Influence of subalin probiotic on Blue Frost fox intestinal lymphoid tissue formation

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Abstract. For research, Blue Frost fox puppies were used at the age of 2 months. 2 groups of animals of 30 heads in each group were formed by the analog method. The experimental animal group received subalin courses with the basic diet according to the scheme: 5 days of probiotic in the amount of 10 - 20 * 10^7 CFU/kg, for two months in courses with 10 days break, only 4 courses.

1 Introduction

The use of biologically active substances in the fight against negative phenomena in the animals' bodies because of external stress factors' influence, as well as infectious agents of bacterial and viral etiology are relevant in the livestock industry.

Biologically active substances are used along with the improvement of existing breeds and selection methods of young breeder in fur-farming to reduce the cost value and increase the efficiency of fodder use; these substances contribute to reducing the oxidation processes' intensity of fodder fats and correcting metabolic processes in the body of cage fur animals and normalize the balance of gastrointestinal tract microbiota [8].

The large-scale study of probiotic preparations' influence on the animal body in the fur-farming industry and obtaining positive results of their application on different fur animals types were indicated in works on minks by Tinaev N.N and Emelianenko P.A. [9], nutria feeding by Barabash B., Sheleshchuk O. [2], silver foxes by Goryachev A.A. et al.

Particular attention is paid to the manifestation of economical (zootechnical) traits, such as: fertilization, fecundity of females, growth and preservation of young, quality of skins, etc.

One of the promising areas of probiotic biopreparations reconstruction is the use of bacteria from the Bacillus genus [12, 13, 14]. Due to its high adaptive and antagonistic properties, wide distribution in nature particularly in forages and external objects with which animals contact most closely, these spore-forming bacteria are an important component of

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transient microflora. Subalin probiotic includes a recombinant strain producing interferon [3, 4,]. Bacteria of the recombinant strain retained high antagonistic activity of the parent and acquired the ability to prolong interferon synthesis (within 1-4 days) in the gastrointestinal tract . This provides a high degree of antiviral and immunomodulating activity. According to studies in assessing the balance of the gastrointestinal tract microbiota [5], high antagonistic activity of the probiotic strain was established in relation to streptococcal and intestinal groups of microorganisms (diameters of growth delay zones 22-25 mm). The microbiota Blue Frost fox puppies has the highest sensitivity to the preparation's action (diameters of growth delay zones 19-25 mm). When studying the biocenosis dynamics during subalin feeding, a significant increase in the resident microorganisms' concentration of the gastrointestinal tract was found: bifido- and lactobacilli; also, it is a pronounced antagonist against pathogenic and opportunistic microorganisms.

The purpose of this study was to establish the regularities of subalin influence on the development of lymphoid tissue of the Blue Frost fox intestine in the postnatal period.

2 Materials and methods

For research, Blue Frost fox puppies were used at the age of 2 months. 2 groups of animals of 30 heads in each group were formed by the analog method. The experimental animal group received subalin with the feed according to the scheme: 5 days of probiotic in the amount of 10 - 20 * 10^7 CFU/kg for 5 days with 10 days break (4 courses).

The material for the study was the sets of the gastrointestinal tract (from the pyloric stomach part to the distal rectum part) obtained during the slaughter of Blue Frost fox in technological conditions. The age of fur animals was dated according to the record of pedigree journals of zootechnical animal farm accounting.

The main methods of morphological study of lymphoid tissue were macroanatomical and morphometric. According to the method of T. Gelman

The resulting intestine sets were washed in running water for 30-40 minutes, stained with 1% Harris' hematoxylin solution, differentiated with 3% acetic acid solution. The small intestine (duodenal, jejunum, ileum) and colon (cecum, colon and rectum) were straightened, its length was measured, then it was cut along the mesenteric edge and the width was measured, then planar whole mounts were prepared by the method of T. Gelman [10].

The total number of single or platelet-grouped lymphoid nodules, their number per 1 cm^2 of the mucous membrane and in each lymphoid platelets in 11 visual fields were determined on whole mounts of the intestinal tube in transmitted light in its own plate of the mucous membrane and in the submucosa. Morphometry was carried out considering the size, shape, topography, local location features, the distance between all Peyer's platelets [11].

Measurements of lymphoid formations were made by microcaliper, measuring the length and width of the intestinal tube - millimeter ruler.

Digital materials were processed using the statistical software package “Statgraphics” and “HG”. Statistical processing of digital materials was carried out using a package of statistical programs “Statgraphics” and “HG”. The reliability of statistical samples" indicators was assessed according to the Steudent criterion (G.F. Lakin, 1981). Validation criterion ($P<0.001$, $P<0.01$, $P<0.05$).

Experimental part on animals was carried out in accordance with the basics of experimenting in animal husbandry, methodical instructions on the production of scientific and economic experiments on fur animals' feeding. Studies were performed in compliance with the international principles of the Helsinki Declaration on humanist attitude to animals, the principles of humanity set out in the European Community Directive (86/609/EC), with observance of the “Rules for work using experimental animals” [Rules for work using

experimental animals. Annex to the Order of the Ministry of Health of the USSR No. 755 dated 12.08.1977].

3 Results of the study

In the study of the small intestine in 8 month old Blue Frost fox puppies, it was found that in the proximal duodenum part there were lymphoid formations in the form of a ring (Fig. 1, 2).

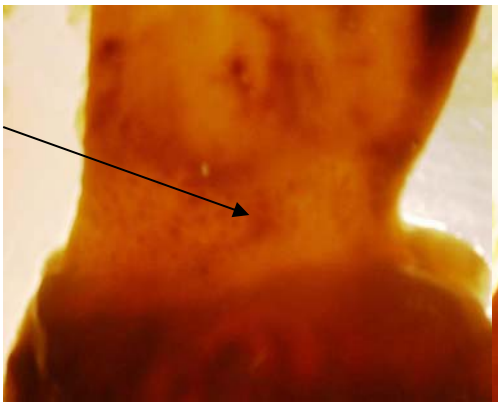

Fig. 1. Lymphoid ring in the proximal duodenum part of the control animal group (arrow).

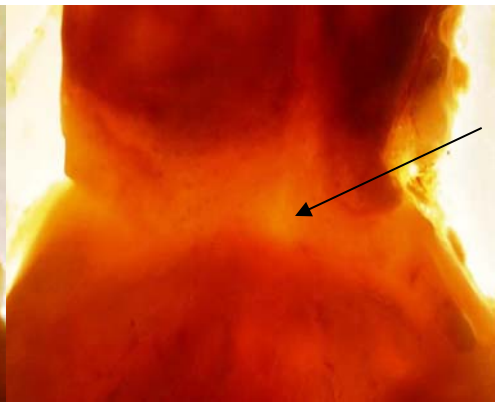

Fig. 2 Lymphoid ring in the proximal duodenum part of the experimental animal group (arrow).

The area of the lymphoid ring in animals of the experimental and control groups amounted to 2.9 and 2.8 $cm^{2,}$ respectively (Fig. 1, 2). At the same time, the density of lymphoid nodules in the lymphoid duodenum ring of the experimental group was 15.48 cm^2, and in the control group - 29.64 $cm^{2.}$

The obtained data on the anatomic-topographic features' study of lymphoid tissue in Blue Frost fox puppies was compared with established norms. The area of different intestine sections of experimental and control groups was compared with the results of the study and established norms by Sintsova N.A., (2005), indicated in table 1.

Table 1. Relative area of intestinal tube sections in Blue Frost fox puppies.

Bowel segment cm^2 (n=6)	Experiment	Control	Norm
Duodenum	81.5 ± 6.4**	61.15 ± 9.8	84.43 ± 3.95
Jejunum	691.6 ± 58.3**	633.0 ± 25.72	538.44 ± 28.23
Ileum	58.0 ± 2.12**	49.2 ± 2.95	25.60 ± 2.12
Cecum	29.25±3.74	24.52±5.25	32.98±2.87
Colon	126.5±3.8*	118.7±2.6*	91.24±6.86
Rectum	40.4±1.5*	51.4±2.3*	57.62±6.91
Small intestine area	828.5±21.75*	745.35±26.25*	648.67±31.75
Total colon	198.24±11.87*	192.63±10.34*	181.84±12.34
Area of all sections	1027.25±25.5*	919.97±15.6*	830.31±38.83

Note: differences between the foxes' groups are reliable at *-P<0.05, ** - P<0.01, ***- P<0.001

When analyzing the data of table 1, it was found that the area of the duodenal section was 7.4% larger, and the jejunum area was significantly larger by 9.25% compared to the control, and in comparison with the norm - by 20%, the ileum section area - by 17% compared to control and 22% compared to the norm.

In the study of anatomic-topographic features of lymphoid formations' distribution in the form of grouped lymphoid nodules clusters (table 2), in the small intestine wall of the

experimental group animals' lymphoid platelets area was 24% (P<0.01) less than in the control group animals, also the number of lymphoid nodules reliably (P<0.05) decreases in the experimental group by 23.8%. At the same time, the average distance between lymphoid platelets in jejunum decreases by 18% (P<0.05) in the experimental group's animals.

Lymphoid tissue in the ilium wall is represented by a linguiform platelet from 90 to 150 mm long with width from 24 (widest part) up to 5 mm. At the same time, it was established that the area of the linguiform platelet and the number of lymphoid nodules in it in experimental group's animals was reliably higher by 28% compared to control, respectively.

Table 2. Morphometry of grouped lymphoid formations, the distance between them in the small intestine wall and the number of lymphoid nodules.

Indicators	Grouped lymphoid formations of the colon wall			Linguiform platelet of the ileum wall	
	Total area	Total nodules	Average distance	Total area	Total nodules
Experiment	21.38±2.57**	3634.6	11.24 ± 0.43*	24.73 ± 3.78*	4204.1 ±354.4**
Control	28.01 ± 2.57**	4767.7	13.07 ± 0.59*	19.3 ± 1.16*	3281 ±625.2**
Norm	26.75	4760.66	14.89 ± 2.76	27.56 ± 3.32	4125 ± 495.0

Note: differences between the foxes' groups are reliable at *-P<0.05, ** - P<0.01, ***- P<0.001

As it can be seen from table 3, single lymphoid nodules were found in the cecum located diffusely in their own plate, both in the submucosa in the control group animals and in experimental group puppies. The density of lymphoid nodules in the submucosa at 1 cm^2 in experimental group animals was 4 times higher than control (P<0,05).

When determining single lymphoid formations in the colon wall, the density of lymphogranular complexes per 1 cm^2 was 3.0±0.7 in the animals of the control group, in the experimental - 2.2±0.44 (Table 3).

In the proximal rectum part in animals of experimental groups in their own plate and in the submucous layer, significantly fewer single lymphoid formations were noted in comparison with control by 70% (P< 0.05), in distal part - by 68% (P<0.05).

Table 3. Density of single lymphoid formations per 1 cm^2 in the colon wall.

Intestine sections	localization	experiment	control	Norm
Cecum	own platelet	2.4±0.7*	3.4±0.54*	3.64±0.4
	submucosa	1.6±0.54*	0.4±0.17*	
Colon	submucosa	2.2±0.44*	3.0±0.7*	2.27±0.24
Rectum	proximal part	1.2±0.44*	4.0±0.7*	3.75±0.16
	distal part	22±0.45**	68±0.7**	84.6±24.12**

Note: differences between groups of foxes are valid *-P<0.05, ** - P<0.01, ***- P<0.001

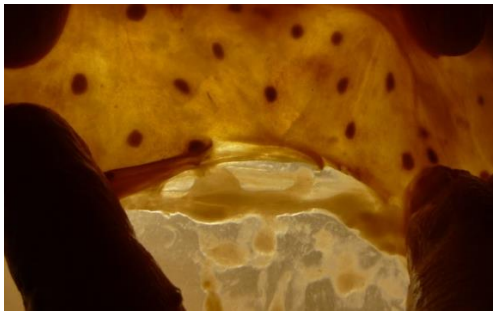

Fig. 3. Lymphoid formations of the cecum of control group animals.

Fig. 4 Lymphoid formations of the cecum of control group animals.

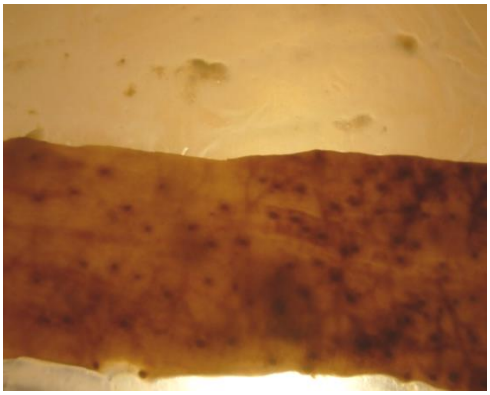

Fig. 5. Lymphoid formations of the rectum of control group animals.

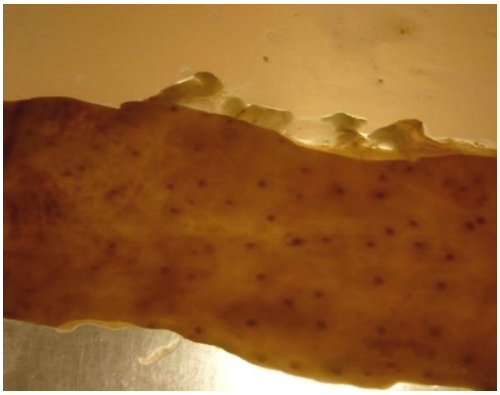

Fig. 6. Lymphoid formations of the rectum of control group animals.

4 Conclusion

Thus, the formation of lymphoid tissue and its anatomic-topographic features in Blue Frost fox puppies occurs during postnatal ontogenesis. The development peculiarities can be influenced by a number of factors: conditions of keeping, feeding and biological preparations use. It has been established that prolonged use of subalin probiotic influences the formation and development of lymphoid tissue of Blue Frost fox puppies' intestines due to a wide range of antagonistic, enzyme properties, as well as the ability to produce *α 2 -interferon* directly into the lumen of the intestine.

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