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Relaxer feed additive as a natural tranquilizer for farmed mink (*Neovison vison*) in stress situations

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Summary

Mink, as carnivorous fur animals, are especially exposed to stress factors during mating, gestation, parturition, and rearing of the young that manifests as aggressive behaviour. So far, preparations applied on mink farms to relieve stress have shown low efficacy. Therefore, we aimed at determining whether the Relaxer feed supplement, prepared from valerian (*Valeriana officinalis* L.) and passion flower (*Passiflora incarnata*) extracts, can be applied to reduce the negative effects of stressors. The study was conducted on mink (*Neovison vison*) of the brown variety on a fur farm (Poland). The study was divided into two stages investigating the effect of the Relaxer additive on adult mink during the mating season (1%, 1.5%, 2% additive) and on young mink weaned from their mothers (1.5%). For all mink groups basic haematological and morphological blood parameters were determined. The animals' condition and health were observed, and behavioural observations were made using a temperament test. The results of blood parameters of mink showed no negative impact of the additive on the animals' health. Statistical differences were observed in red blood cell counts. Analysing the white blood cell count parameters from the first stage of the study, a decrease in white blood cell (WBC) count was observed in the blood of animals of groups K and D1 in successive intakes. In this stage, the lowest cortisol levels were obtained in groups D1 and D2. Analysing the red blood cell parameters of animals at stage II, statistically significant differences were observed. Smaller differences were observed for the studied parameters of the protein-cell image of mink blood. A lower percentage of animals with extreme behavioural types (aggressive and fearful) was found in the experimental group. The feed addition of a mixture of herbs significantly influenced the stress behaviour of mink. Improved behaviour was observed which resulted in improved animal welfare and optimised production parameters. The optimal proportion of the additive in the feed was set at 1.5%.

Keywords: behaviour, feed additive, *Neovison vison*, *Passiflora incarnata*, stress, tranquilizer, *Valeriana officinalis* L.

Stress factors in animals can be diverse. These may be physical or social factors arising from interaction with representatives of the same species or associated with the presence of human beings. These stressors have an additive effect, which means that the effect of multiple stress factors on an animal at a given time can accumulate (4). Stress occurs when the impact of the environment exceeds the body's capacity for self-regulation, especially when the situation becomes unpredictable for animals and is out of their control. Stress results in the release of a number of neurotransmitters, peptides, cytokines, hormones, and other factors into the cardiovascular system and tissues. The most important mediators of the stress response are catecholamines, especially epinephrine (adrenaline) and norepinephrine (noradrenaline). They are produced in the adrenal medulla and by postganglionic fibres of the sympathetic nervous system, from which they pass into the blood. They are joined by glucocorticoids – cortisol and corticosterone, which are secreted by the adrenal cortex following activation of the hypothalamic-pituitary-adrenal (HPA) axis (19). They induce a number of changes in the physiological processes of animals, manifested in circulation or breathing disor-

ders, weight loss, decreased immunity, and increased aggressiveness. The main physiological responses are activation of the sympathetic nervous system and the HPA axis (12, 15). The response of the HPA axis to a stressor may interfere with mammalian reproduction by delaying or blocking development of oocytes (ovarian follicles) and increasing luteinizing hormone (LH), as well as through impairment of oestrus. In males, stress has a negative impact on androgen production and on spermiogenesis and spermatogenesis (18). LH is necessary for specific reactions of Leydig cell membrane receptors that initiate the resumption of testosterone synthesis and secretion (16).

Among carnivorous fur animals, despite many years of breeding selection, some individuals are still wary, fearful and even aggressive. Such animals are highly susceptible to compulsive behaviours, especially stereotypies. Compulsive behaviours appearing in individuals on fur farms are mainly stereotypies directed towards the animal's surroundings and against its own body. In addition to stress, another common cause of this type of behaviour is the anxiety resulting from inadequate stimulation, or none at all, in the animal's living environment (17). This is particularly important in the case of carnivorous fur animals, because these animals are very excitable and require maximum peace and quiet on the farm throughout the year. Mink are especially exposed to stress during mating, gestation, and parturition, as well as rearing of the young. This is manifested as anxiety and fear, and in extreme cases the animals may bite their own offspring, bite off their tails, or pluck their coat.

Preparations used on mink farms to relieve stress have thus far shown little efficacy. Therefore, a study was undertaken to determine whether the Relaxer feed supplement can be used in mink to reduce the adverse effects of stressors.

Material and methods

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Local Ethics Committee on Animal Experimentation of the University of Life Sciences in Lublin, Poland (approval no. 33/2016).

Experimental animals and diet – the first stage of the study. The study was conducted on mink (*Neovison vison*) of the brown variety on a fur farm in south-eastern Poland. All animals were kept in the same kind of cages in a shed system. Animals were kept in a cage sequence: experimental and control. In accordance with guidelines for the welfare of fur animals, the cages were 45 cm high, 30 cm wide, and 90 cm long. Each cage was connected to a wooden nesting box 20 cm high, 28 cm wide, and 23 cm long. All cages were equipped with a shelf on which the animals rested. Adult mink were housed individually, while the young were housed two per cage. The animals received the same feed, balanced for each period in accordance with accepted standards (1), and had uninterrupted access to drinking water. Animals with similar characteristics were selected for the experimental and control groups, with randomization of the sample. The analyses were carried out on animals from the same production group in two stages. Four groups were formed, a control group (C) and three experimental groups (E1, E2 and E3), with 30 animals in each (15 females and 15 males 2-3 years old). In the first stage of the experiment, coinciding with the mating season, from 25 February to 20 March, the feed supplement Relaxer (AGRAID, Szamotuły, Poland) was included in the diet of the animals from the experimental groups. Relaxer is a plant preparation made from valerian (*Valeriana officinalis* L.) extract, as a source of valeric acid, and passion flower (*Passiflora incarnata*) extract, containing vitexin, apigenin and luteolin. The control group (C) – did not receive Relaxer, experimental group (E1) – received 1% Relaxer in its diet, experimental group (E2) – received 1.5% Relaxer in its diet, experimental group (E3) – received 2% Relaxer in its diet.

Blood analysis. Blood was collected for tests three times by clipping the claw, after local anesthesia (ointment with 5% lignocaine). The first blood sampling was performed after 7 days of taking the test preparation supplement, and the next two at 7-day intervals into test tubes with ethylenediaminetetraacetic acid (EDTA). Basic haematological parameters were determined in whole blood. Analyses of morphological parameters were performed using a BS 180 automatic analyser from Cormay (Cormay Ltd., Lublin, Poland). Cortisol levels were determined in plasma. Due to circadian changes in cortisol levels, blood from animals was collected at the same time throughout the experiment, between 8 am and 9 am, when its concentration in the peripheral blood was high. Plasma cortisol levels were determined using a DRG ElizaMat CM apparatus (DRG MedTek, Warszawa, Poland) by ELISA. This is a solid-phase enzyme-linked immunoassay based on the principle of competitive binding. Endogenous cortisol contained in the sample competes with the cortisol-horseradish peroxidase conjugate solution to bind to the coating antibody. After incubation, the unbound conjugate solution is washed away. The amount of bound horseradish peroxidase conjugate is inversely proportional to the cortisol concentration in the test sample. After the substrate solution is added, the colour intensity is inversely proportional to the cortisol concentration in the sample. The absorbance was read at 450 ± 10 nm using a microplate reader within 10 minutes after the stopping solution was added.

Behavioural temperament test. During the experiment, the animals' condition and health was observed, and behavioural observations were made using a temperament test (stick test). At 110 days of age, temperament test was performed on young mink from both groups which was repeated after 14 days. This test has been verified as an indicator of the temperament of American mink that reflects their overall emotional state. It is also mentioned as a measure of animal welfare in the WelFur project, which develops welfare assessment protocols for fox and mink farming (https://www.sustainablefur.com/wp-content/uploads/2018/11/Mink_protocol_final_web_edition_light.pdf, 20). The test assesses the animal's response to an object introduced into

its living space. The test involved the insertion of an object (a wooden stick) into the cage through the mesh (above the nesting box) for 30 seconds. The behavioural response of each individual was determined separately, in triplicate. On the basis of the test, the animals were classified into one of three behavioural profiles: aggressive, fearful, and trusting. Mink were considered to be aggressive if they attacked the object, gripped it with their teeth (with or without a hard bite), or wrenched at the object, attempting to pull it into the cage, with nearly continuous physical contact with the object throughout the test. Mink assessed as trustful showed considerable interest in the object by intensively sniffing and grasping it, while no aggressive behaviour was observed. Mink were considered fearful if they showed little interest in the object (sniffing), while behaviours associated with fear (escaping, vocalization, or defecation) predominated. An individual that was classified in the same behavioural profile in at least two of the three replications was assigned that profile in the final evaluation.

Experimental animals and diet – the second stage of the study. The second stage of the experiment was carried out on young mink in the period around weaning from their mothers. Dosing of the preparation started when young minks start to eat solid food but are still with their mothers (at 5-6 weeks of age) and continued on animals after weaning (7-8 weeks). On the basis of the results obtained in the first stage, a level of 1.5% was selected as the optimal addition of Relaxer to the feed in the second stage. Two groups of 30 animals each were formed, with equal numbers of each sex: control group (C1) – no addition of the Relaxer feed supplement, experimental group (R) – the addition of Relaxer in the amount of 1.5% of daily feed intake.

As in the first stage, morphological blood parameters and cortisol levels were periodically monitored in all experimental animals. Blood for analysis was collected on the 21st day after weaning (1st collection) and after 14 (2nd collection) and 28 days (3rd collection) of feeding with the supplement. The behavioural stick test was also carried out during this period. In addition, the following observations were made during the study period: percentage of mink with various types of behaviour (%), mink body weight before weaning and at slaughter (g), deaths (%), bites of offspring assessed at 6 weeks of age (%), percentage of damaged skins (%). After the skins were dried and combed, they were thoroughly examined for mechanical damage, hair loss, holes bitten through the skin, etc. Three degrees of skin damage were established: 1 – single areas of hair loss up to 0.25 cm^2; 2 – several areas of hair loss up to 0.25 cm^2; 3 – areas of hair loss of 1 cm^2 or more, torn skin, or perforated skin.

Statistical analysis. The results obtained in individual groups and measurements were presented as the arithmetic mean and standard deviation (M ± SD), as well as the arithmetic mean (M) and standard error of the mean (SEM). The normality of the distribution was assessed by the Shapiro-Wilk test. If the distribution was normal, one-way analysis of variance was performed, with differences between groups and blood collections assessed by the Tukey test. The results were analysed using Statistica ver. 13.1. (StatSoft Inc., Tulsa, OK, USA).

Results and discussion

Blood parameters in the first stage of the study. Analysis of haematological parameters of mink blood during the first stage of the experiment showed no negative impact of the additive on the animals' health. The statistical analysis showed numerous significant changes at $P \leq 0.05$ and $P \leq 0.01$. Differences in the parameters were noted both between blood collections and for individual parameters between groups. Statistical differences were observed for red blood cell (RBC) count, but the Shapiro-Wilk test did not confirm this relationship.

Haematocrit (HCT) values differed statistically significantly between groups C, E1 and E2 in the second blood collection, with the highest values recorded in group E2. A similar tendency was observed for mean corpuscular volume (MCV) in collections 1 and 2 in group E2. For this parameter, statistically significant differences were also noted between blood collections in group C. In this group, statistically significant differences in mean corpuscular haemoglobin concentration (MCHC) were also found between the second and third blood collection. Its highest value was recorded in the second sampling from group E1, and the lowest in collections 1 and 2 from group E3. Haemoglobin (HGB) levels differed statistically significantly in the second blood sampling between groups C and E3. The RBC, platelet count (PLT), and mean platelet volume (MPV) were not statistically significant either between groups and or between blood collections (Tab. 1).

Analysis of white blood cell parameters showed a decrease in the white blood cell (WBC) count in groups C and E1 in successive blood collections (Tab. 2). The statistical analysis showed statistical significance for this parameter only in group E3 between the first and second collection, and in the second collection between experimental groups E1 and E3. Lymphocyte (LYM) concentration was statistically significantly different both between successive collections and between groups of animals. The highest value for this parameter was obtained in the blood of the E3 animals in the first blood sampling. As the proportion of the additive in the feed increased, the LYM concentration increased and then decreased (statistically significant differences). WBCs with intermediate characteristics between LYM and the combined value of the other types of white blood cells not classified as lymphocytes or granulocytes (MID) differed statistically significantly in the second blood collection between groups E1 and E3, and in group E2 between collections 1 and 2 (Tab. 2).

The level of cortisol in the first stage of the experiment was markedly varied in successive blood samplings. In the second collection it was lower in animals from groups E1 and E2 than in the control and experimental group E3. Statistically significant differences were demonstrated at $P \leq 0.05$. The highest cortisol concentration was obtained in the second

Tab. 1. Red blood cell parameters in mink blood in the first stage of the study

Parameter	Blood collection	Group				Statystical analysis		
		C	E1	E2	E3			
		M ± SD	M ± SD	M ± SD	M ± SD	M	SEM	P-value
RBC	1	9.62 ± 0.70	8.86 ± 1.72	9.31 ± 0.64	8.80 ± 0.42	9.13	0.21	0.515
	2	9.21 ± 0.36	8.36 ± 0.73	9.26 ± 0.58	8.72 ± 0.56	8.89	0.13	0.039
	3	8.14 ± 1.49	8.88 ± 1.43	8.82 ± 0.54	8.03 ± 0.27	8.47	0.22	0.404
HCT	1	52.40 ± 3.92	46.15 ± 9.43	52.43 ± 3.82	49.13 ± 2.88	49.93	1.24	0.221
	2	48.68 ± 3.10^{a}	43.13 ± 3.99^{ab}	52.43 ± 2.32^{b}	47.73 ± 3.15	48.00	0.92	0.001
	3	46.25 ± 8.30	50.00 ± 5.54	50.63 ± 1.54	44.20 ± 1.27	47.77	1.12	0.122
HGB	1	19.94 ± 1.52	17.92 ± 3.01	19.63 ± 1.04	18.27 ± 1.49	18.90	0.42	0.246
	2	19.02 ± 1.05^{a}	17.47 ± 1.34	18.88 ± 1.20	16.97 ± 1.36^{a}	18.08	0.30	0.021
	3	17.20 ± 1.30	17.50 ± 2.27	18.27 ± 0.74	16.27 ± 1.15	17.31	0.32	0.164
MCV	1	54.58 ± 2.10*	52.08 ± 2.57^{AB}	56.27 ± 1.03^{A}	55.80 ± 0.90^{B}	54.69	0.49	0.003
	2	52.83 ± 1.94^{a}*	51.60 ± 2.84^{b}	56.63 ± 1.81^{ab}	54.63 ± 2.38	53.93	0.59	0.006
	3	56.50 ± 2.37*	56.50 ± 1.67	57.43 ± 0.92	55.03 ± 1.81	56.37	0.38	0.165
MCH	1	20.78 ± 0.89	20.30 ± 0.70	21.10 ± 0.25	20.67 ± 0.49	20.71	0.13	0.194
	2	20.65 ± 0.42	20.93 ± 0.59^{a}	20.43 ± 0.59	19.43 ± 1.39^{a}	20.36	0.20	0.030
	3	21.25 ± 1.06^{a}	19.80 ± 0.98^{a}	20.70 ± 0.65	20.23 ± 0.60	20.50	0.20	0.043
MCHC	1	38.04 ± 0.50	39.04 ± 1.52^{a}	37.52 ± 0.48	37.10 ± 1.49a	37.92	0.27	0.047
	2	39.18 ± 1.09^{AB}*	40.70 ± 2.23	36.13 ± 0.90^{A}	35.53 ± 0.58^{B}	37.89	0.51	0.000
	3	37.70 ± 3.09*	35.07 ± 0.77	36.07 ± 0.78	36.83 ± 1.09	36.42	0.39	0.087
PLT	1	509.20 ± 69.12	450.50 ± 199.57	479.83 ± 18.35	493.67 ± 51.53	482.17	21.95	0.836
	2	459.33 ± 40.83	444.33 ± 30.24	397.67 ± 65.28	425.33 ± 128.81	431.67	15.34	0.549
	3	433.00 ± 54.76	519.33 ± 118.43	469.67 ± 94.00	402.33 ± 135.72	456.08	21.99	0.279
MPV	1	7.70 ± 0.20	8.08 ± 0.31	7.73 ± 0.34	8.03 ± 0.67	7.90	0.09	0.326
	2	7.45 ± 0.49	7.93 ± 0.63	7.78 ± 0.79	7.43 ± 0.20	7.65	0.12	0.361
	3	7.65 ± 0.33	7.97 ± 0.23	7.90 ± 0.38	8.23 ± 0.79	7.94	0.10	0.249

Explanations: a, b and A, B values in rows differ statistically at $P \leq 0.05$ and $P \leq 0.01$; * values in columns differ statistically at $P \leq 0.05$

Tab. 2. White blood cell parameters of mink in the first stage of the study

Parameter	Blood collection	Group				Statystical analysis		
		C	E1	E2	E3			
		M ± SD	M ± SD	M ± SD	M ± SD	M	SEM	P-value
WBC	1	15.43 ± 3.74	13.50 ± 3.43	14.87 ± 0.99	14.50 ± 0.87*	14.54	0.52	0.642
	2	12.82 ± 2.60	13.47 ± 1.20^{a}	11.30 ± 1.99	9.42 ± 2.76^{a}*	11.75	0.53	0.024
	3	11.45 ± 2.10	12.57 ± 1.85	13.33 ± 2.64	11.43 ± 1.75	12.20	0.44	0.353
LYM	1	5.73 ± 1.31*	6.02 ± 0.65	5.33 ± 1.05a	7.40 ± 1.10a*	6.15	0.26	0.014
	2	5.17 ± 1.28	6.37 ± 0.51^{AB}*	4.12 ± 0.77^{A}	3.60 ± 0.33^{B}*	4.82	0.30	0.001
	3	3.45 ± 0.27*	3.63 ± 0.31*	4.17 ± 0.50	4.30 ± 1.48*	3.89	0.17	0.233
MID	1	1.58 ± 0.58	1.50 ± 0.58	1.80 ± 0.28*	1.30 ± 0.15	1.54	0.09	0.279
	2	1.50 ± 0.57	1.67 ± 0.53a	0.97 ± 0.24*	0.87 ± 0.33^{a}	1.25	0.11	0.011
	3	1.35 ± 0.42	1.10 ± 0.18	1.43 ± 0.23	1.03 ± 0.16	1.23	0.06	0.050
GRAN	1	8.13 ± 2.01	5.98 ± 2.78	7.73 ± 1.27	5.77 ± 1.23	6.85	0.43	0.122
	2	6.17 ± 2.13	5.43 ± 2.21	6.23 ± 1.51	4.97 ± 2.44	5.70	0.41	0.684
	3	6.65 ± 1.68	7.83 ± 1.72	7.73 ± 3.06	6.10 ± 2.58	7.08	0.47	0.517

Explanations: as in Tab. 1.

Tab. 3. Cortisol levels in mink blood in the first stage of the study

Blood collection	Group				Statystical analysis		
	C	E1	E2	E3			
	M ± SD	M ± SD	M ± SD	M ± SD	M	SEM	P-value
1	77.80 ± 15.63	79.67 ± 51.55	51.00 ± 33.24	77.00 ± 19.13	71.09	7.02	0.429
2	124.33 ± 76.04[a]	48.00 ± 16.88[ab]	76.00 ± 15.21	120.00 ± 16.58[b]	92.08	10.16	0.010
3	70.00 ± 23.87	49.67 ± 10.33	70.33 ± 26.85	79.27 ± 7.68	67.32	4.28	0.082

Explanations: a, b values in rows differ statistically at $P \leq 0.05$

blood collection in the control and experimental group E3, and the lowest in experimental group E1 (Tab. 3).

Blood parameters in the second stage of the study. Analysis of the RBC parameters of animals in the second stage of the experiment showed statistically significant differences, especially between collections within the same group of animals. Only the RBC was statistically significantly lower in the control group (C1) in the third blood collection compared to experimental group R. In addition, in the control group there were significantly statistical differences between the RBC, HCT, mean corpuscular haemoglobin (MCH), and MCHC. A gradual increase in HGB levels was also noted in successive blood collections. This relationship was confirmed statistically for the control group (C1) for the first and second blood collection. In the experimental group (R), statistically significant differences were obtained in successive blood collections for MCV and MCHC. In addition, a statistically significant increase in HCT between the first and second blood sampling was obtained in the experimental group (R), (Tab. 4).

Much smaller differences were observed for the WBC parameters of the mink included in the second stage of the experiment (Tab. 5). Only the LYM count in the control group (C1) was statistically significantly higher between the first and second blood samplings (Tab. 5). In the second stage of the experiment, the cortisol concentration in the mink blood was lower in the experimental group than in the control group in all blood samplings. However, the levels were not shown to be statistically significant at $P \leq 0.05$ or $P \leq 0.01$ (Tab. 6). Throughout the experiment, in all groups of mink the blood parameters tested were within the reference values for the species (2, 7, 9).

Tab. 4. Red blood cell parameters in mink blood in the second stage of the study

Parameter	Blood collection	Group		Statystical analysis		
		C1	R			
		M ± SD	M ± SD	M	SEM	P-value
RBC	1	5.70 ± 2.36*	7.09 ± 0.29	6.33	0.55	0.228
	2	8.08 ± 0.70*	8.24 ± 0.55	8.16	8.16	0.676
	3	8.14 ± 0.36[a]*	8.53 ± 0.18[a]	8.38	0.10	0.040
HCT	1	33.35 ± 14.00*	39.74 ± 3.48*	36.25	3.22	0.349
	2	46.03 ± 4.52*	45.98 ± 3.00*	46.01	1.06	0.982
	3	50.10 ± 3.28*	52.47 ± 2.03	51.52	0.86	0.173
HGB	1	12.37 ± 4.70*	15.06 ± 0.34	13.59	1.09	0.237
	2	17.47 ± 2.03*	17.33 ± 1.23	17.40	0.46	0.893
	3	16.20 ± 0.80	17.38 ± 0.83	16.91	0.31	0.218
MCV	1	57.67 ± 1.34	56.08 ± 4.96*	56.95	1.02	0.468
	2	56.97 ± 1.13	56.00 ± 1.39*	56.48	0.38	0.216
	3	60.85 ± 2.81	61.48 ± 2.55*	61.23	0.80	0.738
MCH	1	21.93 ± 0.89*	21.30 ± 0.62	21.65	0.24	0.213
	2	21.58 ± 0.70*	21.08 ± 0.57	21.33	0.19	0.206
	3	19.73 ± 0.17*	20.33 ± 0.69	20.09	0.19	0.739
MCHC	1	37.95 ± 2.22*	38.20 ± 3.60*	38.06	0.84	0.891
	2	37.98 ± 0.61*	37.62 ± 1.70*	37.80	0.36	0.629
	3	32.40 ± 0.93*	32.22 ± 1.62*	32.29	0.42	0.420
PLT	1	450.17 ± 132.45	461.00 ± 74.79	455.09	31.68	0.875
	2	489.33 ± 85.12	451.00 ± 83.25	470.17	23.88	0.547
	3	496.50 ± 120.95	654.33 ± 169.59	591.20	52.44	0.262
MPV	1	7.33 ± 0.60	7.12 ± 1.02	7.24	0.23	0.674
	2	8.78 ± 1.05	8.42 ± 0.99	8.60	0.29	0.224
	3	8.80 ± 0.96	7.97 ± 1.11	8.30	0.34	0.201

Explanations: as in Tab. 1.

Zootechnical observations results. Observations of the animals' condition and health were also carried out during the experiment, at critical times in the production cycle. The peak of lactation in mink occurs on the 21st day after parturition. After this period, the milk yield of females gradually decreases and the young begin to be interested in solid food. At four weeks of age, young mink begin to consume feed, which results in increased demand for drinking water. Mink kits are not yet able to drink water from drinkers and therefore compete with each other to lick saliva from the mother's muzzle. This often leads to biting, which may even result in the death of offspring. On the 21st day of rearing with the mothers, the feed supplement was added to the daily feed ration in the amount of 1.5%. At 37-38 days of age the animals were carefully examined for the presence of bites (Tab. 7).

Bites in the form of scabs and fresh wounds were observed in both

Tab. 5. White blood cell parameters of mink in the second stage of the study

Parameter	Blood collection	Group C1 M ± SD	Group R M ± SD	Statystical analysis M	SEM	P-value
WBC	1	7.15 ± 3.38	9.20 ± 2.66	8.08	0.94	0.300
	2	9.72 ± 1.49	10.53 ± 1.69	10.13	0.46	0.397
	3	7.08 ± 0.79	7.38 ± 1.50	7.26	0.39	0.810
LYM	1	3.78 ± 1.51*	4.68 ± 1.39	4.19	0.44	0.335
	2	6.50 ± 1.29*	6.20 ± 1.35	6.35	0.37	0.702
	3	4.75 ± 0.53	4.53 ± 1.11	4.62	0.28	0.523
MID	1	0.72 ± 0.32	0.86 ± 0.38	0.78	0.10	0.515
	2	0.80 ± 0.18	0.93 ± 0.30	0.87	0.07	0.373
	3	0.55 ± 0.13	0.58 ± 0.12	0.57	0.04	0.965
GRAN	1	2.67 ± 1.56	3.66 ± 1.04	3.12	0.42	0.256
	2	2.33 ± 0.71	3.42 ± 1.09	2.88	0.30	0.069
	3	1.88 ± 0.33	2.25 ± 0.90	2.10	0.23	0.318

Explanations: as in Tab. 1.

Tab. 6. Cortisol levels in mink blood in the second stage of the study

Blood collection	Group C1 M ± SD	Group R M ± SD	Statystical analysis M	SEM	P-value
1	35.95 ± 26.65	13.48 ± 6.18	25.74	6.80	0.101
2	37.96 ± 32.13	17.34 ± 13.77	27.65	8.13	0.224
3	58.85 ± 5.00	48.96 ± 17.30	52.92	5.17	0.380

Tab. 7. Litter size and percentage of kits with bites

Group (♀)	Mean litter size (n)	Litters without bites (%)	Kits with bites (%)
C1 (n = 30)	6.0	20.0	30.1
R (n = 36)	5.8	66.7	8.5

Tab. 8. Types of behaviour in young mink (%)

Group/sex		Day	A	F	T
C1	♂	0	20.8	12.5	66.7
		14	12.5	8.3	79.2
	♀	0	16.7	16.7	66.6
		14	12.5	8.3	79.2
R	♂	0	16.7	12.5	70.8
		14	16.7	16.7	66.6
	♀	0	20.8	12.5	66.7
		14	20.8	12.5	66.7

Explanations: A – aggressive, F – fearful, T – trusting

Tab. 9. Average body weight of young mink (g) on successive weighing days

Group/sex	A ♂	A ♀	F ♂	F ♀	T ♂	T ♀
C1	824	612	2966	1510	3912	1854
R	816	722	3006	1528	3880	1846

Explanations: as in Tab. 8.

groups of animals. Bites were found on the head, neck, and shoulder blade area, and less often on the side of the trunk. In the group of females with kits receiving the Relaxer additive, the percentage of kits with bites was 21.6 p.p. lower than in the control group (C1). No fatal bites were observed in either group during the study period.

In the experimental group, after 14 days the percentage of animals with extreme behavioural types (aggressive and fearful) was lower than at the start the study. In the control group (C1), the percentage of animals with aggressive and fearful behaviour did not change when the test was repeated after 14 days (Tab. 8).

The body weight of young mink was monitored throughout the second stage of the study. The animals were weighed three times: at weaning (I), one month after weaning (II), and at slaughter (III). The body weight of the young mink was similar in both groups. The statistical analysis ($P \leq 0.05$ and $P \leq 0.01$) showed no significant differences in male and female body weight between groups in any weighing period (Tab. 9).

Skins obtained from animals from both groups from stage 2 were evaluated as well. The average skin length, measured from the tip of the nose to the base of the tail, was greater within each skin size in the experimental group (R) than in the control group (C1). The differences in skin length between groups affected the percentages of skins of each size. There were 7% more size 40 skins in the experimental group (R) than in the control group (C1), (Tab. 10).

In the control group (C1), 18.6% of skins were damaged in various degrees, as compared to only 6.8% in the experimental group (R). In the group of animals receiving Relaxer (group R), there was no serious skin

Tab. 10. Mink skin size (%)

Sex/skin size		C1 %	C1 cm	R %	R cm
C1	40	50	96.7	57	98.6
	30	39	92.8	36	93.4
	00	11	86.2	7	86.5
R	0	83	79.4	81	81.5
	1	17	74.5	19	75.1

Tab. 11. Mink skin damage

Degree of skin damage	C1	R
1	9.6	5.1
2	4.8	1.7
3	4.2	–

damage affecting suitability further for processing (Tab. 11).

Stereotypical behaviours are an important aspect of mink farming. Many authors consider them one of the indicators of welfare assessment as they show the animals' relationship with the conditions of their environment (4). The relationship between stereotypical behaviour and stress hormone levels is unclear (11). If welfare is defined as 'the condition of an animal reflecting its ability to cope in the conditions of its environment', the question arises as to whether stereotypical behaviours are a sign of 'coping' or rather of helplessness. Mink are nervous animals and thus can often be in a state of chronic stress. This can lead to numerous anomalies in their behaviour, resulting in self-harm, external and internal injuries, disturbances in reproduction, and increased mortality in the young. According to current knowledge, stress arises in tissues and organs as a result of neurohormonal processes in which the main role is attributed to the autonomic nervous system and the hormones of the hypothalamus, pituitary gland, and adrenal glands (5). The Relaxer additive used in the experiment is made from valerian (*Valeriana officinalis* L.) extract as a source of numerous chemical structures, including valerenic acid, valeranone, valepotriate degradation products, lignans, and borneol and its esters, as well as passion flower (*Passiflora incarnata*) extract, a source of vitexin, apigenin and luteolin. The carrier is extruded wheat, a source of easily digestible energy, even for small animals whose digestive system is not yet fully developed. The manufacturer emphasizes that owing to the high glycaemic index, digestibility, and microbiological stability of extruded wheat, it can effectively replace sweet whey or lactose. The results of studies on the effect of valerian root on humans indicate that the sedative and sleep-promoting properties of *V. officinalis* are due to its effect on mechanisms associated primarily with the GABAergic system (14). Gamma aminobutyric acid (GABA) is the most important inhibitory neurotransmitter, assigned an important role in stress and anxiety reactions. Through secondary suppression of noradrenergic and dopaminergic effects, this acid is also responsible for sleep. In patient studies, even low levels of aqueous valerian extract inhibited GABA uptake and stimulated its release from nerve endings. Isolated valerenic acid also affected the GABA system by inhibiting the enzymatic degradation of γ-aminobutyric acid. This is because an increase in the GABA concentration results in the opening of the chloride channels of the neuronal membrane and an increase in the flow of chloride ions through the membrane into the cell, which reduces neuron excitability and inhibits the response to polarizing stimuli. Other research (13) indicates that valerian extracts have a strong affinity for the GABAA/benzodiazepine receptor complex.

Studies have been conducted on animal models on how the central nervous system (CNS) is affected by extracts from *V. officinalis* raw plant material, essential oil, and its components, such as valerenic acid, valerenal, and valeranone, as well as valepotriates and their degradation products. Hölzl (8) carried out an assessment of previous pharmacological studies of commercial extract of *V. officinalis* and individual substances isolated from the raw material. The methods used included a variety of techniques: tests of the spontaneous mobility of experimental animals, prolongation of thiopental-induced sleep, neurophysiological methods: electroencephalography (EEG), the 2-deoxyglucose technique (2DG) according to Sokołów, which measures glucose demand in various parts of the brain, and receptor binding tests. In *in vivo* studies with rat cortical synaptosomes, the acetate fraction (obtained by extraction of the aqueous extract with ethyl acetate), containing valerenic acids and lignans but without valepotriates or volatile components of the oil, was found to cause a fourfold increase in the GABA concentration in the synaptic cleft (13, 14). In further tests, methylene chloride extract from the raw plant material was observed to reduce the need for glucose (mainly in various regions of the cerebral cortex), which suggests an inhibitory effect on neurons and is consistent with the previously described sedative effect of valerian.

The second component of Relaxer is the passion flower: *Passiflorae herba*. This herb contains trace amounts of indole alkaloids alkaloids – up to 0.09%, flavonoids – 0.4-2% dry weight (DW), passiflorine, cyanogenic glycosides, phytosterols, and mineral salts. The raw plant material has a calming effect. Animal experiments have shown that the passion flower counteracts the stimulating effect of amphetamine, hence its use to treat drug addiction and alcoholism in humans. Most often it is used in states of nervous stimulation, in cardiac disorders caused by emotional stimuli, in mild seizures of central origin, for symptoms associated with menopause, and as an auxiliary treatment for intestinal and coronary spasms (3, 6). Passiflora is believed to be an effective remedy with calming and anticonvulsant properties, while not causing sedation. A calming effect without drowsiness is highly beneficial for individuals struggling with stress and anxiety.

The calming effect of passion flower also results from its effect on the benzodiazepine receptors of the GABA system. A study on patients with generalized anxiety found no significant differences in efficacy compared to oxazepam, a benzodiazepine drug (6, 10). A study in children diagnosed with Attention-deficit/hyperactivity disorder (ADHD) compared the effectiveness of passion flower and methylphenidate, a strong stimulant conventionally used in this disorder. The treatment effects were practically the same. According to the authors, this is due to the simul-

taneous calming effect and inhibitory effect on the enzyme monoamine oxidase, resulting in an increased concentration of dopamine. This aspect of its activity additionally improves well-being, because a reduction in monoamine oxidase increases the availability of hormones of happiness (6).

At various times throughout the breeding cycle mink are exposed to stress, which should be mitigated and limited in order to improve the animals' welfare and achieve measurable production benefits. This study using the Relaxer additive in the diet of mink found that the supplementation significantly influenced stress behaviour and production parameters. The results indicate that the optimal 1.5% proportion of the additive in mink feed calmed the animals and reduced their negative stress responses. When the preparation was used, a smaller percentage of bitten and injured kits was obtained during the period of rearing with their mothers. Scars from bite wounds, depending on their size and depth, have a substantial impact on the quality of skins and their degree of damage. In the case of mechanical processing of the skins (fleshing), it is in these places that the skin tears or hair is missing. To sum up, the use of this preparation in the diet of mink can have a positive effect by reducing stress, which will translate into improved animal welfare and optimization of production results.

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