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IMPROVEMENT OF FERRET FUR PELT QUALITY BY USING PRODUCTS OF RECYCLING RAW MATERIALS OF ANIMAL ORIGIN

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Abstract

The waste materials of manufacture of basic animal products are adverse anthropogenic factors affecting environment and, at the same time, they are a source of valuable biologically active substances which can be used. We developed a protocol for complex application of biologically active recycling products, collagen and keratin, obtained by original patented technologies from recycled waste materials of leather and textile industries in combination with melatonin (original modified preparation) for using in ferret breeding. The preparation Melacoll is a stabilized complex of melatonin and collagen. Melacoll has a prolonged effect (up to 4 months) and the toxicity class 4. The keratin-containing substance quality (more than 95 % keratin) makes it possible to use this additive as a protein supplement to fodder. In combined application of Melacoll and keratin, we observed the uniform and statistically reliable weight gain of animals during 4 months exceeding the indices obtained for both small and large doses of keratin (from about 0.59 kg to 2.63±0.13 kg, $t_{obs} > t_{table}$). By month 5 this index decreased which showed completion of animal development and achieving the slaughter weight one month earlier than the standard slaughter terms. Higher concentrations of implanted Melacoll promoted the increase in pelt size. In farm trials, application of Melacoll and keratine led to a reliable 12 % increase in pelt size as compared to the control. The pelt lifetime defects (e.g. broken guard hair) in Melacoll-implanted ferrets was the greatest (37.5 %) whereas in the animals fed with keratin as the fodder additive these defects were the smallest (12.5 %) due to the standard diet enriched with sulphur-containing amino-acids which are responsible for hair strength. Complex use of keratin and Melacoll reliably increased hair density while Melacoll itself did not exert any marked effect on this trait. Optimal concentration of the proposed keratin-containing supplement to the ration in raising slaughter ferret youngsters is 0.6 %, and that of Melacoll is 1 ml of 6 mg/ml substance.

Keywords: fur animal breeding, raising slaughter ferret youngsters, animal raw materials, wastes, recycling, keratin, collagen, melatonin, pelt quality

Ggreen economy aims at protecting the environment, developing an environmentally friendly industry and increasing resources through innovative non-waste technologies for processing raw materials with the maximum balance of valuable components in the products [1]. Secondary raw materials of animal origin are, on the one hand, rather powerful factor of negative anthropogenic environmental impact, on the other — a reserve of valuable biologically active substances that can be used to create various biolgicals for different purposes [2-5]. Keratin, a sulfur containing protein unique in its composition and properties can be extracted from wool and feather recycling waste [6, 7], untanned leather waste products may be a source of collagen [8, 9], and wastes of meat processing industry may serve as raw material for melatonin. a hypophysis hormone. These products are used in the pharmaceutical and cosmetic industries [10-13]. Keratin is a hair-treating agent for certain diseases of human hair [14], is used as a kera-

tin-based wound dressing with curative effect [15], and it is also studied in connection with the application in other areas of biology and medicine [16-19]. In Russia, the use of enzymatic hydrolysis of keratin as a fodder additive for fur animals has been suggested [20]. Foreign studies of melatonin, especially its pharmaceutical forms having a prolonged effect, are mainly associated with medical uses (effects on biorhythms, treatment of sleep disorders) [21, 22]. In Russia, melatonin preparations with prolonged effect for acceleration of fur animal maturation have been developed (RF patents Nos. 2040897, 2096044, 2122787) and are widely used.

Application of products obtained by processing secondary protein-containing raw material can be expanded. For example, in fur farming, it remains relevant to search for preparation that can positively affect the quality of down-and-fur raw materials and semi-finished products. The most attractive formulations are based on natural substrates, combining biological activity and relatively low cost.

We proposed a scheme for the complex usage of biologically active products, collagen and keratin, obtained by innovative original patented technologies based on recycling leather and textile waste, in combination with melatonin (the original modified preparation) and showed that their using in the fur-farming improved both general animal condition and pelt quality.

Our subjective was to estimate effects of products of processing secondary protein-containing waste on fur and pelt quality in ferret youngsters.

Techniques. Collagen was extracted from hide split (the recyclable waste basically not used in lather industry) according to the description (RF Patent No. 2129805). Solubilized keratin was extracted from sheep wool, the recyclable waste of wool processing in the textile industry, by alternating alkaline and acid treatments to purify fibrillary keratin from the accompanying substances followed by its dissolving in a slightly alkalescent medium (RF patent No. 2092072). Collagen served as a fodder additive.

Melatonin (a pituitary hormone, the product of the processed meat waste, code 931684 according to the All-Russian Classifier of Products, a group of intermediates products of synthetic medicines) with prolonged effect was obtained by the original method based on the commercial preparation Melatonin Powder (N-Acetyl-5-Methoxytryptamine) (Shanxi Sangherb BioTech Inc., China). The obtained collagen was a stabilizing agent. Melatonin (6 mg/ml) was immobilized on collagen via incorporation into collagen matrix hydrogel by physico-chemical adsorption [23, 24]. Castor oil (N.A. Semashko Moschempharm, Russia), polyvinyl acetate (PVA) (TEX, Russia), polyvinyl alcohol (PVA) BC-05 (Liwei Chemical Co. Ltd, China), Kuraray Poval® 18-88 (Kuraray Co. Ltd, Japan), as well as a mixture of PVA BC-05 and Kuraray Poval 18-88 in the ratio 1:1 were used as organic copolymers that promote the gradual substance dissolving in the animal's body with a dosed release of the active component. Collagen and melatonin without binding agent was a control. Stability of melatonin-collagen complex, named conventionally as Melakoll, was estimated by the melatonin amount which diffused through the semipermeable membrane for dialysis against the blood-substituting solution (BSS, Russian Research Institute of Hematology and Transfusiology, St. Petersburg). Optical density of the dialysate, which contain diffused melatonin, was measured by a photocolorimeter UFC-2 (Zagorsk Optical and Mechanical Plant, Russia) in the range of λ = 315-980 nm (optical path length 5 mm), in which the working wave length was determined for better monitoring of the melatonin output from complex. Melatonin levels in the dialysate were measured by the calibration graphs. Acute toxicity of the Melakoll complex was assessed on white mongrel mice (males, n = 5,

in control group m = 16.7±0.8 g, in test group m = 16.9±0.9 g) in accordance with State Standard 12.1.007-76 "Harmful substances. Classification and general safety requirements" and the requirements set out in State Standard 32296-2013 "Methods of chemical products testing on the human body. The basic requirements of the assessment acute toxicity test, while intragastric intake by the fixed dose method". The experiments were carried out in accordance with the of the Geneva Convention protocols and the principles of proper laboratory practice (National Standard of the Russian Federation State Standard R 53434-2009), and also according to the recommendations of The Guide for the Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996)

For farm test (carried out in Russian Sable Enterprise, Moscow region), 104 males of black ferret (*Mustela putorius*) youngster of cage housing, age 2.5 months at the beginning of the experiment, were divided into groups (8 animals each). At the beginning of the experiment, 1 ml of Melakoll, as an accelerator of vital biorhythms, were subcutaneously injected to ferrets from groups I-V into the **nape** of the neck. In groups I, II, III, IV and V, the drug concentration was 3; 6; 9; 12 and 15 mg/ml, respectively. Group VI was a control where animals were injected with PVA in combination with collagen, that is, with an immobilization matrix and a linking agent. Throughout the experiment, groups I-VI, as well as group VII (control animals that did not receive either Melakoll or keratin) were fed according to the norms adopted in the farm. Animals of groups VIII-XIII, in addition to basic diet, received keratin for 5 days with 5-day intervals during the whole experiment to exclude overfeeding because of high (95 %) keratin content in the feed additive [25-28]. Keratin supplement dosages in groups VIII, IX, X, XI, XII and XIII were 0.2; 0.4; 0.6; 0.6 (average for the remaining experienced groups); 0.8 and 1 % of feed per animal, respectively. Additionally, at the beginning of the experiment, the ferrets of group XI were injected with 1 ml of Melacoll, the melatonin concentration of 6 mg/ml selected based on the RF patents Nos. 2040897, 2096044 and 2219910.

During the monitoring period, the animals were weighed individually with an accuracy of 0.01 kg. At the end of the experiment, the ferrets were slaughtered. Pelts have been subjected to the primary processing (skinning, fleshing, correction and conserving). The pelts were removed using a cut along the rump, with the head fur, paws and tail remained. The pelts were cleaned of the meat prunes, of bones from paws and tail, of cartilages from the ears, and of tendons, then degreased without damaging the hair roots, corrected the hair outward, with longitudinal stretching (without overstretching) (State Standard 11146-65 "Undressed leather of the white and black ferret. Technical specifications"), and air-dried.

The pelts were sorted in accordance with the requirements of State Standard 11146-65 "Unprocessed leather of white and black ferret. Technical specifications", with regard to grade, size, defects, color. The grade was determined organoleptically by totality hair commercial features (puffiness, gloss, density, length and softness, fur maturity) and skin appearance. For determining pelt size, the length from the interpupillary line to the tailhead and the width in the pelt middle were measured, the obtained values were multiplied and the size was assigned as large, medium or small (State Standard 11146-65). Defect-free pelts were either without or with defects which did not significantly change the quality. If the defects exceeded the area of tolerance, the pelts were referred to a group with a small, medium or large defect.

Rump hair density was determined by direct counting hair roots at horizontal sections of 1 cm^2 pelt area or per microscope field of view (mm^2). In direct calculation, a piece (1 cm^2) was cut out, then, fixing pelage with a thread to

form a beam. Guard, awn and then downy hairs were selected from the beam with tweezers; guard and awn hairs were counted apiece, downy hairs were counted by hundreds. An average for 3 samples was calculated. Microscopic histologic section were prepared according to the standard method (S.A. Kasparians et al., Methodological Recommendations for Determining Quality Indicators for Tanning and Fur-and-Meat Raw Materials. Moscow, 1986, in Russ.), stained with hematoxylin and eosin, fixed with Canadian balsam under covered glass and viewed at a 10-fold magnification (a microscope Micromed Eureka 40x-1280x with a digital ocular (OOO Observational devices, St. Petersburg). Micrometric ruler scale division was 8.4 μm, the field of view was 0.7 mm^2. The arithmetical mean was determined for the number of hair follicles in 10 fields of view of the microscope with recalculation for 1 cm^2.

During statistical processing, the arithmetical mean (*M*) and standard error of the mean (±SEM) were calculated. The statistical significance of the mean differences was assessed by the Student's *t*-test [29-31].

Results. The quality of products in fur farming depends on the balanced and biologically proper value of feeds and biostimulators. The collagen-stabilized form of melatonin with a prolonged effect was supposed to be used as biorhythms regulator to accelerate animal growth while solubilized keratin should serve as a protein fodder additive to stimulate dense pelage formation.

The collagen hydrogel, used as the basis for the melatonin preparation, had neutral pH 6.5-7.0 and averaged 1.3 % of mass fraction of solid substances. The counts of microorganisms in the substance did not exceed the values allowed by Sanitary Regulations and Standards 2.3.2.1293-03 "Hygienic requirements for the use of food additives" (approved by the Chief State Sanitary Physician of the Russian Federation, April 18, 2003, http://docs.cntd.ru/document/901862338). Concentration of immobilized melatonin (6 mg/ml) corresponded to that of preparations which accelerate pelage maturation (RF patents Nos. 204897, 2096044, 2122787).

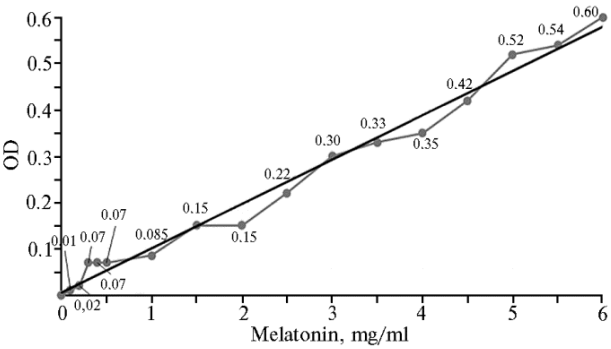

Fig. 1. Calibration graphs of optical density (OD) **depending on melatonin concentration** (λ = 315 nm).

The comparison of calibration graphs for melatonin solutions in BSS (data not given) showed that it is possible to reliably estimate melatonin concentration at 315 nm (Fig. 1).

The greatest stability showed sample No. 6, conventionally named Melacoll, from which 13.3 % of the initial amount of melatonin was released during the observation. A decisive role in this was played by copolymers, a mixture of two polyvinyl alcohols (Table 1).

1. Dynamics of melatonin releasing from the stabilized collagen complexes depending on copolymers

Composition	Days					
	10		20		30	
	melatonin concentration in dialysate					
	mg *M*±SEM)	%	mg (*M*±SEM)	%	mg (*M*±SEM)	%
1. Collagen + melatonin (CM)	0.40±0.01	6.7	1.00±0.01	16	1.50±0.01	25.0
2. CM + castor oil	0.25±0.01	4.2	1.00±0.01	16	1.20±0.01	20.0
3. CM + PVA	0.20±0.01	3.3	0.50±0.01	8.3	1.00±0.01	16.0

Continued Table 1

4. CM + PVOH VA-05	0.30±0.01	5.0	0.50±0.01	8.3	0.90±0.01	15.0
5. CM + PVOH Kuraray Poval® 18-88	0.40±0.01	6.7	0.60±0.01	10.0	0.90±0.01	15.0
6. CM +PVA mixture	0.20±0.01	3.3	0.50±0.01	8.3	0.80±0.01	13.3
7. Collagen + PVOH mixture	0.00	0	0	0	0	0

N o t e. PVA — polyvinyl acetate, PVOH — polyvinyl alcohol.

2. Average weigh (g) **of white mice organs in testing acute toxicity of Melacoll by a fixed dose method** (M±SEM, n = 5)

Organ	Control	Test
Heart	0.178±0.004	0.179±0.005
Lungs	0.276±0.013	0.274±0.012
Liver	1.899±0.060	1.910±0.061
Kidney	0.502±0.020	0.509±0.022
Spleen	0.240±0.013	0.243±0.014

Four hours after the laboratory mice received intragastric Metacoll, their general condition (i.e. a decrease in activity, lack of appetite, a need for water) indicated stress but pathologies did not develop. In 24 hours, the activity of the mice became normal, the appetite returned completely. On days 7 and 14 and hereafter, the animals were active, ate feed completely, no deviations in their condition were noted. No one mouse died during the experiment. At 2.5 months after the experiment beginning, the animals were slaughtered for pathological examination (Table 2). However, the difference in organ weight in the control without Melacoll and in the test with Melacoll was insignificant: t_{obs} = 0.01; t_{table} = 2.3 (i.e., $t_{obs} < t_{table}$, P > 0.05). These results indicate that Metacoll has no pronounced toxic effect on animals and can be attributed to substances of the toxicity class 4 in accordance with State Standard 12.1.007-76 "Harmful substances. Classification and general safety requirements".

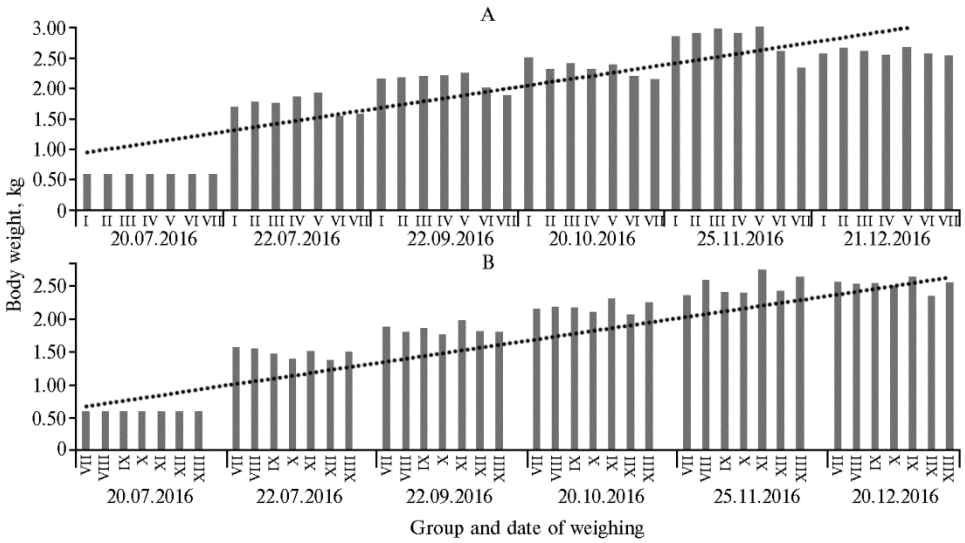

Fig. 2. Dynamics of weight gain of black ferret (*Mustela putorius*) received Mellacol injection (A) **and dietary keratin additive** (B) (a farm trial, Russian Sable Enterprise, Moscow Province, 2016). For description of groups and preparations used, see the section «Techniques». Controls: group VI (injection of mixture of collagen and PVA) and group VII (animals which did not receive either Melacoll or keratin). Dotted lines reflect an increment trend.

A characteristic feature of keratin composition is a high proportion of sulfur-containing amino acids cystine and cysteine which are extremely important for pelage formation in fur farm animals, especially in growing youngsters. The denser fur, the higher the animal's need for dietary sulfur-containing amino acids [28, 32, 33]. So, intact keratin should favorably affect fur formation. However, it should be noted that the level of digestible protein in the diet of fur animals is 8-11 g per 100 kcal [27, 32]. This amount avoids the toxic effects of ni-

trogen-containing products of protein decomposition [32]. At the same time, the lack of digestible protein decreases the hair tolerance to damaging, as a rule, mechanical, effects [32].

The resulting product was a homogenous liquid, gray beige in color (from light to dark tones), pH 6.5-7.5; dry matter ratio was 3.0-5.0 % and keratin content, as per total nitrogen, reached 95-98 % of dry matter. Microbiological purity of the final product was high, with microbial contamination less than 10^1 CFU/cm^3, yeasts, moldy and yeast-like fungi were absent. In farm trial, we used this substance in small doses which are not significantly modifying the protein ratio of 0.23-1.13 g of keratin per 156 g feed portion, but raising amount of sulphur-containing amino acids.

At different Melacoll dosage applied at the beginning of the trial, there was a direct correlation for the first four months between the used dose and the monthly increase in the body weight of ferret youngsters (Fig. 2, A). During this time, the body weight, as compared to the initial values, increased approximately 5-fold in the experimental groups, whereas only 3.6-fold in the control. At month 5 of life, the body weigh of the experimental animals decreased by an average of 0.5 kg and was almost equal to that in the control group. It should be noted that during each month there was no statistically significant difference between the weight of animals received minimum and maximum dose of Melacoll. Thus, on weighing dates (July 20, 2013, August 22, 2016, September 22, 2013, October 20, 2016, October 25, 2013, December 20, 2016), the t_{obs} values in groups I and V were 0.01, 0.30, 2.08, 1.62, 1.20 and 0.90, respectively, at t_{table} 2.14. At the same time, the difference in the monthly weight gain was statistically significant (Table 3).

The obtained data indicates that Melacoll accelerated animal development for almost a month, since continuation of the experiment negatively affected ferret weight in the experimental groups by the end of the 5th month.

3. Statistical significance of monthly differences in body weight gain of black ferrets (*Mustela putorius*) received injection (min/max) **of Melacoll** (a farm trial, ferret cages, Russian Sable Enterprise, Moscow Province, 2016)

Weighing date	Student's *t*-criterion	
	group I (min), *tobs.*	group V (max), *tobs.*
20.07-22.08	16.39	11.80
22.08-22.09	4.25	2.17
22.09-20.10	5.08	2.67
20.10-25.11	2.35	3.88
25.11-20.12	1.88	0.86

$T_{tab.}$ 2.14

N o t e. Design of the experiment and application of tested preparations are described in section «Techniques».

When using keratin as a feed additive (see Fig. 2, B), the growth acceleration for the first 2 months was more pronounced at low doses than for larger doses. Over time, the difference in the body weight of animals which got small and large doses of keratin (groups VIII and XIII) became statistically unreliable. The values of t_{obs} 0.39 < t_{table} 2.14 (P > 0.05) in October and t_{obs} 2.0 < t_{table} 2.14 (P > 0.05) in December indicated the effect of small doses.

Cumulative effect of Melacoll and keratin (group XI) is a matter of special interest. The weight gain in the test animals was statistically significant for 4 months at *tobs.* of 16.59, 5.16, 2.29 and 2.58, respectively, vs. $t_{tab.}$ 2.1. In this, the increment exceeded the values which were obtained for keratin only, regardless of the dose used, and the maximum effect, like for Melakoll, was recorded for month 4.On month 5, the body weight in this group decreased by 3 %, but the differences were statistically insignificant (t_{obs} 1.58 vs. t_{table} 2.14). It indicates that the animals have grown to slaughter weight in November, that is, 1 month before the expected date. A similar trend in weight loss was also observed in the groups where pure keratin was used as a protein additive.

Our findings are in line with the available publications on feasibility of pro-

longed-release melatonin and keratin supplements for feeding young animals in fur farming [2, 4, 20, 28]. At the same time, according to our data, the combined use of these two biologicals has more pronounced effect.

Melatonin, by stimulating animal maturation, positively affects the size and weight of pelts [28, 32]. Melacall had similar effect (Table 4). An increase in the concentration of implanted Melacoll resulted in a larger fur pelt size, with the largest found in group V where the concentration of melatonin was the maximum. For implantation of collagen and PVA mixture (control group VI), the pelt size was practically the same as in group VII (untreated control, t_{obs} 1.11 < t_{table} 2.14, P > 0.05), which confirms the existing opinion on the functional role of melatonin in hair biology of fur animals [27, 28, 32]. While keratin feeding, the largest pelt size was found in groups VIII and XIII (912 and 918 cm^2, respectively), and in group XI where keratin was used together with Melacoll (998 cm^2). Differences between this indexes in groups VIII and XIII were not statistically significant (t_{obs} 1.21 < t_{table} 2.14, P > 0.05), which confirms our hypothesis about the advisability of using small doses of keratin.

In group XI, the differences in pelt size as compared to control (12 %) were significant (t_{obs} 2.89 > t_{table} 2.14, P > 0.05), unlike groups VIII and XIII in which only keratin was administered and the increase in pelt size compared to the control was 7-9 % (t_{obs} 2.21 < t_{table} 2.14, P > 0.05). In this, the pelt size in the group XI was significantly larger than in group VIII (t_{obs} 2.99 > t_{table} 2.14, P > 0.05) and in group XIII (t_{obs} 2.96 > t_{table} 2.14, P > 0.05). In evaluation according to the State Standards, all pelts were assigned to the grade 1 (full-haired with high, frequent, shiny hair and thick fluff, with a fluffy tail). All pelts exceeded 600 cm^2 in size (see Table 4), that is, they were characterized as large.

4. Pelt grade and size in black ferrets (*Mustela putorius*) which received Melacoll and keratin separately and jointly (a farm trial, ferret cages, Russian Sable Enterprise, Moscow Province, 2016)

Group	Grade	Size, cm^2 (M±SEM)	Size category	Defects			Imperfect pelts, %
				small	medium	large	
Melacoll injected							
I	1	930±39	Large	5.6	No	No	5.6
II	1	947±40	Large	16.7	No	No	16.7
III	1	951±32	Large	5.6	No	No	5.6
IV	1	961±48	Large	12.0	No	No	12.0
V	1	1023±30	Large	0	16.7	No	16.7
Controls							
VI	1	869±42	Large	16.7	No	No	16.7
VII	1	861±30	Large	5.7	16.7	No	22.4
Dietary keratin supplement							
VIII	1	912±38	Large	16.6	No	No	16.6
IX	1	889±35	Large	No	11.1	No	11.1
X	1	908±36	Large	No	5.5	No	5.5
XI (+ Melacoll injection)	1	998±32	Large	No	No	No	0.0
XII	1	872±47	Large	9.0	No	No	9.0
XIII	1	918±34	Large	7.0	No	No	7.0

Note. For description of groups and preparations used, see the section «Techniques». Controls: group VI (injection of mixture of collagen and PVA) and group VII (animals which did not receive either Melacoll or keratin).

One of the important indicators of the pelt quality is the kind of defect [20, 28]. Imperfections which reduce pelt quality may occur during animal's lifetime depending on nutrition and keeping conditions, effects of environment and physiological state of animal, or these may result from unprofessional actions or negligence of personnel at slaughtering and during primary processing of raw materials [20, 25]. Particular interest for us was the studying of lifetime defects, which include broken guard hair, bald patches, scrapes, poor hair, bites, and tangled hair [25, 26]. Of the above-mentioned, the guard hair damage, bald patch

and scrapes were found (Table 5). The lifetime defects of pelts were the most frequent (37.5 %) in animals of groups II, IV and V which received implanted melatonin. In groups VIII-XIII fed with keratin as a protein supplement to the basic diet, the lifetime defects reached a 25.0 % level. At Melacoll implantation, guard hair damage occurred. In control groups, 25 % pelts were characterized by guard hair damage, whereas in the pelts from animals that received keratin this defect was found only in two groups, one pelt per each (see Table 5). In our opinion, this may be due to the better availability of sulfur-containing amino acids responsible for hair strength [20, 27].

5. Frequency of lifetime defects of pelts in black ferret (*Mustela putorius*) which received Melacoll and keratin separately and jointly (a farm trial, ferret cages, Russian Sable Enterprise, Moscow Province, 2016)

Group	Pelts with lifetime imperfections, psc/%		
	broken guard hair	bald patches	scrapes
Melacoll injected			
I	1/12.5	0	0
II	2/25.0	0	0
III	1/12.5	1/12.5	1/12.5
IV	3/37.5	0	0
V	3/37.5	0	0
Controls			
VI	2/25.0	0	0
VII	2/25.0	1/12.5	0
Dietary keratin supplement			
VIII	1/12.5	0	1/12.5
IX	0	1/12.5	0
X	0	1/12.5	0
XI (+ Melacoll injection)	0	0	0
XII	1/12.5	1/12.5	0
XIII	0	0	1/12.5

N o t e. For description of groups and preparations used, see the section «Techniques». Controls: group VI (injection of mixture of collagen and PVA) and group VII (animals which did not receive either Melacoll or keratin). In each group, 8 pelts were examined.

6. Fur density in black ferret (*Mustela putorius*) which received Melacoll and keratin separately and jointly (a farm trial, ferret cages, Russian Sable Enterprise, Moscow Province, 2016)

Group	Direct count on rump, psc/cm^2 (M±SEM, n = 3)	Histological estimates of hair follicle number	
		per field of view (M±SEM, n = 10)	average, pcs/cm^2
Melacoll injected			
I	6304±530	44.1±1.6	6300
II	6340±543	44.3±1.7	6328
III	6410±541	44.9±1.7	6414
V	6533±653	45.7±1.4	6529
Controls			
VI	6069±509	42.5±0.9	6071
VII	6111±511	42.8±1.8	6114
Dietary keratin supplement			
VIII	6840±548	47.8±1.6	6828
X	6832±583	47.8±1.4	6829
XI (+ Melacoll injection)	7141±614	49.9±1.8	7129
XIII	6870±587	48.1±2.3	6871

N o t e. For description of groups and preparations used, see the section «Techniques». Controls: group VI (injection of mixture of collagen and PVA) and group VII (animals which did not receive either Melacoll or keratin). For the differences between groups I and VII, II and VII, III и VII, V и VII, and VI and VII t_{obs} are 0.04, 0.05; 0.07; 0.09; 0.01 at t_{table} 2.77, respectively.

The commercial value of fur pelts is mainly due to the fur density which is crucial for fur puffiness and thermal conductivity, as well as for the suitability of pelts for manufacturing valuable finished fur products. We estimated this index in the groups with the maximum Melacoll and keratin effects, as it followed from body weight dynamics, pelt size, and the kinds of pelt defects (Table 6). Melakoll did not exert a marked influence on the fur density, which was confirmed by an assessment of the statistical significance of differences (see Table

6). It should be noted that some scientists opine against the use of melatonin as a stimulant, because according to their data, this leads to a decrease in fur quality deterioration because of unnatural growth acceleration [32, 33].

Keratin increased the fur density by 12 % compared to the control (t_{obs} 4.1 > t_{table} 2.77, P > 0.05). In the combined using keratin and melakoll, the hair density increased significantly by 16 % compared to the control (t_{obs} 3.1 > t_{table} 2.77, P > 0.05). The results of direct fur counting correlated well with the histological estimates of hair follicle number. The main factors of fur density are the hair number per bundle, the number of grouped bundles and their distribution. Histological comparison of pelt horizontal sections in experimental group XI and control group VII (Fig. 3) revealed a greater number of bundles in group XI and confirmed the fact that the number of bundles and hairs depend both on the hereditary features and on the external factors, such as feeding and keeping conditions, which affect realization of the animal genetic potential.

Thus, the combined use of keratin and melatonin-containing Melacoll mutually enhanced the effect of each biostimulant. Keratin enriches the diet with sulfur-containing amino acids that activate hair growth, while melatonin, due to accelerated biorhythms, contributes to a better eating and digestion. These factors ultimately ensure the acceleration of animal growth and positively affect the pelt quality.

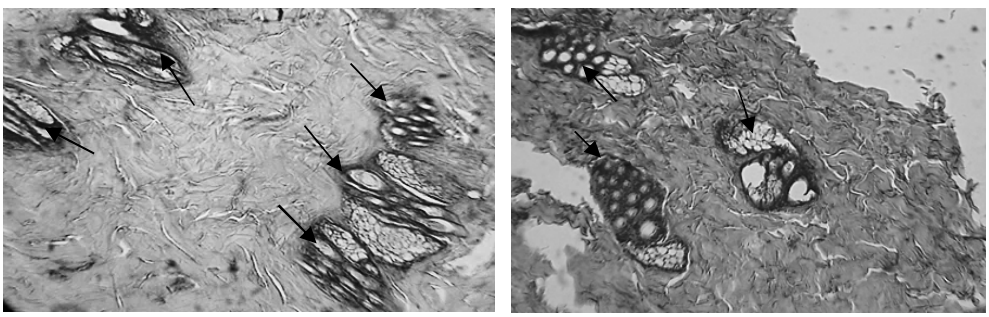

Fig. 3. Horizontal section of a pelt in black ferret (*Mustela putorius*): a combined use of injected Melacoll and dietary keratin (group XI, on the left) and animals which did not receive these preparations (group VII, on the right) (a farm trial, ferret cages, Russian Sable Enterprise, Moscow Province, 2016). Hematoxylin and eosin staining, a magnification of ×10; arrows note hair bundles.

So, the obtained results lead to the following conclusions. An original melatonin-collagen complex with prolonged effect, conditionally named Melacoll, was produced from unusable wastes and used to stimulate the growth of ferret youngsters. Also, the effect of keratin-containing substance was tested, the quality of which allows us to use it as a protein fodder additive. An increase in concentration of the implanted Melacoll increases the pelt size, whereas keratin shows positive effect at low doses. In combination of Melacoll and keratin, the resultant effect exceeds that of each preparation separately. There is an even, statistically significant gain in body weight for 4 months. During this period, the animal growth is completed and they reach a pre-slaughter stage. In the farm trials, the combined use of Melacoll and keratin significantly increased pelt size and quality, as followed from estimates of fur density and defects. The largest number of pelt lifetime defects (37.5 %), in particular, guard hair damage, was noted in administering Melacoll without keratin additive. Direct counting and histological study confirmed that the combined use of keratin and Melacoll significantly increases the fur density, although Melacoll itself does not noticeably affect expression of this trait. Dietary keratin (0.6 % of feed weight) and 1 ml injection of Melacoll (6 mg/ml) are optimal to accelerate growth of the ferret youngsters.

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