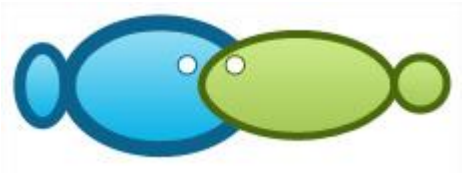

Digestibility of productive carp feeds under the effect of mannan oligosaccharide

[1]Olha P. Dobrianska, [1]Yurii M. Zabytivskyi, [2]Olga V. Deren

[1] Lviv Research Station of the Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine, Velykyi Lubin, Ukraine; [2] Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine. Corresponding author: O. P. Dobrianska, olya_dobryanska@ukr.net

Abstract. Carp is one of the main objects in Ukrainian aquaculture. The basis of the diet of carp in an intensive cultivation technology consists of components with low digestibility. Therefore, finding the means to increase feed conversion in fish rearing process is the topical issue. Applying modern technological methods made it possible to identify and isolate unique sugars present in the cell wall of a specific yeast strain *Saccharomyces cerevisiae*. These developments allowed producing a prebiotic of a new generation of mannan oligosaccharides – Actigen® (Alltech Inc., USA). According to the spectrum of action of mannan oligosaccharides, after getting into the digestive tract of animals the prebiotic normalizes mucin production that promotes the development of a healthy surface of the intestinal villi needed for a better absorption of nutrients. This, in turn, contributes to the improvement of the digestive tract functioning, to an effective stimulation of the immune system and, consequently, increases the productivity during fish cultivation. This work presents the results of studies of the activity of digestive enzymes in the intestine of age -1+ carp, which were fed with a feed based on cereals (Control) and a feed supplemented with the prebiotic at a rate of: 0.025% (Experiment 1), 0.05% (Experiment 2) and 0.075% (Experiment 3). The activity of α-amylase in fish of Experiment 3 was found to be higher by 14.33 and 9.92% compared to Experiment 1 and Experiment 2, respectively. Prebiotic at a rate of 0.075% stimulated carbohydrase activity of carp intestine. The use of prebiotics at a rate of 0.025 and 0.05% of the basic diet does not create stress, which would cause a compensatory effect of transaminases, while an increase in the concentration of the supplement to 0.075% causes a decrease in amino acid metabolism in the carp's intestinal cells. Alkaline phosphatase (AP) activity in intestinal cells probably does not change under the action of different concentrations of the experimental supplement in the feed. The carbohydrase system is responsible for the efficiency of cleavage and transportation of carbohydrate hydrolysis products and of the enzymes, which are integral proteins. Since it has the ability to accelerate the processes of carbohydrate hydrolysis and transport, the recorded increase in its activity make it possible to use feed resources in fish farming more efficiently.

Key Words: carp, prebiotic, enzymes, α-amylase, alanine aminotransferase, alkaline phosphatase.

Introduction. Productivity and quality of fish products depend first of all on the physiological state of the fish organism, which, in its turn, is largely defined by the condition of the digestive system and the composition of the intestinal microflora (Chernikova & Ponomarenko 2016). Taking into account that the efficiency of feed consumption and the feed conversion ratio depend on the activity of the processes of disintegration of feed components and their subsequent absorption in the intestinal tract, fish growth depends directly on the efficiency of digestion processes (Dekhtiarov et al 2008).

To increase the activity of the protective intestinal microflora in animal husbandry and fish farming, farmers often use prebiotic products, which have a positive effect on the productivity and survival of animals, and have a stimulating effect on their immune system by suppressing opportunistic pathogenic microflora (Ringo et al 2010; Bentea et al 2014). In this context, Actigen (Alltech, Inc production) is a promising preparation, which is a "second" generation prebiotic containing mannose. Actigen is a highly purified, concentrated and specifically controlled fraction of carbohydrates, namely mannan oligosaccharides, which are obtained from the exterior walls of cells of *Saccharomyces cerevisiae* (Spring et al 2015; Chernikova & Prokopenko 2017).

One of the first studies of this product in aquaculture was to assess the effect of Actigen on the survival of pangasius artificially infected with bacteria *Edwardsiella ictaluri* (Hung 2011). The results of the studies conducted with catfish and rainbow trout showed that the addition of actigen at a ratio of 0.08–0.12% in the composition of an artificial feed led to a 13.7% increase in productivity, improved feed conversion and immune status parameters, in particular, lysosome activity and leukocyte count (Hung et al 2012). There was a positive effect of adding Actigen at a ratio of 0.04% to the diet of Nile tilapia on the growth rate (Abdel-Tawwab 2012), as well as on feed digestibility and fish productivity when using 0.08% prebiotic in feeds for Siberian sturgeon (Sverinciuc et al 2017).

Field studies showed that when Actigen was added to the main diet of age -1+ carp at a ratio 0.05%, the average weight of fish exceeded the control group by 21.3%. At the same time, fish productivity increased by 31.0%, while feed costs for growing age -1+ carp decreased by 1.3 times (Dobrianska et al 2019).

According to the biological properties of Actigen and the results of studies of its use in aquaculture, the aim of this work was to determine the activity of intestinal enzymes (α-amylase, alanine aminotransferase (ALT) and alkaline phosphatase (AP)) of age -1+ carp, fed with feeds containing different concentrations of the studied supplement.

The α-amylase activity was studied based on the fact of high carbohydrase activity in carp that allows them to effectively use high-carbohydrate feeds (Al-Tameemi et al 2010; Zabytivskyi 2002). However, such feeding causes an increased risk of pathogenic microflora development, which actively uses carbohydrates for its growth (Mulyani et al 2018). The study of α-amylase activity in the carp intestine will help to assess the indirect effect of different quantities of mannan oligosaccharide, contained in feed, on carbohydrate hydrolysis.

The formation of a sorption layer of mannan oligosaccharides has a direct effect on the activity of intestinal microflora that is reflected on the processes of detoxification in the digestive tract. The activity of transferases, in particular ALT, is an important indicator of the state of internal homeostasis of enterocytes, intestinal cells, which ensure the synthesis of enzymes for membrane and intracellular digestion (Butt & Volkoff 2019).

AP activity assessment is important for the assessment of the intensity of phosphate cleavage from organic compounds, which occur during the digestion process. Especially, high amounts of this enzyme are contained in the apical part of enterocytes, due to their integral nature (Gisbert et al 2018). Since the studied prebiotic supplement creates a separate structure over the glycocalyx layer, it is important to study the activity of the processes induced by AP under the action of different quantities of prebiotic in the carp intestine.

Material and Method. The object of the study was age -1+ scaled carp. The experiment was performed in 2018 in the conditions of four 150 m^2 analogous ponds with one source of water supply, which were stocked with age -1 scaled carp with an average weight of 55–58 g and a stocking density of 1000 fish ha^{-1}. Experimental feeding lasted 60 days during the growing season. Carp of the experimental groups additionally received Actigen at ratios of 0.025% (Experiment 1), 0.05% (Experiment 2) and 0.075% (Experiment 3), added to the main feed by wet granulation. The control group of fish received a balanced feed, which included (%): 50–wheat grain, 30–sunflower meal, 10–meat and bone meal, 9–barley bran, 1–chalk.

Experimental works were performed in accordance with the rules of the European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes (European Convention 1986).

The hydrochemical regime of ponds, the dynamics of changes in the content of organic matter and nutrients that are important for the development of natural food supplies, as well as for the oxygen and temperature regimes were monitored during the growing season (Dobrianska et al 2019). The amount of feed was calculated according to the fish growth rate and water temperature.

At the end of the experiment, fish were caught and a complete pathological

autopsy of five individuals from each group was performed with the collection of intestines for the analysis, according to the purpose. Fish for analysis were taken after their intestines were emptied of feed. The water temperature, at which the experimental carp were kept before sampling, was 22°C. To analyze the activity of enzymes, the medial part of the intestine after the first loop was dissected in the cold, by separating it from adjacent tissues, and then it was placed in liquid nitrogen at a temperature of minus 196°C and stored in a freezer at a temperature of minus 80°C. After thawing, tissues were homogenized in a Ringer's solution for cold-blooded animals (0.65%–NaCl, 0.014%–KCl, 0.02%–$NaHCO_3$). The enzyme activity was determined in the supernatant according to the methods described below.

The α-amylase activity was determined by the Caraway method based on a decrease in the optical density of the substrate solution after its hydrolysis by an enzyme (TU U 24.4-13433137-050:2006 2016).

The ALT activity was determined by the Reitman-Frankel method with the use of the color reaction of the hydrolysis product with 2,4-dinitrophenylhydrazine. The enzyme activity was calculated based on the intensity of colored hydrazine of pyruvic acid (TU U 24.4-13433137-047-2003 2016).

The AP activity was determined by the reaction with phenylphosphate, the result of which consisted of phosphate and phenol. Phenol with 4-aminophenazone in the presence of sodium periodate forms a colored compound, the intensity of which is used to calculate the enzyme activity (TU U 24.4-13433137-047-2003 2016). All calculations were performed per unit of protein in a reaction mixture determined by the Lowry method (Lowry 1952).

The obtained results were processed by variation statistics using correlation and regression analysis in MS Excel 2007 and Statistica-6. The significance of differences was defined at $p<0.05$.

Results and Discussion. Table 1 illustrates α-amylase activity in the intestine of experimental fish. The use of prebiotic was found to result in changes in α-amylase activity. In the control group, the activity of this enzyme was the highest: 9.14±0.77 mg of starch x sec^{-1} x mg^{-1} of protein.

Table 1

The α-amylase activity, mg of starch x sec^{-1} x mg^{-1} of protein

Group of fish	*M*	*σ*	*m*	*Cv*
Control	9.14	1.13	0.77	12.35
Experiment 1	6.98*	1.50	1.31	21.55
Experiment 2	7.26*	0.70	0.50	9.63
Experiment 3	7.98*	1.45	0.96	18.21

* **–** the difference with the control is significant, $p<0.05$.

Addition of lower concentrations of prebiotic into the feed resulted in a decrease in amylase activity in the intestines of age -1+ carp. E.g., an application of 0.025% prebiotic caused a decrease in amylase activity, relatively to the control group, by 23.60% ($p<0.05$). An increase in its content in the feed to 0.05% in Experiment 2 resulted in an increase in the activity of this enzyme, relatively to Experiment 1, by 4.01%. The highest amylase activity among the experimental groups was in Experiment 3, where the 0.075% prebiotic concentration was used. In this group, amylase activity was 7.98 mg of starch sec^{-1} x mg^{-1} of protein, and it was higher by 14.33 and 9.92% compared to Experiment 1 and Experiment 2, respectively. At the same time, it was lower than in the control group by 12.69% ($p<0.05$). Thus, the carbohydrase activity in the carp intestine increased as the quantity of the added supplement increased. Apparently, this was due to the activity of the beneficial microflora, which colonized the sorbent and performed a partial hydrolysis of this oligosaccharide. In general, the functional state of the digestive system of the fish in Experiment 1 and Experiment 2 was higher than in the control group.

ALT activities in the conditions of the three experiments are presented in Table 2. The transamination reaction is quite active in enterocytes, in the process of amino acid synthesis. The content of this enzyme in the blood is a marker of damage to the hepatobiliary system of the liver, the cells of which have its highest concentration (Kamyshnikov 2009). According to a number of studies, ALT activity increases rapidly in the mammalian liver cells, as a compensatory detoxification reaction, due to the toxicosis caused by stress factors (Ferents 2016; Kopylchuk et al 2017). Similarly, stressors in intestinal cells also cause an increase in the synthesis of this enzyme. In Experiment 3, the use of prebiotic was found to probably affect the activity of ALT in enterocytes, by reducing it.

Table 2

ALT activity, mmol of sodium pyruvate x h^{-1} x mg^{-1} of protein

Group of fish	*M*	*σ*	*m*	*Cv*
Control	1.97	0.09	0.06	4.50
Experiment 1	1.81	0.13	0.11	7.40
Experiment 2	1.88	0.44	0.34	23.56
Experiment 3	1.51*	0.13	0.11	8.69

* – the difference with the control is significant, $p<0.05$.

In Experiment 2, the average value of ALT activity in the intestine of age -1+ carp, which consumed the prebiotic supplement at a ratio of 0.05%, did not differ significantly from those obtained in the control group. In Experiment 1, the activity of this enzyme seemed to decrease by 8.12%. In Experiment 3, a decrease in ALT activity by 23.35% ($p<0.05$) was observed. Thus, the use of the studied concentrations of prebiotic in the composition of the main diet of age -1+ carp did not generate stress, which would cause a compensatory effect of transaminases. The concentration of the studied supplement at a ratio of 0.075% caused a decrease in amino acid metabolism in the intestinal cells of carp.

The values of AP activity in the carp intestine in experimental and control groups are presented in Table 3.

Table 3

Alkaline phosphatase activity, μmol of phenyl phosphate x sec^{-1} x mg^{-1} of protein

Group of fish	*M*	*σ*	*m*	*Cv*
Control	15.49	4.73	3.41	30.57
Experiment 1	14.79	2.49	1.85	16.85
Experiment 2	15.81	2.36	1.81	14.90
Experiment 3	16.04	2.00	2.24	12.48

This enzyme is intracellular and participates in the dephosphorylation reaction during energy and plastic processes. The studies of the effect of negative factors on fish body demonstrated ambiguous functional activity of this enzyme in different tissues. E.g., under the action of glyphosate on the body of age -1+ carp, the AP activity in liver cells decreased by 5.9 times compared with the control group, while AP content in the blood increased by 1.9 times (Zhydenko et al 2015). This trend was observed under conditions of the destruction of hepatocyte membranes due to toxicosis in percids. The action of phenol and potassium dichromate in perch liver cells, on the contrary, resulted in an increase in AP activity that was explained by the adaptive properties of fish body (Prychepa 2016).

In our study, the AP activity in intestinal cells probably did not change under the effect of different concentrations of experimental supplement in the feed. There was only a tendency to increase in this parameter, in Experiment 3, which may indicate an adaptive response of the digestive tract in the form of phosphorylation activation.

Conclusions. The study of the digestive enzymes in the intestine of age -1+ carp showed that the supplementation of the main feed with a prebiotic based on mannan-oligosaccharides at ratios of 0.025, 0.05 and 0.075% by weight did not cause pathology in the functioning of intestinal enzymes. By analyzing the activity of the selected enzymes, we can conclude that in this context the optimal supplement concentration was at a ratio of 0.05%.

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Conflict of interest. The authors declare no conflict of interest.

References

Abdel-Tawwab M., 2012 Interactive effects of dietary protein and live bakery yeast, *Saccharomyces cerevisiae* on growth performance of Nile tilapia, *Oreochromis niloticus* (L.) fry and their challenge against *Aeromonas hydrophila* infection. Aquaculture International 20:317-331.

Al-Tameemi R., Aldubaikul A., Salman N. A., 2010 Comparative study of α-amylase activity in three Cyprinid species of different feeding habits from Southern Iraq. Turkish Journal of Fisheries and Aquatic Sciences 10:411-414.

Bentea M., Şara A., Ani A., Barbu A., 2014 The effects of prebiotic products in fish nutrition. Bulletin USAVM Animal Science and Biotechnologies 71(2):271-272.

Butt R. L., Volkoff H., 2019 Gut microbiota and energy homeostasis in fish. Frontiers in Endocrinology 10(9), doi: 10.3389/fendo.2019.00009.

Chernikova A., Ponomarenko N., 2016 [Use of prebiotics on the basis of mannan-oligosaccharides in feeding of chickens-broilers]. Ukrainian Black Sea region agrarian science 2(90):155-160. [In Ukrainian].

Chernikova G., Prokopenko E., 2017 [Slaughter quality of broiler-chickens by prebiotic Actigen using]. Agrobiodiversity 1:50-53. [In Ukrainian].

Dekhtiarov P. A., Yevtushenko M. Y., Sherman I. M., 2008 [Fish physiology]. Agricultural Education, Kyiv, 341 p. [In Ukrainian].

Dobrianska O., Deren O., Hryhorenko T., 2019 [Productive indices of age-2 carp after application of a prebiotic in their feeding]. Ribogospod Nauka Ukraine 4(50):95-108. [In Ukrainian].

Ferents N. M., 2016 [Features of transaminase activity in blood and liver in experimental pneumonia under immobilization stress and influence of corvitin on them]. Achievements of Clinical and Experimental Medicine 1:82-84. [In Ukrainian].

Gisbert E., Nolasco H., Solovyev M., 2018 Towards the standardization of brush border purification and intestinal alkaline phosphatase quantification in fish with notes on other digestive enzymes. Aquaculture 487:102-108.

Hung L. T., 2011 Building new aquafeeds: Feeding for health and performance in Tra catfish (*Pangasiaodon hypophtalamus*). The Alltech 28th Annual International Symposium, Lexington, Kentucky.

Hung L. T., Trung N. L., Thuy D. N., 2012 Effects of actigen on growth performances and fish health improvement of Tra catfish (*Pangasiaodon hypophtalamus*). The Alltech 28th Annual International Symposium, Lexington, Kentucky, 10 p.

Kamyshnikov V. S., 2009 [Clinical laboratory tests from A to Z and their diagnostic profiles]. MEDpress-Iinform, Moscow, 304 p. [In Russian].

Kopylchuk G. P., Voloshchuk O. M., Balandyuk O. V., 2017 [Biochemical markers of the functional liver state in rats with toxic hepatitis under the conditions of germanium citrate administration]. The Animal Biology 19(1):59-64. [In Ukrainian].

Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., 1951 Protein measurement with the Folin phenol reagent. Journal Biological Chemistry 193(1):265-275.

Mulyani Y., Nyoman I., Aryantha P., Suhandono S., Pancoro A., 2018 Intestinal bacteria of common carp (*Cyprinus carpio* L.) as a biological control agent for *Aeromonas*. Journal of Pure and Applied Microbiology 12(2):601-610.
Prychepa M. V., 2016 [Features adaptation native perch for the actions of environmental factors]. PhD thesis, Institute of Hydrobiology of the NAAS of Ukraine, Kiyv, 196 p. [In Ukrainian].
Ringo E., Olsen R. E., Gifstad T. O., Dalmo R. A., Amlund H., Hemre G.-I., Bakke A. M., 2010 Prebiotics in aquaculture: a review. Aquaculture Nutrition 16:117-136.
Spring P., Wenk C., Connolly A., Kiers A., 2015 A review of 733 published trials on Bio-Mos®, a mannan oligosaccharide, and Actigen®, a second generation mannose rich fraction, on farm and companion animals. Journal of Applied Animal Nutrition 3:1-11.
Sverinciuc C., Benţea M. I., Şara A., 2017 The effects of some fodder additives on growth performance of Siberian sturgeon (*Acipenser baeri*). Agriculture - Science and Practice 101-102(1-2):105-109.
Zabytivskyi Y. M., 2002 [Carbohydrase activity of the intestine of frame and scaly breeds of carp of the Lubin intrabreed type under the influence of copper]. Scientific Notes of Ternopil Pedagogical University 2(17):105-109. [In Ukrainian].
Zhydenko A. O., Mishchenko T. V., Krivopisha V. V., 2015 [Response of carp fish to the action of glyphosate]. The Scientific Issues of Ternopil Volodymyr Hnatiuk National Pedagogical University 3–4(64):227-230. [In Russian].
*** European Convention, 1986, European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasbourg.
*** TU U 24.4-13433137-047-2003, 2016 [Instruction «Alkaline phosphatase» to a set of reagents for determining the activity of alkaline phosphatase by reaction with phenylphosphate (endpoint)]. [In Ukrainian].
*** TU U 24.4-13433137-047-2003, 2016 [Instruction of «ALT» to a set of reagents for determination of alanine aminotransferase activity by the Reitman-Frenkel method]. [In Ukrainian].
*** TU U 24.4-13433137-050:2006, 2016 [Instruction «Alpha-amylase» to a set of reagents for determining the activity of α-amylase (diastase) by the amyloclastic method of Caraway]. [In Ukrainian].

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Authors:
Olha Petrivna Dobrianska, Lviv Research Station of the Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine, 81555, Velykyi Lubin, Ukraine, e-mail: olya_dobryanska@ukr.net
Yurii Mykhailovych Zabytivskyi, Lviv Research Station of the Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine, 81555, Velykyi Lubin, Ukraine, e-mail: yurafish@ukr.net
Olga Volodymyrivna Deren, Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine, 03164, Kyiv-164, Obukhivska St, 135, Ukraine, e-mail: derenj@ukr.net

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