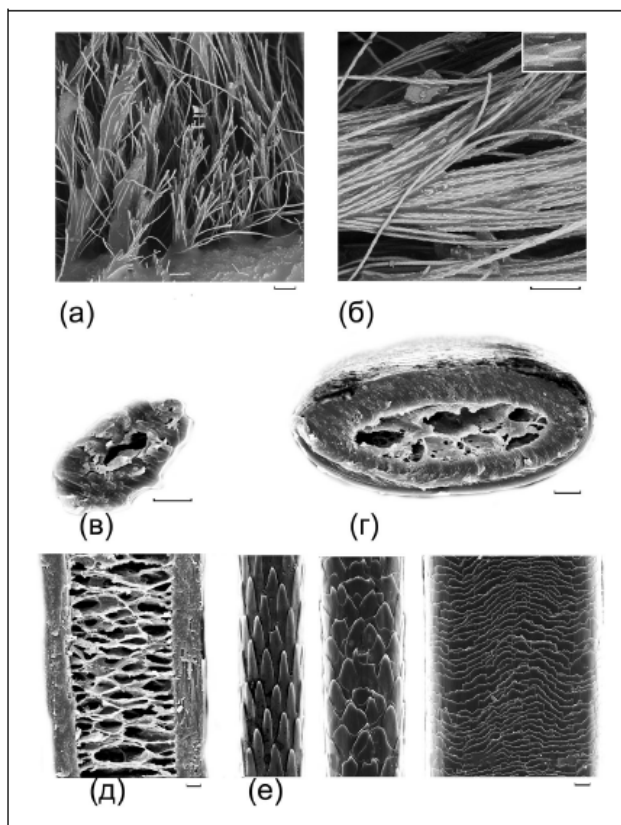


Fig. 1: Microstructure of the chest guide hairs from an American mink adult male. (a) –general view of the guard hairs and underfur bundles. (б), inset-spear-like cuticle of underfur. (в) – a cross section of the guide hair at the base of the shaft. (г) – the same in the granna; (д) –medulla on a longitudinal cut of the granna. (e) – cuticle pattern from the base of the shaft to its granna (from left to right). SEM. Scale: а, б –100 μm; в, г, д, е – 10 μm

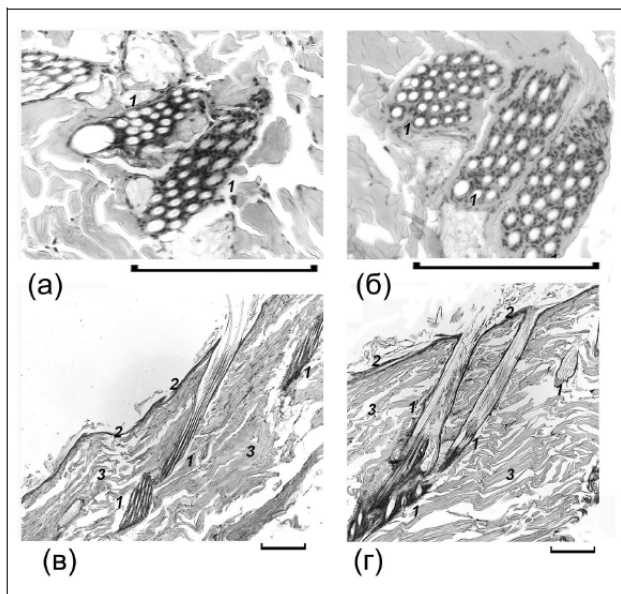


Fig. 2: Comparative morphology of hair bundles in the chest skin from control (а, б) and experimental American mink adult males (в, г). а, б– oblique section. в, г – longitudinal section. 1–underfur bundles, 2–epidermis, 3–dermis. Photomicrograph. Hematoxylin-eosin, magnification а, б–×400, в, г–×100. Scale 200 μm

Conclusion

Thus, our research has shown that the use of hydrolysate (a protein feed additive) has a positive effect:

- On the commercial characteristics of mink pelts, because the animals consume additional amino acids
- On the size of mink pelts and the quality of furs
- On the thickness of the papillary layer and on deeper embedment of hair bundles
- On the enlargement of hair bundles and on an increase in the number of underfur hairs in each bundle, i.e., it contributes to fur density and strength
- On the hair length without coarsening them

The dose of hydrolysate equal to 4-mL/day is more effective. At the same time, we were unable to identify the effect of the feed additive on development of the skin glandular apparatus and on the epidermis thickness, which is probably related to endocrine regulation of their activity.

The results that we obtained show how commercial characteristics of the breeding stock of American minks can be rapidly restored in case of unforeseen losses of these animals, including losses caused by coronavirus.

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Author's Contributions

Pavel Nikolayevich Abramov: Responsible executor, 20%.

Olga Fedorovna Chernova: Responsible executor for laboratory research, 20%.

Nikolai Aleksandrovich Balakirev: head of research, 20%.

Seidfatima Mirovna Borunova: Responsible executor, analysis of results, 20%

Dastanbek Asylbekovich Baimukanov: the author of the idea, the head of the event, generalization, preparation of the manuscript, 20%.

Ethics

All the principles of scientific ethics have been observed during the research work, there is no conflict of interest.

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