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https://doi.org/10.52973/rcfcv-e362916
Revista Científica, FCV-LUZ / Vol. XXXVI

# Growth, body composition and digestive enzyme activities of rainbow trout fed with dietary Beta-glucan containing mannan oligosaccharide

# Crecimiento, composición corporal y actividades de enzimas digestivas en truchas alimentadas con una dieta de beta-glucano que contiene manano oligosacáridos

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## ABSTRACT

This study investigated the impact of a yeast containing the prebiotic β-glucans, on the growth parameters and enzyme activities of rainbow trout (*Oncorhynchus mykiss*) at various dosage levels. The fish were randomly placed in plastic tanks (120 x 50 x 32 cm) in three trial groups and replicates, with an average weight of 73.37 ± 0.21 g (n = 90). Three different diets were prepared; 0 g.kg-¹ in group G-1 (control), 0.5 g.kg-¹ of the prebiotic (mannan oligosaccharides + β-glucan) in group G-2 and 1 g.kg-¹ ratio prebiotic (mannan oligosaccharides + β-glucan) were added to group G-3. The fish were fed these experimental feeds for 60 days. By the end of the experiment, the G-2 group had achieved the highest average live weight and weight gain, with values of 155.38 ± 0.19 g and 81.76 ± 0.16 g respectively. Feed conversion ratio, specific growth rate, protein efficacy ratio and survival rate showed the best values (1.13 ± 0.03, 1.24 ± 0.003, 1.91 ± 0.001, and 93.33 ± 3.33, respectively) were observed in the G-2 group ($P < 0.05$). Pepsin activity was also significantly higher in the G-2 group (163.94 ± 2.23 U/mg protein; $P < 0.05$). Similarly, the highest trypsin, amylase, and lipase activities were recorded in the G-2 group, with values of 1.09 ± 0.05, 5.31 ± 0.22, and 4.38 ± 0.11 U/mg protein, respectively ($P < 0.05$). No significant differences were detected among the groups with respect to muscle proximate composition ($P > 0.05$). Both β-glucan–supplemented yeast groups positively influenced the growth performance and digestive enzyme activities of trout. However, the group receiving 0.5 g.kg-¹ β-glucan supplementation exhibited significantly superior results compared to the other groups ($P < 0.05$).

**Key words:** Prebiotic; *Oncorhynchus mykiss*; growth rate; feed conversion; protein efficiency.

## RESUMEN

En esta investigación se analizaron los efecto de una levadura que contiene el prebiótico β-glucano, sobre los parámetros de crecimiento y las actividades enzimáticas en la trucha arcoíris (Oncorhynchus mykiss) a diferentes proporciones. Los peces se colocaron aleatoriamente en tanques de plástico (120 x 50 x 32 cm) (73,37 ± 0,21 g; n = 90) en tres grupos experimentales con réplicas. Se prepararon tres dietas diferentes: 0 g/kg en el grupo G-1 (control), 0,5 g/kg manano oligosacaridos + β-glucano en el grupo G-2 y 1 g/kg de prebiótico manano oligosacaridos + β-glucano en el grupo G-3. Los peces fueron alimentados con estas dietas experimentales durante 60 días. Al final del experimento, los mayores valores de peso y ganancia de peso fueron 155,38 ± 0,19 g y 81,76 ± 0,16 g, respectivamente, en el grupo G-2 al comparar el peso vivo promedio y la ganancia de peso. El índice de conversión alimenticia, la tasa de crecimiento específico, el índice de eficiencia proteica y la tasa de supervivencia mostraron los valores más altos en el grupo G-2 con 1,13 ± 0,03; 1,24 ± 0,03; 1,91 ± 0,001; y 93,33 ± 3,33, respectivamente ($P < 0,05$). La mayor actividad de pepsina se observó en el grupo G-2 ($P < 0,05$) con 163,94 ± 2,23 U/mg proteína. Las actividades más altas de tripsina, amilasa y lipasa también se encontraron en el grupo G-2 con 1,09 ± 0,05; 5,31 ± 0,22; y 4,38 ± 0,11 U/mg proteína, respectivamente ($P < 0,05$). No hubo diferencias significativas entre los grupos en cuanto a los componentes nutricionales del músculo ($P > 0,05$). Se ha determinado que la adición de levadura con el prebótico β-glucano afecta positivamente tanto los parámetros de crecimiento de la trucha como todas las actividades enzimáticas, pero el grupo suplementado con 0,5 g/kg de β-glucano mostró diferencias significativamente mayores ($P < 0,05$) que los demás.

**Palabras clave:** Prebiótico; *Oncorhynchus mykiss*; tasa de crecimiento; conversión alimenticia; eficiencia proteica.

Recibido: 27/02/2026 Aceptado: 28/04/2026 Publicado: 22/05/2026
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## INTRODUCTION

Feed costs constitute the majority of production expenses in aquaculture, and fish meal has the highest share in feed input. In order to ensure the production of fishmeal obtained by hunting, as caught fish stocks began to decrease, the sector began to search for alternative protein sources and alternative feed additives to accelerate mixed feed studies and confirm sustainability [1].

As a result of the data obtained in the light of microbiological, biotechnological and engineering studies carried out with modern technology, it is aimed to ensure more production in less unit area and to produce this valuable protein source with the least mortality rates of enterprises through sustainable cultivation [2]. For this purpose, the use of antibiotics in the sector for the prevention and treatment of diseases and for increasing body resistance has begun, so that over time, antibiotic use has become routine practices due to its effects on the growth performance of individuals with high body resistance [3].

The damage caused by the antibiotics and other chemicals used to the natural ecological environment has been ignored for a long time. However, over time, these antibiotics used not only caused the development of bacterial resistance in animals, but also began to threaten human health with the residues they left in animal products. This is why the use of antibiotics in animal feed is prohibited in the European Union since 2006 [3].

Although aquaculture research on prebiotics is increasing day by day, it still cannot reach the desired intensity. Additionally, to studies on the growth and immune system performance of fish species that were fed prebiotic additives, many studies have attempted to observe that these oligosaccharides have a positive effect on the beneficial bacteria in the intestinal flora and have digestive and immune system regulating effects [4].However, in the light of these studies, factors such as the type, age, and application dose of the fish to be applied prebiotics should be taken into consideration. Because prebiotics have different effects or do not cause any reactions depending on the species [4].

Prebiotics are mainly effective in regulating intestinal flora and in the activity of probiotics. In other words, it is defined as a fermented and non-digestible component that allows specific changes in activity and / or composition in the gastrointestinal flora which benefit the host's health and well-being [5].

Recently, there has been a great interest in the use of prebiotics as a functional food to regulate the composition of the gastrointestinal microbiology to benefit the host health [5, 6]. The host should have beneficial and systemic effects and must be hydrolyzed in the colon microflora and have a positive effect on the host. Prebiotics affect the composition and activity of microflora in organisms. They regulate bowel movements and increase the absorption and bioavailability of minerals such as calcium and magnesium. They also have a positive effect on blood cholesterol and triglyceride levels and reduce the risk of developing intestinal and extraintestinal infections by limiting the proliferation of pathogenic microorganisms [5].

There are positive aspects such as strengthening the immune system. According to previous studies, various types of prebiotics and their combinations have been shown to effectively enhance the growth performance of different aquatic species. Among them, mannan oligosaccharides (MOS), galactooligosaccharides, fructooligosaccharides, and other oligosaccharides are the most generally used prebiotics in aquaculture today [4, 7, 8].

One example of a prebiotic mixture is Immunogen (β-glucan + MOS), a commercially available product proven to boost the growth of common carp (*Cyprinus carpio*) [9]. MOS is obtained from the cell wall of the yeast. The cell wall of this yeast consists of 30 % mannan, 30 % glucan and 12.5 % protein. MOS is derived from the amino acids on the surface of the yeast cell wall and provides a binding site to the bacteria. Pathogen organisms follow to the MOS instead of following to the intestinal epithelial cells, thus moving in the intestinal tract without being colonized [6, 9, 10].

After the ban of the use of antibiotics as a feed additive, many researchers have intensified their research on natural additives which may be an alternative to antibiotics. One of these natural substances was β-glucan obtained from the cell wall of Saccharomyces cerevisiae [1]. The use of β-glucans as a feed additive to enhance the immune system and to reduce the risk of death against bacterial and viral diseases that are fatally infected and to support the immune and performance of animals, and draw attention as a good alternative in light of the studies [1].

In a study on Nile tilapia (*Oreochromis niloticus*), MOS was added to feed rations at 0, 2, 4, and 6 g.kg-1 and research was conducted on survival, growth and strengthen the immune system. Compared with the control group, fish fed the MOS-supplemented diet did not show significant differences in weight gain, body length, survival rate, or feed conversion ratio (FCR). Nevertheless, these fish demonstrated a significantly enhanced resistance to infection by Streptococcus agalactiae [11].

Another study conducted on seabass (*Dicentrarchus labrax*) fish with MOS-supplemented feeds, MOS addition significantly reduced the lipogenic enzyme activity in the liver and more regular morphological structure of the hepatocytes. This could be linked to improved energy utilization and feed efficiency, as well as reduced feed intake [12].

The cell walls of yeasts contain high levels of two substances: β-glucan and MOS. Their immunostimulant properties and ability to reduce harmful organisms by strengthening the intestinal barrier are well known [13]. As a result of feeding, the findings showed that there was a significant improvement in feed conversion and the activities of amylase, lipase, and protease were higher in fish fed beta glucan and MOS mixture than in the control group in carp [13].

A study was conducted on flounder (*Platichthys stellatus*) to observe their feeding behavior, growth rate, and physiological parameters when fed a diet supplemented with β-glucan/MOS for 56 days. Individuals fed with β-glucan/MOS supplemented feed showed significant ($P < 0.01$) growth and development compared to the control groups [14].

For many years, the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have been widely utilized in biotechnological applications, particularly in the production of food and beverages. In recent years, however, non-conventional

yeast species have gained increasing attention, with *Pichia guilliermondii* emerging as an important alternative [15].

This material was used for the first time in this study for trout. Functional foods such as β-glucan and MOS, a prebiotic additive, can rise efficiency in the feed industry and meet the needs of the fish farming sector by falling antibiotic use, developing digestive activity and improving feed efficiency. The main objective of in this research was to value the effects of addition a mixture of β-glucan and MOS to extruded trout feed on growth performance, fish body chemical composition, and digestive enzymes. The demonstration of the economic benefits of this approach was also observed.

## MATERIALS AND METHODS

### Fish experiment design and growth performance

In this study, nine semi-recirculated rearing tanks (120cm x 50cm x 32cm) were used. Each tank contained 10 fish (10/0.19 $m^3$). The 90 trout used in the study were randomly selected from a stock of 400 fish and placed in the. A total of 90 fish were weighed (73.37 ± 0.21 g) individually before being placed in experimental tanks. The experiments were conducted with triplicate.

Three different diets were prepared considering the energy requirements of the trout during the study period. Diets used for this experiment: Group G-1 (control): 0 $g.kg^{-1}$, Group G-2: 0.5 $g.kg^{-1}$ prebiotic (MOS + β-glucan) Group G-3: 1 $g.kg^{-1}$ prebiotic (MOS + β-glucan). A crude protein content of 46–47 % was achieved in test feeds formulated using fish meal, corn gluten, fish oil, trace minerals, vitamin supplements, antioxidants, binding agents, and beta-glucan. Prepared feeds of β-glucan + MOS mixed (ADM Alliance Nutrition, USA) contribution were used. prebiotic contained 38 % protein, 1.5 % crude oil, 10 % cellulose and 8 % moisture. A yeast mannan, is a proprietary from yeast (*Pichia guilliermondii*) supplement for all life stages and classes of livestock, companion animals, and poultry. It is a coproduct of citric acid manufacturing.

Due to the use of high-quality raw materials and the single production site has a consistent composition and is cost effective [16], this product does not contain viable microbial cells. The yeast used contained 16 % MOS and 16 % β-glucan. The design and chemical composition of the research diets are shown in TABLE I. Nitrogen-Free Extract was calculated as the remainder of crude protein + crude lipid + ash. Calculated based on standard physiological energy values: 19 $kJ.g^{-1}$ for protein, 36 $kJ.g^{-1}$ for lipid and 15 $kJ.g^{-1}$ for carbohydrate [17].

**TABLE I**

***Formulation (g.kg-1 diet) and chemical composition of the experimental diets.***

| Feed Content (g.kg-1) | G-1 | G-2 | G-3 |
|---|---|---|---|
| Fish meal | 500.00 | 500.00 | 500.00 |
| Fish oil | 100.00 | 100.00 | 100.00 |
| Corn gluten meal | 230.00 | 229.50 | 229.00 |
| Dextrin | 100.00 | 100.00 | 100.00 |
| Vitamin mix | 20.00 | 20.00 | 20.00 |
| Mineral mix | 20.00 | 20.00 | 20.00 |
| Binder (CMC) | 30.00 | 30.00 | 30.00 |
| CtriStim® (MOS+β-glucan) | 0.00 | 0.50 | 1.00 |
| **Feed composition (g.kg-1)** | | | |
| Dry matter | 947.95 | 947.92 | 947.90 |
| Crude protein | 473.84 | 473.71 | 473.59 |
| Crude lipid | 206.58 | 206.54 | 206.50 |
| Crude ash | 119.92 | 120.11 | 120.30 |
| Nitrogen-Free Extract (NFE) | 147.61 | 147.56 | 147.51 |
| Other | 52.05 | 52.06 | 52.07 |
| Gross Energy ($MJ.kg^{-1}$ DM) | 18.45 | 18.44 | 18.43 |
| P:E ratio ($g.MJ^{-1}$) | 25.68 | 25.68 | 25.68 |

Group G-1 (control): 0 g.kg-1, Group G-2: 0.5 g.kg-1 prebiotic (MOS + β-glucan) Group G-3: 1 g.kg-1 prebiotic (MOS + β-glucan). CMC: Carboxymethyl cellulose; MOS: mannan oligosaccharides, P:E ratio protein:energy

Fish fed ad *libitum* two times for morning (09:00-10:00 h) and afternoon feeding (17:00-18:00 h) during the 60 day grow out period. The experiment was conducted in a climate-controlled laboratory at the Fish Culture Unit. The water temperature was kept between 13 ± 0.3 °C, and a 12-h light : 12-h dark photoperiod was maintained throughout the study. Dissolved oxygen concentrations were measured every other day using a YSI Model 58 oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA) and maintained at ≥ 6 $mg.L^{-1}$ throughout the experiment. The pH was monitored twice weekly using an electronic pH meter and recorded 7.89 ± 0.65 (Fisher Scientific, Cincinnati, Ohio, USA). Total nitrate ($NO_3$), levels were determined weekly and recorded 0.70 ± 0.21 $mg.L^{-1}$ using a Merck Spectroquant® Nova 60A (Germany) system.

### Biometric parameters

In order to analyze the growth indices of the rainbow trout, all fishes from each tank was determined once every 15 d during the 60-d experiment, at least 12 h after the last feed. The fish were weighed individually by a digital scale (ME503 T/A00, Switzerland) after they had been anesthetized. At the end of the experimental period, body weight gain (BWG), percent body weight gain (PBWG), specific growth rate (SGR), FCR, protein efficiency ratio (PER), and survival rate were determined according to the equations provided below. Growth performance was evaluated using the formulas cited in the literature and listed below [18, 19].

BWG = Wt - W0

PBWG = (Wt-W0)/W0×100

SGR = (lnWt-lnW0) / t ×100

FCR = Feed given (g) / wet weight gain (g)

PER = Wet WG (g) - protein intake (g)

Survival rate = (Nt / N0) / 100

In the above formulas, Wt denotes to the mean final body weight (g), whereas W0 indicates the mean initial body weight

(g). The sign t represents the length of the experimental period expressed in days. N0 and Nt denote the number of fish at the beginning and at the end of the trial, respectively.

## Economic profit analysis

Feed cost was incorporated into the economic evaluation of the experimental groups. The cost of feed required to produce 1 kg of biomass was calculated based on the feed price and FCR. The Economic Conversion Ratio (ECR) and Economic Profit Index (EPI) were calculated according to the following formulas:

ECR (US.$/kg) = Feed cost (US.$/kg) x Feed conversion ratio (kg diet/kg fish),

The Economic Profit Index [EPI (US$ /fish) = final weight (kg/fish) x fish sale price (US$ /kg)-ECR (US$/kg) x weight increase (kg)] [20].

## Sampling biochemical analyses and digestion enzyme assay

At the end of the trial, 27 fish in total (3 fish from each tank, corresponding to 9 fish per treatment) were randomly selected from every treatment group. The fish were then dissected, and tissue samples were obtained for biochemical analysis.

Fish fillets were subjected to proximate composition analysis as follows: moisture content was determined by drying the samples at 60 °C until a constant weight was achieved; crude protein was measured by determining Kjeldahl nitrogen (×6.25) using an automated distillation unit (Velp DK20, DK6, UDK126; Italy) crude lipid was extracted with a chloroform/methanol solvent system; and ash content was quantified by incineration in a muffle furnace (Protherm Furnaces 110, Germany) at 550 °C for 18 h [21].

To evaluate the effect of yeast-based β-glucan on digestive enzyme activity, six fish were sampled from each tank at the end of the experiment, and crude enzymatic extracts were subsequently prepared according to experiment protocol [22]. Pepsin activity (E.C.3.4.23.1; Enzyme Commission, Pepsin A) was assayed using 2 % hemoglobin from bovine blood in HCl as a substrate. Trypsin activity (E.C.3.4.21.4; Enzyme Commission, Trypsin) was measured [23] and the measurements modified using benzoyl-DL-arginin-p-nitroanilide (BAPNA, Sigma) as a substrate [24]. Amylase activity (E.C. 3.2.1.1; Enzyme Commission, Alpha-amylase) was assayed by the dinitrosalicylic acid (DNS, Sigma®) procedure [25] using 1 % soluble starch (Sigma) as a substrate. Lipase activity (E.C.3.1.1.3; Enzyme Commission, Lipase) was measured spectrophotometrically (Analytikjena-SPECORD 50, Germany) [26]. Tissue protein content was determined following the method described by Lowry *et al*. [27].

## Statistical analysis

All data were subjected to one-way ANOVA (SPSS 22.0). Duncan test was also used to compare means and significance was accepted at probabilities of 0.05 or less.

# RESULTS AND DISCUSSION

After a 60 d study, the effects of adding different rates of yeast-derived beta-glucan to the feed on final weight, weight gain (WG), live weight gain (LWG), daily live weight gain (DLWG), percentage live weight gains (LWG %), SGR, protein efficiency ratio (PER) and survival rate were investigated, and the data are presented in TABLE II.

**TABLE II**

***Growth performance, feed utilization data and muscle proximate composition of trout fed with different levels of β-glucan supplementation on feeding trial (n = 3) Different letters within a same line denote significant differences (P < 0.05). Values are expressed ±***

| | G-1 | G-2 | G-3 |
|---|---|---|---|
| Initial weight (g) | 73.18 ± 0.12$^{a}$ | 73.62± 0.40$^{a}$ | 73.32 ± 0.11$^{a}$ |
| Final weight (g) | 133.31 ± 0.26$^{a}$ | 155,38 ± 0,19$^{b}$ | 153.62 ± 0.15$^{b}$ |
| Weight gain (g) | 60.13 ± 0.17$^{a}$ | 81.76 ± 0.16$^{b}$ | 80.48 ± 0.08$^{b}$ |
| LWG % | 82.17 ± 0.20$^{a}$ | 111.05 ± 0.19$^{b}$ | 110.03 ± 0.19$^{b}$ |
| DWG (g) | 0.99 ± 0.003$^{a}$ | 1.35 ± 0.003$^{b}$ | 1.33 ± 0.003$^{b}$ |
| SGR | 0.97 ± 0,007$^{a}$ | 1.24 ± 0.003$^{b}$ | 1.23 ± 0.003$^{b}$ |
| FCR | 1.47 ± 0.12$^{a}$ | 1.13 ± 0,03$^{b}$ | 1.14 ± 0.003$^{b}$ |
| PER | 1.43 ± 0.02$^{a}$ | 1.91 ± 0.001$^{b}$ | 1.90 ± 0.003$^{b}$ |
| Survival rate | 86.67 ± 3.33a | 93.33 ± 3.33$^{b}$ | 93.33 ± 3.33$^{b}$ |
| Muscle composition % | | | |
| Moisture | 73.08 ± 0.23$^{a}$ | 71.71 ± 0.31$^{a}$ | 71.88 ± 0.20$^{a}$ |
| Crude protein | 20.07 ± 0.10$^{a}$ | 21.30 ± 0.16$^{a}$ | 20.93 ± 0.32$^{a}$ |
| Crude lipid | 4.40 ± 0.01$^{a}$ | 4.30 ± 0.17$^{a}$ | 4.25 ± 0.16$^{a}$ |
| Crude ash | 2.27 ± 0.13$^{a}$ | 2.06 ± 0.59$^{a}$ | 2.71 ± 0.36$^{a}$ |

SEM of three replicates in each groups

Optimum growth was observed on G-2 diet, whereas the control diet (G-1) produced the lowest growth rate. Fish fed with yeast glucan diets final weight, WG, LWG (%) and SGR increased significantly ($P < 0.05$) when compared control diet no glucan added G-1 diet. There were significant changes in survival between control and yeast based glucan added diets ($P < 0.05$). The lowest FCR was obtained at 0.5 g.kg$^{-1}$ glucan (G-2) supplementation. Fish fed the control diet showed a higher FCR. On the other hand, yeast based glucan supplementation improved nutrient utilization; moreover, fish fed G-2 diet showed highest PER. Yeast based glucan supplementation did not significantly affected muscle fish composition (TABLE II).

It is well established that when prebiotics are added to fish and shellfish diets, they grow better, use food more efficiently, are stronger against disease and have higher digestive enzyme activity [28]. In the large intestine, an increased production of short-chain fatty acids and lactic acid is stimulated by the fermentation of dietary prebiotics, which in turn stimulates the growth of beneficial bacteria. This improves growth performance and the health of the host.

The current investigation revealed that the growth rates of fish were enhanced when they were fed diets comprising yeast-derived glucan. Significantly higher growth rates ($P < 0.05$) and higher weight gain were shown by fish fed the yeast-based glucan 0.5 g.kg$^{-1}$ diet than with other treatments.

The yeast glucan supplementation groups gained more weight than the control group. Live weight gain percentage ranged from 82 % to 111 %, and was significantly affected by the yeast-based glucan. This study showed that adding 0.5 g.kg$^{-1}$ beta-glucan yeast to trout diets could provide an effective

prebiotic supplement without negatively affecting fish growth performance.

In former research, the effects of MOS on the growth performance of aquatic species have been investigated, showing varying results. For example, MOS supplementation had no encouraging effect on growth parameters in Atlantic salmon (*Salmo salar*) [29] and cobia larvae (*Rachycentron canadum*) [30] thus, the inclusion of prebiotics in feed rations was not recommended.

However, in another study on Oreochromis niloticus, fish were fed diets containing MOS at levels of 0, 2.5, 3.5, and 4.5 g.kg-1 for sixty d. The results showed that fish fed the diet supplemented with 3.5 g.kg-¹ MOS exhibited improved growth, feed conversion ratio, protein efficiency ratio, and nutrient composition [8]. Similarly, in *D. labrax* fed diets containing 2 and 4 g.kg-¹ MOS, a better growth rate was obtained, with approximately 10% higher growth compared to the control group [31]. In rainbow trout, fish fed with 2 g.kg-¹ MOS also demonstrated better growth performance than the control group [32].

In agreement with these results, Persian sturgeon, *Acipenser persicus*. [33], large yellow croaker *Pseudosciaena crocea* [34], *Lobeo rohita* fingerlings [35], white shrimp *Litopenaeus vannamei* [36], juvenile rainbow trout (*Oncorhynchus mykiss*) [37], Nile tilapia, *O. niloticus* [38], Showed differences in the effects of MOS, which may be attributed to factors such as yeast strain, fish species, or feeding duration [39]. In our study, MOS supplementation had positive effects on growth parameters at both doses, with the 0.5 g/kg-¹ MOS (G-2) level showing the most pronounced effect. However, the effective dose of MOS in the diet may vary depending on the feeding duration, fish species, age, nutritional status, and physiological condition.

The bio-economic values of trout fed with feed containing beta-glucan-enriched yeast are presented in TABLE III.

**TABLE III**
***Bio-economic analysis of trout fed with feed containing yeast-derived β-glucan.***

| | G-1 | G-2 | G-3 |
|---|---|---|---|
| FCR | 1.47 | 1.13 | 1.14 |
| Feed cost ($/kg) | 1.3065 | 1.3089 | 1.3112 |
| ECR | 1.92 ± 0.02 [a] | 1.47 ± 0.004[b] | 1.49 ± 0.004[b] |
| EPI | 0.40 ± 0.002[a] | 0.49 ± 0.001[b] | 0.48 ± 0.001[b] |

Different letters within a same line denote significant differences (P < 0.05).
Values are expressed ± SEM of three replicates in each groups

When the ECR values were examined at the end of the research, when the values of yeast-derived beta glucan feeds were compared, it was seen that the best values were 1.47±0.004 in the G-2 group and 1.49 ± 0.004 in the G-3 group. A statistical difference was determined between these two groups and the G-1 group (P < 0.05).

When examined in terms of EPI, the lowest was found to be 0.40±0.002 in the G-1 group, and the highest was 0.49 ± 0.001 in the G-2 group. Statistically, it was determined between the groups fed with β-glucan-derived yeast and the control (P < 0.05) and the most profitable feed group was determined to be the G-2 group. At the end of the research, a profitability of 0.45 $/kg was determined in terms of feed cost and accordingly, a feed cost profit of 23.43 % was calculated for 1 kg of feed. Feed input constitutes approximately 40-50 % of the trout production cost. When examined from this perspective, the annual feed cost brings a profitability of 9.2-11.5 %.

At the end of the 60-day study, the effects of feeding trout with different amounts of yeast-derived beta-glucan on stomach pepsin activity (A), intestinal trypsin (B), amylase (C) and lipase (D) activities are presented in FIG. 1.

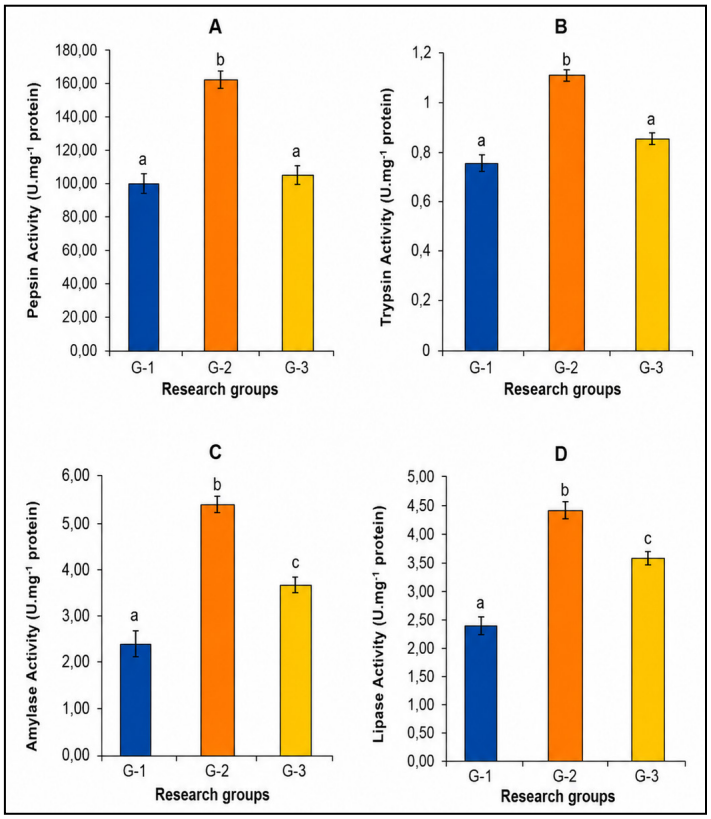


**FIGURE 1.** Pepsin activity in stomach tissues (A), Trypsin (B), Amylase (C) and Lipase (D) activities (U.mg-1 protein) in intestine tissues of rainbow trout fed different levels of β-glucan supplementation. Each value represents the mean ± SEM. (n = 6). The different letters indicate significant (P < 0.05) difference between diet groups

Subsequent to being administered varying quantities of yeast supplementation over a 60-day period, the mean pepsin and trypsin digestive enzyme activities of the G-2 treatment group exhibited a significant discrepancy from those of the control and G-3 groups (FIGS. 1A and 1B). However, fish fed with yeast based glucan added groups were significantly different than control group for amylase and lipase activity (P < 0.05) (FIGS. 1C and 1D).

The results of the analysis of digestive tract enzyme activity showed that diets supplemented with beta-glucan yeast significantly increased the activity of gastric pepsin, intestinal trypsin, lipase and amylase enzymes. The highest digestive enzyme activities were generally observed in the G-2 group. Pepsin, trypsin, amylase, and lipase activities in this group were significantly higher compared to the other groups (P < 0.05).

No direct studies have been found on the effect of yeast-based prebiotics used as feed additives on enzyme activity. However, other research has shown that different prebiotic

supplements and feeding regimes can influence digestive enzymes. For example, feeding white seabream (*Diplodus sargus*) and meagre (*Argyrosomus regius*) with diets containing 1–2% brewer's spent yeast (BSY) (*Saccharomyces pastorianus*) affected amylase, lipase, and protease activities differently between species, White seabream showed lower proteolytic but higher amylase and lipase activities than meagre. In white seabream, enhanced amylase and protease activities were observed in the pyloric caeca and intestine, and lipase activity was increased in the pyloric caeca, following BSY administration. In meagre, an improvement in amylase activity in the pyloric caeca was seen with the 2 % BSY diet [40].

Similarly, common carp fed diets with 0.5–1.5 g.kg-¹ Biomin® IMBO synbiotic showed increased trypsin and chymotrypsin activities, while α-amylase, lipase, and alkaline phosphatase were not significantly affected [41].

Another study, conducted over 60 day in rainbow trout, investigate the effects of replacing fish meal with a nucleotide-supplemented prebiotic on growth and digestive enzymes. The results indicated that the prebiotic supplementation positively influenced both growth and digestive enzyme activities. At the end of the study, pepsin, trypsin, and amylase activities were found to be significantly different in all prebiotic-supplemented groups compared to the control group [42].

Studies examining how prebiotics affect enzyme activities in fish are still limited. Fish fed with 100 mg.kg-¹ of prebiotic xylooligosaccharides (XOS) showed better growth performance than those fed with 50 or 200 mg.kg-¹ XOS. Similar to this research, no positive correlation was observed between the XOS level and fish growth. Moreover, there was no significant difference in final weight gain between the 200 mg.kg-¹ XOS group and the control group, suggesting that higher XOS levels might have negatively affected nutrient digestibility, leading to reduced growth performance [43].

The microbial communities in the fish digestive system play a crucial role in maintaining host health and improving the balance of the normal gut flora [44]. In the studies, it was determined that prebiotic additives increased the fermentation of short-chain fatty acids in the intestine. Digestive enzymes increase the efficiency of feed use in fish [45]. The characterization of these enzymes provides information on the digestive capacity of fish. It also provides information on the enzymes' ability to hydrolyse carbohydrates, proteins and lipids. [46].

Prebiotics increase intestinal viscosity and increase the efficiency of the digestive system in many organisms [47] and enhance the solubility and absorption of minerals such as calcium, magnesium, and iron ions [48], and the bioavailability of trace elements [48], by lowering the intestinal pH.

As is common knowledge, prebiotics have very valuable effects on the digestive systems, most notably with the morphological and microbiological systems of organisms [47]. Therefore, fish fed with prebiotics tend to develop better enzyme activity due to the modulation of the beneficial microbial population, which enhances trypsin, amylase, and lipase activities. Consequently, it is thought that growth efficiency also improves in relation to increased enzyme activity [49].

## CONCLUSION

In this study, the growth performance, enzyme activities, and feed cost of rainbow trout were investigated by adding a prebiotic substance consisting of a mixture of MOS and glucan, used as a yeast source, to the feed at different levels (0, 0.5 and 1 g.kg-1). When the results were analyzed, the best growth parameters and digestive enzyme activities were obtained in the group (G-2; 0.5 g.kg-1) fed with feed prebiotic.

Considering that enzymes are the most important substances for the digestive function to occur, the results obtained suggest that the appropriate amount of prebiotic supplementation stimulates enzyme activity showed better digestion, thereby enabling to achieve better growth values. However, further studies are needed to determine whether prebiotics can be used in different fish species or in different growth stages of the same species, and if so, in which stages and in what quantities.

## ACKNOWLEDGMENTS

This study was supported by Mersin University, Department of Scientific Research Projects (BAP-2017-2-TP2-2081). The authors would like to thank ADM Alliance Nutrition for supplying yeast supplement free of charge.

### Conflict of interest

The authors declare no conflicts of interest regarding this article's research, authorship, and/or publication.

### Ethical approval

This research was conducted following the approval of the animal experiment by the Mersin University Local Ethics Committee (Decision number: 14/46/2016).

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