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The effect of pro-, pre- and synbiotics on the health of mink, morphometric parameters of their digestive tract, and microbiological analysis of its contents

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The aim of the study was to analyse the effect of selected feed additives (pro-, pre- and synbiotics) on the health of mink, morphometric parameters of their digestive tract, and microbiological identification of its contents. The observations were made on pastel mink assigned to the following dietary treatments: group I – standard farm feed without supplements, group II – probiotic-supplemented feed, group III – prebiotic-supplemented feed, and group IV – synbiotic-supplemented feed. The feed additives were found to affect the growth and weight gains of the mink. Body weight at winter fur priming ranged from 1.45 to 2.54 kg in females and from 2.70 to 4.20 kg in males. During the rearing period (weaning to slaughter), the highest weight gains were observed in the group receiving prebiotics – on average 760 g in females and 1970 g in males. Analysis of the morphometric parameters of the digestive tract showed highly significant differences between means for the groups, except for the weight of the lungs and spleen and the length of the stomach. The mean concentration of total bacteria in the intestinal contents was similar in groups II and III (8.5-9.4 x 10^4 CFU/g), higher in group IV, and highest in the control group (5.9 x 10^6 CFU/g). Among the bacteria identified, *Corynebacterium* was dominant in all groups. The total fungal count in the intestinal contents was lowest in the mink receiving prebiotics. The dominant fungi were *Candida*, particularly *Candida glabrata*. The proportion of fungi of this genus varied between groups from 84.5% to 89.5%. The other fungi identified in the digesta were *Rhizopus* spp. and *Aspergillus* spp.

KEY WORDS: diet, feed additives, morphometric and microbiological analysis

An appropriate diet for a given animal species plays an enormous role in maintaining the animal's health, as well as in the treatment of gastrointestinal diseases. This is particularly important in European Union Member States, in which a ban on the use of feed antibiotics. was imposed in 2000 (Casewell et al., 2003). This has increased interest in natural feed additives having a beneficial effect on the health and productivity of animals.

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Animal health depends primarily on a well-functioning immune system that is able to counteract negative factors from the external environment. The use of suitable feed additives – pro-, pre- and synbiotic substances – can increase the body's defence potential, which translates into improved health. According to Kolanowski (1999), the task of modern feed, besides ensuring proper development, is to increase the body's efficiency, slow down degenerative processes, and prevent the onset of certain chronic and infectious diseases. This is particularly important in carnivorous fur-bearing animals, in whose diet the European Union permits the use of animal by-products. According to dietary recommendations for fur-bearing animals, the presence of pathogenic bacteria and toxins in feed is not permitted, and the total bacterial count may not exceed 6 million/g of feed (Gugołek et al., 2011). According to Gliński and Kostro (2002), improper feeding may result in intoxication and metabolic disorders, which are among the main causes of losses in mink breeding (about 70%).

Studies carried out in other animal species, e.g. poultry (Award et al., 2009), farm animals (Augustyniak and Nawrotek, 2014), carnivorous fur-bearing animals (Gugołek, 2002; 2014 and Winiarska and Gąsiorek, 2016), mono- and polygastric animals (Mizak et al., 2012), and companion animals – cats and dogs (Wincewicz, 2011), showed that the use of new-generation feed additives positively affected the functioning of selected segments of the digestive system. According to O'Hara and Shanahan (2006), the intestines play a decisive role in the immune system of the entire body because the intestinal mucosa contains 80% of all immunocompetent cells. Brzozowski (2016) demonstrated that the digestive tract of the neonate is sterile, and is colonized by various strains of bacteria when it begins to drink its mother's milk. Among bacteria colonizing the gastrointestinal tract of farmed animals, we can distinguish beneficial bacteria (*Bifidobacterium* spp. and *Lactobacillus* spp.), potentially harmful microbes (*Escherichia coli*), and pathogenic bacteria (*Clostridium* and *Staphylococcus*). Contamination of the environment and feed with mycotoxins, stress factors, an unsuitable diet, and medications can have an adverse effect on the intestinal microbiota. Disturbance of intestinal homeostasis can lead to abnormal immune reactions that impair the body's efficiency, resulting in problems with digestion and nutrient absorption. According to Gugołek et al. (2011), good health is ensured by a well-chosen diet – a balanced feed ration that is able to correct dietary deficiencies and satisfy the high feed requirements of animals in various physiological states and periods of rearing.

The beneficial effect of new-generation feed additives on immune function is manifested in part by an increase in the number of bacteria acidifying the intestinal environment (mainly lactic acid bacteria). The practical use of pro-, pre- and synbiotics as feed additives, besides increasing the absorption of nutrients contained in feed, involves regulation of the intestinal microbiota and elimination of pathogenic strains of bacteria and fungi colonizing the digestive tract.

The aim of the study was to analyse the effect of selected pro-, pre- and synbiotic feed additives on health, morphometric parameters of the digestive tract, and microbiological identification of its contents in mink whose feed contains animal by-products.

MATERIAL AND METHODS

The study was carried out on a farm with carnivorous fur-bearing animals, located in south-eastern Poland. The observations were conducted on 423 mink of the pastel variety obtained from 96 females.

The animals were divided into experimental groups. The experimental factor was feed with the addition of pro-, pre- and synbiotic substances influencing the development of bacteria. The feed

additives were used in the amount of 0.2 g/mink/day, as recommended by the manufacturer. The experimental design was as follows:

Group I – control, feed normally used on the farm with no additives

Group II – experimental, feed normally used on the farm with an added probiotic

Group III – experimental, feed normally used on the farm with an added prebiotic

Group IV – experimental, feed normally used on the farm with an added synbiotic

All animals were fed according to standards for the species, and the proportions of the feedstuffs were as follows:

- feedstuffs of animal origin – 80% (10% fish waste, 20% turkey bones, 50% poultry slaughter waste)
- feedstuffs of plant origin – 10% (2% soybean oil, 8% extruded wheat)
- vitamin additives – 1.5%
- haemoglobin – 1.5%
- water 7%

During the reproductive season, the females were mated in a 1-8-9 system. After being weaned from their mothers at the age of about 7 weeks, young mink were kept in pairs in identical cages (0.9 x 0.35 x 0.4 m) in a shed system.

During the experiment, the body weight of the animals was monitored every month and their weight gains were calculated. At the beginning of December, 20 males were slaughtered according to the applicable procedure for the species. Slaughter always took place at the same time, two hours after morning feeding.

After skinning, the length of the carcass was measured from the occipital squama to the base of the tail. The body cavity was opened and the internal organs were taken out and cleaned. The intestines were separated from the stomach and the mesentery was removed. The organs, i.e. the liver, heart, lungs, spleen, kidneys, stomach and digestive tract, were separated and weighed. The individual parts of the digestive tract length were laid out on a moist, impermeable surface, and their length was measured with a measuring tape. The length of the oesophagus was measured from the larynx to the stomach, and the small intestine from the duodenum (where it connects to the pylorus) to the ileocecal valve. The length of the large intestine – the colon and rectum – was measured from the ileal orifice to the anus. The measurements were used to calculate the total length of the intestines and the length of the digestive tract, and the ratio of the length of the intestines and the length of the digestive tract to the length of the carcass was calculated for comparative purposes. A pH meter (Matthaus ph-CPU) was used to determine the pH of the contents of the stomach and intestines.

For the microbiological analysis, biological material (intestinal contents) was collected, cooled, and transported to the laboratory in sterile containers. Then weighted samples (20 g) of the digesta from each feeding group were placed in sterile bottles containing 180 ml of Ringer's solution. The samples were homogenized for 5 min, and the material was allowed to sediment for another 15 min. A series of decimal dilutions was prepared from the resulting suspension in sterile Ringer's solution. Each dilution was surface-plated in the amount of 0.1 ml on a previously prepared microbiological medium. Each sample was prepared in duplicate to ensure reliable results and eliminate sampling errors.

The following were determined in the intestinal contents: total count of aerobic mesophilic bacteria on nutrient agar for 48 h at 37°C; total count of fungi and moulds on Sabouraud agar for 5-

7 days at 25°C; coliform count on Endo LES agar for 18-24 h at 37°C; *E. coli* count on m-FC agar for 24 h at 37°C; and total count of anaerobic bacteria (*Clostridium* spp.) on TSC agar base (bioMérieux), using oxygen absorbers (GENbag Anaerobic, bioMérieux) and indicator strips of oxygen consumption.. The material was incubated for 24 h in anaerobic conditions (Anaerostat). The presence or absence of bacteria of the genus *Salmonella* was determined by plating the material on SS agar. The colonies were evaluated macroscopically to identify bacteria and yeast-like fungi and then transferred to Sabouraud or enriched agar. The colonies were evaluated macroscopically, and Gram staining was performed. The final identification was made using API tests (bioMérieux Polska). Filamentous fungi were analysed macro- and microscopically using a microculture and a key for identification of fungi (Tsuneo Watanabe, 2010). All tests were performed according to standards PN-EN ISO 7218 and PN ISO 4832.

The results were analysed by one-way analysis of variance (ANOVA). Significance of differences between means was estimated using Duncan's multiple range test. The computations were performed using Statistica 13.1 with the following linear model:

$y_{ij} = \mu + a_i + e_{ij}$,
where:
y_{ij} – observed value of feature,
μ – mean value of feature in population,
a_i – effect of experimental treatment,
e_{ij} – sampling error.

RESULTS AND DISCUSSION

Meeting the nutritional needs of animals is the most important environmental factor that can be used to influence their body condition, production results, reproductive parameters, and in the case of mink, the size and quality of their skins. It is currently believed that to achieve success in breeding, it is not sufficient to supply animals with energy and nutrients (protein, carbohydrates, fats, vitamins, and mineral compounds) in a suitably balanced feed ration that meets the highest quality standards. The widespread use of antibiotics and their adverse side effects have raised interest in the use of new-generation feed additives in the diet of animals and people. In the light of current research, health and healing properties are ascribed to these substances (pro-, pre- and synbiotics), as presented in studies by Bengmark (2001), Collins and Gibson (1999), Gugołek (2014), Kapka-Skrzypczak et al. (2012), Kolanowski (1999), Nowak et al. (2010), and Ochmański and Barabasz (1999). According to Wincewicz (2011), dietary supplementation with appropriate bacterial species having confirmed properties can help to maintain homeostasis of the digestive tract and increase tolerance to unfavourable stimuli. It also influences the course of digestion and absorption of nutrients from feed.

Probiotics prevent excessive development of pathogenic microbes in a natural manner, contributing to stabilization of the population of intestinal microorganisms and to enzyme activity in the digestive tract. In this way they ensure optimal digestion and better utilization of feed (Grela and Semeniuk, 1999).

Gibson and Roberfroid (1995) showed that prebiotics have a beneficial effect on the host by stimulating the development of the normal bacterial biota colonizing the digestive tract, particularly the large intestine. They do not exhibit healing properties, but they perform a prophylactic function, positively influencing the health of mink. Gugołek (2014), Jorgensen (1988), and Winiarska and

Gąsiorek (2016) reported that prebiotics modified the intestinal microbiota; reduced the pH of the intestinal contents; increased absorption of mineral compounds, lipid and carbohydrate metabolism, and resistance to bacterial infections; and also helped to prevent infections, diarrhoea and constipation. Synbiotics, which are a combination of a prebiotic and a probiotic, exert a synergistic effect that enhances their individual beneficial effects. The use of a prebiotic together with a probiotic is an alternative means of prophylaxis which both impedes reproduction of pathogens and stimulates the body of the host, increasing its resistance to bacterial infections. The use of a synbiotic usually also increases the rate of growth and improves feed conversion.

Animals were mated from 1 to 20 March, and the young were born following 45-day gestation, at the end of April and beginning of May. The fertilization rate in the mink herd was 89.4% (Table 1). The percentage of females that whelped was 87.5%, while unmated and dead females together amounted to about 3%. The percentage of infertile females was relatively high, at 9.4%. Among the females that whelped, six destroyed their litters. A total of 509 mink kits were obtained, including 476 born alive – on average 5.7 live-born kits per litter among females that whelped. The average number of mink reared per female in the herd was 4.4. The largest litters numbered 10-12 kits. Mortality among kits during the suckling period was about 10.4%, while the percentage reared was 83.1%.

Table 1

Reproductive performance of mink

Females for mating		Females					Number of young				Mortality during maternal nursing period	Percentage of young reared (reared-to-born ratio)
		unmated	infertile	dead	whelped	litter-destroying	total born	live-born	stillborn	reared		
head	96	2	9	1	84	6	509	476	33	423	53	
%	100	2,1	9,4	1	87,5	7,1	5,3* 6,0**	5,0* 5,7**	0,3* 0,4**	4,4* 5,0**	10,4[#]	83,1

*Counted for females intended for mating **Counted for females that whelped

[#]Counted in relation to the total number of young born

The use of animal by-products is permitted in mink feeding. These can potentially be a source of pathogenic microbes. Pregnant females and young animals are particularly susceptible to such infections. Śmielewska-Łoś et al. (2001) reported that the diet and its components can be a source of bacteria (*Salmonella* and *E. coli*), viruses (Aujeszky's disease), parasites (toxoplasmosis) and toxins (botulism). Symptoms of various types of bacteriosis in females include infertility caused by inflammation of the reproductive organs, embryo death, abortion, stillbirths, and deaths in the first few days of life. Kopczewski et al. (2005, 2006), in a study of the causes of unsuccessful reproduction of foxes and mink, determined that most early deaths are caused by bacterial infections or behavioural disorders in females. In the present study, the percentage of infertile females was high – twice as high

Table 2

Body weight (mc) and body weight gains (pmc) of mink by month of rearing (kg)

Mounth		Group I		Group II		Group III		Group IV		SEM*
		♀	♂	♀	♂	♀	♂	♀	♂	
July	mc	1,01	1,73	1,05	1,64	1,1	1,65	1,07	1,69	0,031
August	mc	1,21 A	2,07 C	1,38 B	2,24 D	1,51 B	2,45 E	1,34 B	2,39 E	0,052
	pmc	0,2	0,34	0,33	0,6	0,41	0,8	0,27	0,7	
September	mc	1,40 A	2,54 C	1,65 B	2,91 D	1,73 B	3,22 E	1,66 B	3,11 E	0,074
	pmc	0,39	0,81	0,6	1,27	0,63	1,57	0,59	1,42	
October	mc	1,60 a	3,18 C	1,73	3,14 Cc	1,80 b	3,46 D	1,73	3,33 d	0,081
	pmc	0,59	1,45	0,68	1,5	0,7	1,81	0,66	1,64	
November	mc	1,55 A	3,19 C	1,78 B	3,34 CEc	1,86 B	3,62 D	1,82 B	3,54 DEd	0,04
	(min-max)	(1,45-1,76)	(2,88-3,64)	(1,58-2,00)	(2,70-4,12)	(1,62-2,20)	(3,20-4,24)	(1,60-2,54)	(3,00-4,10)	0,098
	pmc	0,54	1,46	0,73	1,7	0,76	1,97	0,75	1,85	

Means in rows with different letters are significantly different, separately for males and females:
a – $P \leq 0.05$; A – $P \leq 0.01$
* SEM – standard error of the mean calculated from the formula $SEM = s:\sqrt{n}$
where
s – standard error
n – number of observations

as the average for pastel mink in Poland in herds subject to use and breeding value assessment (4.6% of females in 2016-18), while deaths were 0.6% lower. The percentage of mink reared during the study period was also 6.3% lower than in herds subject to assessment by the National Animal Breeding Centre (KCHZ) (2017, 2018, 2019).

After being weaned from their mothers, the young mink were divided into four groups according to the feed additive used in the diet. The body weight of the kits at weaning was uniform for each sex between groups (Table 2). In subsequent months, differences in body weight were confirmed between groups for females and males (August and September $P \leq 0.01$; October and November $P \leq 0.01$ and $P \leq 0.05$). On reaching winter fur maturity, the females weighed from 1.45 to 2.54 kg and the males from 2.70 to 4.24 kg.

Based on the body weight of the animals, the average monthly weight gains were calculated. They were found to be higher in the experimental groups in August and September, but in the controls in October (Table 2). The females in group I were characterized by uniform weight gains, on average 0.20 kg per month, except for November. Greater fluctuations were observed in the females in the remaining groups (II-IV) – from 0.05 to 0.41 kg on average. In the males from the control group, weight gains increased with age (up to October), while weight gain in the experimental groups was lowest in October and November. For the entire rearing period, the greatest weight gain was noted in the group III mink, which had a prebiotic added to their diet – on average 760 g for females and 1970 g for males.

The average body weight of mink attained during winter fur maturity was high, corresponding to the 'very large' size in the Conformation Evaluation Standard (2009), except for about 9.1% of females from group I (control), which were classified as large. According to the above-mentioned standard, mink of the pastel variety are classified as very large if they reach a body weight above 2700 g in the case of males and above 1500 g in the case of females.

The body weight of the males at slaughter ranged from 2.0 to 3.4 kg, with an average of 2.70-2.90 kg (Table 3). The average carcass weight was similar in all groups, while the weight of the skin was similar in the experimental groups and about 20.0-25.6% lower in the control group. Differences in the weight of individual organs in the groups were relatively small, reaching 5.5 g in the case of the heart, lungs, and spleen and 10.0 g for the kidneys and stomach. Greater differences were noted for the digestive tract – about 16.0 g – and the liver – about 31.0 g. The differences in the weight of individual organs between groups were statistically confirmed ($P \leq 0.01$ and $P \leq 0.05$), except for the lungs and spleen.

Morphometric measurements of individual organs and segments of the digestive tract carried out by Kowalska et al. (2014, 2015) and Kulawik et al. (2013) indicate substantial differences between farmed and wild animals. This is largely due to the type of food they eat, the rhythm and quantity of food intake, and seasonal changes in the number of intestinal bacteria or parasites. In the present study, highly significant and significant differences were found between groups for all measurable features of the digestive tract except for the stomach, and in the case of all organs except the lungs and spleen.

One of the most important organs of the digestive system is the liver, which accounts for 1% to 5% of body weight, depending on the species. In farmed mink it is usually 2.2% to 3.0%, which corresponds to a weight of about 50 g in males and about 30 g in females (Gugołek and Gugołek, 2019). In the present study, liver weight was higher, ranging from 3.5% to 4.3% of the body weight of the mink, depending on the diet. The individuals in group III had the highest liver weight – on average about 126 g.

The length of individual segments of the digestive tract varied depending on the diet used. The greatest differences were noted for the length of the large and small intestine and the total length of the digestive tract (Table 4). The average spread of the measurements was 5.4 cm in the first case, i.e. 46.2%, 71.0 cm in the second case, or 26.9%, and 65.8 cm in the last case – 21.5%. These differences were statistically confirmed ($P \leq 0.01$ and $P \leq 0.05$). The small intestine and the entire digestive tract in December were longest in group IV, 5.7% and 5.1% shorter in group III, and the shortest in group I (26.9% and 21.5% shorter than in group I). The reverse tendency was observed for the length of the large intestine, which was longest in the control group I. Statistically highly significant differences were confirmed for the pH of the stomach and intestines of the mink at slaughter. These differences were 4.7-5.9 and 4.8-6.5, respectively. The pH of the stomach was highest in group II and the pH of the intestines in group I, while both were lowest in group III.

Due to the presence of hydrochloric acid in the stomach, there is an acidic environment whose effect consists in disinfection of food, protein denaturation, and activation of certain enzymes. In the present study, the pH of the stomach contents at slaughter ranged from 4.4 to 5.9, and the pH determined in the laboratory ranged from 2.3 to 3.6, revealing a deficiency of hydrochloric acid. If the stomach does not produce enough digestive juices containing pepsin, the food cannot be fully digested. In conditions of hypochlorhydria, it is supply of iron to mink that is most difficult, because iron is mainly contained in meat, which is difficult to digest. Impaired absorption of magnesium, zinc (problems with the skin and coat), and calcium may occur as well.

Analysis of measurements of the digestive system has been undertaken by Vhile et al. (2005), Szymeczko (2001), and Gugołek and Gugołek (2018), who showed differences in the length of the intestines between various animal species, which is closely linked to their diet. In foxes and mink, the ratio of the length of the intestines to body length is 3-5:1, while among herbivores it ranges from 10:1 in rabbits to 25:1 in sheep. In mink, the ratio of the length of the digestive tract to body length differs depending on sex – from 4.2 to 5.1 in females and from 5.2 to 5.9 in males. In the present study, the ratio of intestinal length to body length was 4.3-5.5, while the ratio of the length of the digestive tract to body length was 5.1-6.3. These results correspond to those obtained in the present study for groups I, II and III. Only in group IV, which received a synbiotic, were the ratios higher in both cases.

The overall condition of farm animals and their usability depends largely on the functioning of the digestive tract. The digestive tract is the primary habitat for microorganisms in every living organism, and its microbiota changes during the animal's life depending on its species, age, and physiological state; on the segment of the digestive tract, its structure, pH, and digestive enzymes; and on feeding, immune mechanisms, and medication. The digestive tract, especially the intestines, provides favourable conditions for the development of bacteria: an abundance of food, moisture, and a suitable temperature (36-40°C in mink). A major difficulty in microbiological diagnostics is to distinguish the commensal (physiological) microbiota from pathogenic microbes, especially when we still know so little about the microbiota of the digestive tract of fur-bearing animals. In the present study, microbiological analyses were used to determine the size of selected populations of the intestinal microbiota of farmed mink. The total bacterial count determined in the intestinal contents ranged from 8.5×10^4 to 5.9×10^6 CFU/g (colony-forming unit, which specifies the number of microbes in test material; Table 5). It was highest in group I, where the animals received feed without additives influencing the development of bacteria. The number of mesophilic bacteria was lowest in

Table 3
Post-slaughter measurements

	Weight (kg)			Body/carcass lengh (cm)	Organ (g)						
Group	Mink	Skin	Carcass		Heart	Liver	Lungs	Spleen	Kidneys	Stomach	DT*
I	2,73	0,64	2,09	47,4	18,5 Aa	95,2 Aa	32	13,6	22,3 A	69,2 a	251,0 Aa
II	2,84	0,81	2,03	47	18,3 Aa	107,0 ACbc	28,6	19	31,6 B	72,1 a	246
III	2,93	0,86	2,07	49	21,4 B	125,8 BCd	32,2	16,4	23,3ACa	75,4 Ab	235,3 B
IV	2,9	0,8	2,1	49	19,7 Ab	120,5 Bd	31,6	18,7	30,3 BCb	66,3 B	239,3 b

*DT – digestive tract
Means in columns with different letters are significantly different: a – $P \leq 0.05$; A – $P \leq 0.01$.

Table 4
Measurements of the digestive tract

	Digestive tract (cm)					pH		Intestine length to body/carcass length ratio	Digestive tract length length to body/carcass length ratio
Group	Oesophagus	Stomach	Small intestine	Large intestine	Total length	Stomach	Intestines		
I	26,4 A	9,4	193 A	11,7 A	240,5 Aa	5,85 A	6,51 A	4,3	5,1 : 1
II	22,0 B	9	215 B	6,3 B	252,3 Ab	5,91 A	5,78 B	4,7	5,4 : 1
III	25,0 Ca	9,3	249 C	7,3 B	290,6 B	4,67 B	4,80 C	5,2	5,9 : 1
IV	26,0 ACb	9,3	264 D	7,0 B	306,3 C	5,07 C	5,12 C	5,5	6,3 : 1

Means in columns with different letters are significantly different: a – $P \leq 0.05$; A – $P \leq 0.01$

groups II and III – 2.9 x 10^4 CFU/g and 4.9 x 10^4 CFU/g, respectively. The level of mesophilic bacteria in group IV was 3.8 and 2.2 times as high as in groups II and III, respectively. The concentrations of mesophilic coliforms and *Enterobacteriaceae* bacteria were higher in the control group than in the experimental groups. Among the experimental groups, the lowest number of coliforms was noted in the group receiving probiotics and prebiotics, while the number of *Enterobacteriaceae* was lowest in group III, receiving a prebiotic. The numbers of *E. coli* ranged from 1.9 x 10^4 CFU/g to 4.7 x 10^4 CFU/g, depending on the feeding group, and were highest in the group receiving a synbiotic. The genus *Corynebacterium* was dominant among bacteria identified in all groups. *Actinomyces neuii* also stood out in group I – *10%.*

Table 5

Concentration of microorganisms in the intestinal contents of milk (CFU/g)

Parameter	Intestinal contents			
	Group I	Group II	Group III	Group IV
Total fungal count	3,9 x 10^1	6,2 x 10^1	1,8 x 10^1	6,4 x 10^1
Total bacterial count	5,9 x10^6	8,5 x 10^4	9,4 x 10^4	1,9 x 10^5
Aerobic mesophilic bacteria count	1,7 x10^7	2,9 x 10^4	4,9 x 10^4	1,1 x 10^5
Enterobacteriaceae bacteria	3,0 x10^5	3,6 x10^5	1,8 x10^5	3,0 x10^5
Coliform bacteria count	3,2 x10^6	2,1 x 10^4	2,9 x 10^4	5,9 x 10^4
E. coli count	2,2 x 10^4	1,9 x 10^4	2,6 x 10^4	4,7 x 10^4
Anaerobic bacteria count	4,3 x10^4	3,3 x 10^2	6,4 x 10^1	1,8 x 10^3
Identified bacteria	dominance of *Corynebacterium*			
	Actinomyces neuii – 10%	*Actinomyces neuii – 10%*	*Cellulomonas* sp. – 8%	*S. enterica* subsp. *arizonae* – 5%

In a healthy animal, each part of the intestine is colonized by specific microbiota, which lives in symbiosis with the host. In the microbiota of the digestive tract of sucklings, which mainly consume milk, acidophilic bacteria predominate. The transition from a milk diet to a mixed diet containing more protein increases the number of proteolytic, base-producing bacteria. The presence of bacteria of genera such as *Lactobacillus*, *Enterococcus*, and *Bifidobacterium* is highly beneficial in both the upper and lower digestive tract and results in good overall health and good feed utilization. There are several dozen species of microbes in the digestive tract of mink, although some authors estimate the total number of species occurring in the intestines at over 400. The qualitative composition of this microbiota fluctuates, because with each food ingested a new set of microbes is introduced. According to Brzozowski (2016), the approximate proportions of the bacteria colonizing the digestive tract of healthy animals are as follows: *Enterococcus faecium* (more than half), *Lactobacillus acidophilus* (10-20%), *E. coli* (about 1%) and other species (about one-third). In the

present study, *Corynebacterium* species were the dominant bacteria among those identified in the intestinal contents of mink in all groups.

The composition of the gut microbiota changes in sick animals with symptoms of infection or diarrhoea. According to Brzozowski (2016), a symptom of unfavourable changes is an increase in the proportion of *E. coli* accompanied by a decrease in the numbers of *Enterococcus faecium* and *Lactobacillus acidophilus*. Other pathogens can also be present in the digestive tract, e.g. *Salmonella*, *Staphylococcus*, and some species of *Bacillus* and *Clostridium*.

In the present study, the average concentration of total fungi in the intestinal contents was lowest in group III – 1.8 x 10^1 CFU/g; twice as high in group I; and similar and much higher in groups II and IV – 6.2-6.4 x 10^1 CFU/g.

The macro- and microscopic identification of fungi based on available tests and a key showed that the dominant fungi in the intestinal contents were of the genus *Candida*, predominantly *Candida glabrata* (Table 6). The proportion of fungi of this genus in all groups ranged from 84.5% to 89.5%. The other fungi identified in the digesta were *Rhizopus* spp. (3.5% to 14.7%) and *Aspergillus* spp. (4.4 to 6.1%).

Candida glabrata is a yeast-like fungus that occurs in physiological conditions in animals and humans. It does not cause infections in healthy individuals, but in those with impaired immunity it can foster the development of infections. The most favourable conditions for its development are found in the digestive tract, where it harmlessly supports digestion and repels attacks by bacterial pathogens. Its numbers depend mainly on suitable pH (acid-base balance) and competition in the form of other, friendly microbes.

Table 6

Fungi identified in the intestines of mink (%)

Intestinal contents (% in samples)							
Group I		Group II		Group III		Group IV	
Candida sp.	86,46	*Candida sp.*	89,50	*Candida sp.*	85,33	*Candida sp.*	84,56
Rhizopus sp	9,17	*Aspergillus sp.*	5,43	*Rhizopus sp.*	14,67	*Rhizopus sp.*	9,33
Aspergillus sp.	4,37	*Rhizopus sp.*	3,52			*Aspergillus sp.*	6,11

To sum up, the feed additives used had a positive effect on the health, growth and body weight gains of the mink. During winter fur maturity the females weighed from 1.45 to 2.54 kg, and the males from 2.70 to 4.20 kg. During the rearing period (from weaning to slaughter), the animals in group III gained the most weight – on average 760 g for females and 1970 g for males. The analysis of the morphometric parameters of the digestive tract showed highly significant differences between the means for groups except for the weight of the lungs and spleen and the length of the stomach. A significant increase was noted in the length of the small intestine when the feed additives were used, especially the prebiotic and synbiotic, while the length of the large intestine decreased relative to the control group.

It was also determined that the microbiota of the digestive tract of the mink can be modified by using appropriate feed additives – pro-, pre and synbiotics. Due to the very rapid passage of material

in mink, it is important for the microbes contained in the preparation to have a short generation time. The total bacterial count in the intestinal contents ranged from 8.5 x 10^4 to 5.9 x 10^6 CFU/g and was highest in the control group, without feed additives. The number of mesophilic bacteria (2.9 x 10^4 CFU/g) and the concentration of coliforms were lowest in the group of mink receiving probiotics, while that of bacteria of the family *Enterobacteriaceae* was the highest. The group receiving feed with prebiotics had the lowest counts of total fungi, bacteria of the family *Enterobacteriaceae*, and anaerobic bacteria in the intestinal contents. Compared to the other feeding groups, the animals receiving a synbiotic with their feed had the highest level of total fungi and *E. coli* bacteria. The genus *Candida* predominated among the fungi identified in the intestinal contents, and within this genus, *Candida glabrata*. The proportion of fungi of this genus varied between groups from 84.5% to 89.5%.

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Wpływ substancji pro-, pre- i synbiotycznych na zdrowie, pomiary morfometryczne przewodu pokarmowego oraz wyniki badań mikrobiologicznych treści jelita norek

Streszczenie

Celem przeprowadzonych badań była analiza wpływu wybranych dodatków paszowych: substancji pro-, pre- i synbiotycznych na zdrowie, pomiary morfometryczne przewodu pokarmowego oraz identyfikację mikrobiologiczną treści przewodu pokarmowego norek. Obserwacje prowadzono na norkach odmiany pastel, które żywione były w grupie I – karmą powszechnie stosowaną na fermie bez żadnych dodatków, w grupie II karmą z dodatkiem probiotyku, w III – prebiotyku, a IV – synbiotyku. Stwierdzono wpływ zastosowanych dodatków paszowych na wzrost i przyrosty masy ciała norek. Po osiągnięciu zimowej dojrzałości okrywy włosowej masa ciała samic wahała się od 1,45 do 2,54 kg, a samców od 2,70 do 4,20 kg. Za okres odchowu (odsadzenie-ubój) zwierzęta w grupie III przyrosły najwięcej – samice średnio 760 g, a samce 1970 g. Analiza parametrów morfometrycznych przewodu pokarmowego wykazała wysoko istotne różnice pomiędzy średnimi dla grup, z wyjątkiem masy płuc i śledziony oraz długości żołądka. Średnia koncentracja ogólnej liczby bakterii w treści jelit w grupie II i III była podobna ($8,5\text{-}9,4x10^4$ jtk/g), wyższa w grupie IV i najwyższa w grupie kontrolnej – na poziomie $5,9x10^6$ jtk/g. Wśród zidentyfikowanych bakterii we wszystkich grupach dominowały *Corynebacterium.* U norek otrzymujących paszę z dodatkiem prebiotyku w treści jelit odnotowano najniższy poziom ogólnej liczby grzybów. Przeważały grzyby z rodzaju *Candida*, z dominującym – *Candida glabrata*. Udział grzybów tego rodzaju w poszczególnych grupach był zróżnicowany i wahał się od 84,5 do 89,5%. Pozostałe zidentyfikowane grzyby w treści pokarmowej to: *Rhizopus sp.* i *Aspergillus sp.*

SŁOWA KLUCZOWE: żywienie, dodatki paszowe, badania morfometryczne i mikrobiologiczne