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# Defence mechanisms in fish: frequency of the genus *Lactobacillus* bacteria in the intestinal tract microflora of carps

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The lactoflora of the wall and the content of the digestive tract of carps (*Cyprinus carpio* L.) has been investigated. The 65 fish individuals tested belonged to three age groups: 20 were yearlings, 25 were two and 20 three years old. The intestine of each individual was investigated separately. Bacteria of the genus *Lactobacillus* were detected in the content of the digestive tract of 42 (64.61%) fish and in the wall of the digestive tract of 22 (33.84%) fish. Lactobacilli were found most frequently in the content of the digestive tract throughout its whole length (11) and in the wall of its mid part (9). Analysis of the data allowing to determine the distribution of carp intestine lactoflora strains into groups has shown that both in the digestive tract content and in its wall group II, *i.e.* facultative heterofermentative lactobacilli prevailed. They were found in intestine content of 38 carps and in the wall microflora of 13 carps. Only lactobacilli of II (17) and II + III (10) groups were detected most frequently in the content of carp digestive tract, meanwhile lactoacid bacilli of group II a were detected only in the wall. The obtained results show that the genus *Lactobacillus* bacteria can occur as part of normal digestive tract microflora in carps. They are found as an important constituent part in the microbiocenosis of the content and wall of separate digestive tract segments of carp individuals of different age.

**Key words:** *Lactobacilli*, digestive tract, wall, content, microflora, carps

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## INTRODUCTION

The carp is one of the most important objects of commercial pisciculture. The breeding of carps under conditions of intensive aquaculture causes essential changes in the physico-chemical properties of a pond ecosystem as well as in the structure of planctonic and benthic organism's biocenosis. The unusual (if compared with natural) living and rearing conditions, frequent stressogenic situations often evoke structural changes in the digestive tract microbiocenosis of fish. These changes result in dysbiosis, a complex pathological condition. Their eventual effects are reflected in the indices of fish growing, survival and productivity.

Nowadays the digestive tract ecosystem is considered as the protective system of the organism, and the genus *Lactobacillus* bacteria play a significant role in it.

A macroorganism and its microflora are in the state of a dynamic equilibrium which has settled and strengthened in the course of a long evolutionary development. In a physiologically normal state

the organism and its microflora maintain tight symbiotic relations. The normal microflora of the digestive tract plays the main role in the formation of immunobiological activity of the organism. It is characterized by a clearcut morphokinetic effect, it synthesizes a series of enzymes and vitamins, utilizes nutritive substances with the formation of unsubstituted amino acids such as triptophan [1–3]. Of particular significance is the indigenous, residential, *i.e.* wall microflora and its important constituent part – the lactoflora. It forms a natural ecological barrier which is one of the factors of organism's resistance [4, 5]. Besides, the *Lactobacillus* bacteria are characterized by a direct antimicrobial activity and an influence on other protective mechanisms of the organism [5, 6].

The importance of the functions performed by the microflora and lactoflora under conditions of eubiosis and their essential changes in cases of dysbiosis attract researchers' attention and stimulate a search for the means to normalize digestive tract biocenosis with the aid of bacterial preparations [7–9]. On the other hand, application of antibiotics in

the treatment of diseases of bacterial origin in fish often fails to give the expected result. Therefore justifiable is the ever-increasing interest in probiotics as a means of treatment and prophylaxis of digestive tract diseases, in the possibilities and perspectives of their application [10, 11].

However, the search for the means of treatment of probiotic origin, their testing and application require exhaustive studies of digestive tract microflora.

Scientific publications concerning the digestive tract lactoflora of humans and warm-blooded animals are rather numerous [12, 13], however, data on the digestive tract lactoflora in fish are not abundant [14, 15], besides, more often than not they are inconsistent or just limited to a mere stating of facts.

We failed to find any study results that could be helpful in answering the questions whether the genus *Lactobacillus* bacteria inhabit the digestive tract microflora in all individuals of the same and of different fish species, how they are distributed in the digestive tract, whether they can be part of intestine wall microflora, etc.

The aim of the present work was to elucidate the frequency of lactoflora distribution in commercial carp populations reared in ponds, to study the lactobacilli strain occurrence in separate segments of the digestive tract of young fish, to determine their taxonomic grouping and the distribution of the representatives of these groups in fish intestine microflora.

## MATERIALS AND METHODS

The lactoflora of the content of separate segments of the digestive tract and of intestine wall has been studied in 65 carps (*Cyprinus carpio* L.) obtained from the ponds of Lithuanian fish-breeding farms.

The test fish individuals were visually healthy and belonged to three age groups: 20 of them were yearlings, 25 were two and 20 three years old.

The fishes were dissected under sterile conditions. Intestine content and wall samples were taken from three segments of their digestive tract – the fore, mid and hind parts.

Suspensions and dilutions ( $10^{-2}$ – $10^{-6}$ ) of test substances were prepared using the Haenel, Müller-Beuthow buffer solution [16].

To elucidate the ability of lactobacilli to join intestine wall microflora and in order to obtain unquestionable results, intestine wall homogenates were prepared especially carefully and with great responsibility. With a sterile scalpel the upper layer of the intestine mucous membrane was removed, with only the serous film left. The latter was three times washed with the above-mentioned buffer solution. Then, using fine sterile glass particles it was ground into

a homogeneous mass, diluted and thus prepared for primary sowing [17].

0.05 ml of each test substance was sown. For primary lactoflora sowing, selective MRS-4 and MRS-5 media modified by Lenzner were used [17]. The sown up plates were incubated in anaerostates at a temperature of 24 °C and 37 °C for 5 to 10 days.

The isolated strains of lactobacilli were identified [18] and grouped according to Kandler and Weiss [19].

## RESULTS AND DISCUSSION

Lactobacilli are known to comprise a constituent part of natural intestine microflora in some fish species such as cod (*G. morhua*), saithe (*Pollachius virens*), capelin (*Mallotus villosus*), herring (*C. harengus*), Atlantic salmon (*Salmo salar*), rainbow trout (*Salmo gairdneri*), wolffish (*Anarhichas lupus*) [14, 20–23].

Part of scientific publications report on seasonal variations in the lactoflora quantitative and species composition and note the highest level of this bacterium population in fish intestine to occur in the middle of summer [15, 24, 25].

Attention also has been given to age-related changes in intestine lactoflora population. Lactobacilli were detected in the intestine of 4-day-old Atlantic cod larvae and fry, as well as in the intestine microflora of young Atlantic salmon. All the strains isolated were identified as *L. plantarum* [14, 15].

In the present study, as a result of investigating the content of digestive tract and the lactoflora of intestine wall in 65 carp individuals, the genus *Lactobacillus* bacteria have been found in the microflora of intestine content in 42 (64.61%) carps and of intestine wall in 22 (33.84%) carps ( $P < 0.01$ ).

Lactoflora studies in separate intestine segments have revealed the presence of lactobacilli in the content of the fore part in 24 carps, of the mid part in 25 and of the hind part in 25 carps and in intestine wall microflora in 7, 11 and 7 carps, respectively ( $P < 0.01$ ).

Lactobacilli have been detected most frequently in the content of the digestive tract throughout its whole length (11), *i.e.* in all three segments, and in the wall of the mid part (9) (Table 1).

The lactoflora occurrence in the content of separate parts of intestine and in its wall has been found to be influenced by the age of the carps studied and by related changes in their nutrition. Lactobacilli in yearling carps have been detected more frequently in the content of the fore and mid intestine segments than in the hind part and have been found most frequently localized in the wall of the mid segment ( $P < 0.01$ ).

**Table 1. Lactobacilli frequency in the microflora of different digestive tract segments of carp individuals of different age**

Intestine segments	Number of carps							
	yearlings		aged 2 years		aged 3 years		total	
	content	wall	content	wall	content	wall	content	wall
a	3	2	2	1	1	1	6	4
b	3	4	3	3	1	2	7	9
c	2	2	4	2	2	2	8	6
a+b	1	1	2	1	1	0	4	2
a+c	2	0	1	1	0	0	3	1
b+c	1	0	0	0	2	0	3	0
a+b+c	1	0	5	0	5	0	11	0
Total	13	9	17	8	12	5	42	22

a – fore part of digestive tract, b – mid part of digestive tract, c – hind part of digestive tract.

According to the data of some authors [24, 25], carbohydrates remain accumulated in the mid and hind segments of fish digestive tract. This could be an explanation for a more frequent occurrence of lactobacilli in the wall of the hind segments of carp intestines. Nevertheless, a significant part of the microorganisms under study occur in the fore parts of carp intestine and participate in the process of intensive destruction of nutrient substances in these parts, thus providing favourable conditions for the lactobacilli to feed.

Analysis of the data allowing to determine the distribution of carp intestine lactoflora strains into groups has shown that both in intestine content and wall group II, *i.e.* facultative heterofermentative lactobacilli prevailed (Table 2).

They were found in 38 carps from 42 in whose intestine content lactobacilli were determined, and in 13 carps of 22 they inhabited wall microflora ( $P < 0.01$ ). In 16 carps from 42, in the digestive tract content group III and in 12 from 42 group I lactic acid bacilli were found ( $P < 0.01$ ). The fre-

quency of group I and III lactobacilli in carp intestine wall microflora was nearly the same (Table 2).

The occurrence of separate lactobacilli groups in the content and wall of digestive tract has been found to be age-dependent. In intestine content of yearling and two-year-old carps group III lactobacilli were more frequent than group I, while in three-year old carps group I was more frequent than group III ( $P < 0.05$ ). In the intestine wall of yearling carps group III lactobacilli are more frequent than group II and I, in two-year-old carps group II prevailed, and in three-year-old carps group I was predominant ( $P < 0.05$ ) (Table 2). This implies the existence in carp intestine content and wall of some regulative mechanisms depending on age and age-related changes in the nutrition ration.

It was of interest to elucidate the prevailing lactobacilli grouping in carp digestive tract content and wall. The results showed that most frequent were only group II (17) and II + III (10) lactic acid bacilli. In three carp individuals, the intestine content harboured lactobacilli of all the three groups.

**Table 2. Occurrence of representatives of lactobacilli groups in digestive tract content and wall microflora of carp individuals of different age**

Lactobacilli groups	Number of carps							
	Content			Total	Wall			Total
	yearlings	aged 2 years	aged 3 years		yearlings	aged 2 years	aged 3 years	
I	3	4	5	12	2	2	4	8
II	12	15	11	38	4	6	3	13
III	7	8	1	16	5	1	1	7

I – obligately homofermentative representatives of the genus *Lactobacillus*, II – facultatively heterofermentative representatives of the genus *Lactobacillus*, III – obligately heterofermentative representatives of the genus *Lactobacillus*.

Table 3. Occurrence of separate lactobacilli groups and their combinations in digestive tract content and wall microflora of carp individuals of different age

Lactobacilli groups	Number of carps							
	yearlings		2 years old		3 years old		total	
	content	wall	content	wall	content	wall	content	wall
I	0	1	0	1	1	2	1	4
II	5	3	5	5	7	1	17	9
III	1	4	2	1	0	0	3	5
I+II	1	0	4	1	3	1	8	2
I+III	0	0	0	0	0	0	0	0
II+III	4	0	6	0	0	0	10	0
I+II+III	2	1	0	0	1	1	3	2
Total	13	9	17	8	12	5	42	22

I – obligately homofermentative lactobacilli, II – facultatively heterofermentative lactobacilli, III – obligately heterofermentative lactobacilli.

In carp digestive tract wall, analogously to content, only group II lactobacilli were found most often (9). From the intestine wall of two carps, lactic acid bacilli of all the three groups were isolated (Table 3).

In carps of different age, the frequency of separate lactobacilli groups and their combinations in intestine content and wall differed: in yearlings, in digestive tract content only group II (5) and II+III (4) lactobacilli and in the wall only groups III (4) and II (3) occurred with similar frequency. In two-year-old carps, like in yearlings, in intestine content only group II (5) and II+III (6) lactobacilli occurred at nearly the same frequency, whereas in intestine wall microflora only group II (5) lactic acid bacilli were predominant ( $P < 0.05$ ).

In the intestine content of three-year-old carps, most frequent were only group II (7) lactobacilli, however, in intestine wall only group I (2) lactic acid bacilli were found somewhat more frequently than other groups or their combinations ( $P < 0.05$ ) (Table 3).

The above-presented results show that the genus *Lactobacillus* bacteria can occur as part of normal digestive tract microflora in carps. They are found as a constituent part in the biocenosis of the content and wall of separate digestive tract segments of carp individuals of different age.

The further studies should be aimed at revealing the factors determining the lactobacilli occurrence in the digestive tract of fish, to study the properties of their isolated strains with the perspective to use those most active as probiotics for normalization of intestine microflora in commercial fish.

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#### ŽUVŲ APSAUGINIAI MECHANIZMAI: *LACTOBACILLUS* GENTIES BAKTERIJOS KARPIŲ VIRŠKINAMOJO TRAKTO MIKROFLOROJE

#### S a n t r a u k a

Tirta karpių (*Cyprinus carpio* L.) atskirų virškinamojo trakto atkarpų turinio ir sienelės laktoflora. 65 individai buvo trijų amžiaus grupių: 20 – šiųmetukai, 25 – dvišariai ir 20 – trivasariai karpiai. Kiekvieną žuvį ištyrus atskirai, laktobacilos buvo konstatuotos 42 (64,61%) karpių žarny-

no turinio ir 22 (33,84%) – sienelės mikrofloroje. Dažniausiai pienarūgštės lazdelės aptinkamos karpių žarnyno turinyje per visą virškinamojo trakto ilgį, t. y. visose trijose jo atkarpose (11) ir vidurinės atkarpos sienelėje (9). Tiek žarnyno turinyje, tiek sienelėje vyravo II grupės, t. y. fakultatyvinės heterofermentinės, laktobacilos. Jos buvo rastos 38 karpių žarnyno turinio ir 13 – sienelės mikrofloroje. Tyrimais išaiškinta, kad žuvų virškinamojo trakto turinio mikrobiocenozėje dažniausiai aptinkamos tik II (17) ir II + III (10) grupių pienarūgštės lazdelės, tuo tarpu žarnyno sienelėje vyravo II (9) grupės laktobacilos. Gauti rezultatai rodo, kad *Lactobacillus* genties bakterijų gali būti žuvų normalioje virškinamojo trakto mikrofloroje. Kaip svarbi sudėtinė virškinamojo trakto mikrobiocenozės dalis jos yra randamos įvairaus amžiaus karpių atskirų virškinamojo trakto atkarpų turinio ir sienelės mikrofloroje.

**Raktažodžiai:** *Lactobacillus* genties bakterijos, virškinamasis traktas, sienelė, turinys, mikroflora, karpiai