

Effects of Some Probiotics and Synbiotic Dietary Supplementation on Growth Performance and Digestive Enzymes Activity of Common Carp, *Cyprinus Carpio*

Mohammed S. Qaddoori¹, Salah M. Najim², Khalidah S. Al-Niaeem^{3*}

^{1,2,3}Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Basrah, Iraq.

³kalidah_salim@yahoo.com; ³ORCID ID: <https://orcid.org/0000-0001-7236-6464>

Abstract

The study was conducted to show the effect of using the prepared synbiotic and comparing it with the local Iraqi probiotic and the commercially imported synbiotic as a feed additive in the diets of common carp for the period of 70 days. 75 fingerlings with a starting weight of 11.15 ± 1.00 g, live mass of 55.94 ± 1.5 g and an average length of 9.1 ± 1.5 cm were randomly distributed among five treatments (three replicates for each treatment and five fish for each replicate) and cultured in the closed rotating system. A standard diet was prepared and added to 0.1% each of the Iraqi probiotic (T2), commercial imported probiotic (T3), commercial import synbiotic (T4) and the synbiotic (T5) from lactic acid bacteria (*Streptococcus thermophilus* and *Lactococcus bulgaricus*). The experimental diets were 3% of the body weight and the fish were weighed every 14 days. The results showed the superiority of the fifth treatment (T5) for the studied traits and the results indicated a significant difference ($P < 0.05$), between them and the experiment's parameters in growth criteria, which included a final weight rate of 130.48 g, weight gain of 73.65 g, daily growth rate of 1.05 g/day, specific growth rate of 1.19 g/day, relative growth rate of 129.55%, thermal growth rate of 0.73, condition factor of 1.69 and ration evaluation criteria that the amount of feed included 167.48 g, the feed conversion ratio 2.28, feed efficiency ratio 43.96%, protein intake 50.76 gm, protein efficiency ratio 1.45, protein production value 29.36%. The best protein ratio was 18.28% and the lowest fat percentage was 4.22% in the chemical analysis of body components. The blood parameters which included the enzyme alpha-amylase 61.82 IU/L and lipase 17.89 IU/L.

Keywords: Fish, Common Carp, Growth Performance, Digestive Enzymes.

DOI: 10.47750/pnr.2022.13.S07.027

INTRODUCTION

Aquaculture, which includes the cultivation of aquatic plants and animals (such as fin fish, shellfish and seaweed), is one of the fastest growing food production sectors in the world, with an average annual growth rate of 5.3% during the period from 2001 to 2018, and production increased by more than 600% since 1990 (FAO, 2020). It has an important role in enhancing food production to contribute to food security and human nutrition, despite the growth of aquaculture, the difficulty of feeding the world's ever-increasing population and expected to reach 9.7 billion by 2050 (United Nations, 2019). It is a living reality, which calls for political, scientific and global discussions due to population growth and stagnation in natural fisheries, and this is a challenge to aquaculture as a global production to achieve remarkable goals, and it is estimated to reach 109 million tons in 2030 (FAO, 2020). It is expected to achieve the required additional increase in global production that aquaculture will be the only available solution, but it can produce additional adverse environmental impacts, if its

expansion is not based on sustainable farming systems (Cottrell et al., 2020).

The recirculating aquaculture systems (RAS) allows fish to be cultured in a controlled and controlled environment to reduce direct interactions between production processes and the environment (Ahmed and Turchini, 2021). Intensive culture systems are used to increase production, and here fish in such systems may be exposed to infection and stress, which weakens their health and these systems, may fail due to a lack of resources or difficulty controlling the improvement of culture conditions. When intensive culture systems fail, fish and aquatic organisms stop feeding gradually, this leads to deterioration in their physiological state, which leads to a decrease in immunity (Nie et al., 2021). There are some strategies, including improving the nutritional diet by using feed additives that stimulate growth and the use of balanced feeding programs, as these additives have a role in improving fish productivity and improving their health status (Paray et al., 2021).

The inclusion of appropriate additives to raise the level of

nutritional value provided to fish, such as adding lactic acid bacteria and through the important microbial fermentation process in organisms that follow a diet rich in fiber, fermentation is a simple and cheap process that has been practiced for a long time, and leads to the improvement of the nutritional value of many crops Agriculture (Verni et al., 2019).

Bacterial fermentation of foodstuffs has been used for several centuries to preserve the nutritional value and extend the shelf life, and with the development of modern preservation techniques, the primary objective of the fermentation process is no longer the preservation, but rather to obtain many products distinguished by their taste, flavor, texture and nutritional value, and as lactic acid bacteria were employed on Widely used in the manufacture of many dairy products, meat, vegetables, and fermentation of pastries, this group of bacteria has witnessed great interest due to its positive effects in fermented foods, foremost of which is the inhibitory effect against pathogenic microorganisms, as it produces many inhibitory substances such as organic acids hydrogen peroxide, diacetylcholine, carbon dioxide, bacteriocins and other substances (Linares-Morales et al., 2018; Murray et al., 2020 and Hussain et al., 2021).

The use of the probiotic, the prebiotic and the synbiotic is a suitable alternative to overcome these problems due to its ability to improve the nutritional value by reducing anti-nutritional factors for feed, modifying the immune system and gut microflora, and it has an anti-bacterial role and its ability to stimulate the growth factor when used as food additives to improve Local immunity to the gut, increasing the presence and activity of beneficial bacteria and secretion of digestive enzymes, and reducing harmful bacteria (Dawood, 2021 and Yilmaz et al., 2022). The relationship between balanced feeds and the intestinal health of fish It is expected that awareness of this relationship will help the aquaculture industry, especially fish, to develop appropriate nutritional strategies that ensure good nutrition and protect the health of fish.

The current study aims to show the effect of adding the Iraqi probiotic, the commercial imported probiotic, the commercially imported synbiotic, and the synbiotic prepared after adding it to the cultured carp diets on the productive performance of the fish through their recirculating aquaculture systems.

MATERIALS AND METHODS

Four units of the recirculating aquaculture systems (RAS) were established in each unit containing four basins with a capacity of 30 liters each. Each unit was connected to a special glass basin for filtering and placed with a rack holder measuring (100×100) cm divided into three sections. The rearing basins were placed in the rack The first and second,

while the glass basin for filtering was at the bottom of the basins, with the provision of a backup electric current system (a 5-amp inverter device with a 150-amp battery) and these are the basic needs of the (RAS), which were referred to by (Yamamoto (2017).

The filtration system consists of a glass basin with dimensions (90×30×40) cm³ divided into three sections. The first section consists of a mechanical filter. The first layer is made of polyethylene plastic threads, the second layer is made of a piece of cloth for the purpose of filtration and the third layer is a size mesh. 100 microns placed inside a filter made of plastic with dimensions (25×23×12) cm for the purpose of sedimentation of waste and solid residues from food.

The biological filter consists of biological balls and ceramic (limestone) and a plastic mesh for the purpose of containing them. Nitrogen and works to convert nitrite into nitrate. The surface area of contact for one ball is 550 m²/m³ and the ceramic (limestone) is a hollow ceramic cylinder that leads to the same purpose for the growth of nitrifying and nitrogen-loving bacteria, and the surface area of contact is 131.2 m²/m³ per stone. The third section is a basin of pure water loaded with oxygen with a Chinese type pump to raise the water with a capacity of 50 watts and a flow rate of 4500 liters / hour and a lifting capacity of 2.5 meters (Muhtaliefa et al., 2019).

Experience Fish

Fingerlings common carp fish *C. carpio* were brought from the ponds of the aquaculture unit of the College of Agriculture, University of Basrah in Al-Haritha. They were transported using 25-liter plastic bags containing water. Upon arrival to the laboratory, the fish were sterilized with saturated saline solution to get rid of the pathogens. The fish were randomly placed in plastic tanks with a capacity of 30 liters for the purpose of acclimatization for 14 days. The experimental fish were fed on a laboratory-made control diet.

The fish of the first experiment were distributed in 15 plastic tanks with an average initial weight of 11.15±1.00 g and a live mass rate of 55.94±1.5 g, an average length of 9.1±1.5 cm per tank. The fish were fed with five experimental diets (5 treatments) with three replicates per treatment and five fish for each replicate. The rate of environmental factors for water quality control was measured in table (1). Feed is introduced gradually until reaching 3% of the fish's weight, with two meals per day (8 am, 4 pm). The experiment lasted for 70 days, starting from 5/03/2022.

Table (1): Water characteristics average of the experiment ponds in (R A S)

Temperature (C°)	dissolved oxygen mg/l		NH3 mg/l		NO ₂ µg/l		NO ₃ µg/l		pH		Salinity (‰)	
	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	outlet	Inlet	Outlet	Inlet	outlet	Inlet
23.5	8.20	8.26	0.15	0.10	0.19	0.13	0.47	0.29	7.9	8.1	2.41	2.40

Experience Diets

1. Preparation of the synbiotic: the barley grains were purchased from the market, cleaned of impurities, washed and dried well, and after they were completely dry, they were ground well with a Chinese-made mill and sifted the barley flour with a very fine sieve to obtain the amount of 500 g of barley flour. Mix it well with 3.5 liters of distilled water according to the method Xiang et al. (2019). Then, lactobacillus bacteria (*Streptococcus thermophilus*, *Lactococcus bulgaricus*) manufactured by the Italian company SACCO, with a weight of 0.01 g are added to the mixture and incubated at 30°C for 24 hours. Add 750 ml of the synbiotic to each kg of the standard ration mixture prepared for the fifth treatment (T5).
2. Addition of the Iraqi probiotic in treatment (T2) produced by Dr. Saad Abdul-Hussein, University of Baghdad/ College of Agriculture, containing (*Lactobacillus acidophilus*, *Bifidobacter*, *Bacillus subtilis*, *saccharomyces cervisia*) 1×10^8 CFU/g.
3. Addition of the commercial probiotic Suntypo Bio-gulf in the treatment (T3) made in China with a concession from the French company Golf, containing (*Bacillus subtilis* and Fermentation metabolite) 5×10^9 CFU/g. Addition of the synbiotic mixture from Biomin company Australian to the treatment (T4) containing (*Enterococcus* sp., *Bifidobacterium* sp., *Pediococcus* sp., *Lactobacillus* sp. and Fructo - oligosaccharides (inulin) 2×10^{11} CFU/kg.

The feed materials were brought and ground with a laboratory mill Chinese with a capacity of 650 watts, and the feed materials were mixed for each treatment (Table 2). The first treatment (T1) was the control treatment free of addition, while the additives were added to the second, third and fourth diets (T2, T3, T4) by 0.1%, while the fifth treatment (T5) was the treatment of the prepared synbiotic thread, then it worked in the form of a paste that was placed in a Japanese-origin meat mincer with a diameter of 4 mm and was minced twice to ensure the cohesion and pressure of the bush, then the diets were exposed to the open air for seven days to dry and preserved In nylon bags in the

refrigerator at a temperature of 5 C°.

Table (2): The components of laboratory rations (%) and Chemical analysis of experiment standard diet (%)

Feed material	T1	T2	T3	T4	T5	
Fishmeal	30	30	30	30	30	
Soybean meal	27	27	27	27	27	
Yellow corn	13	13	13	13	13	
Wheat	14	14	14	14	14	
Barley	14	14	14	14	14	
Oil	0.5	0.5	0.5	0.5	0.5	
Vitamins and mineral	1	1	1	1	1	
Salt	0.5	0.5	0.5	0.5	0.5	
Total	100	100	100	100	100	
Addition	0	0.01	0.01	0.01	0.01	
Chemical analysis of experiment standard diet (%)						
Protein	Fat	Fiber	*Carbohydrates (NFE)	Ash	Humidity	*Gross energy Kcal/ 100 g
30.31	8.40	4.79	44.50	7.76	4.25	400.00

*NFE, nitrogen-free extract (100 – (protein + lipid + ash + fiber)) (Wee. and Shu, 1989).

*Gross energy (Kcal/g)= (4.56 * protein + 9.45 * fat + 4.1 * carbohydrate) (A.O.A.C., 2000).

Studied Parameters

- 1- Weight Gain (WG) (g)/ fish
Final Weight (FW) (g)- Initial Weight (IW) (g)
- 2- Daily Gain Rate (D.G.R) (g) (Philipose et al., 2013)
=Final Weight (FW) (g)- Initial Weight (IW) (g))/ (number for days)×100

- 3- Specific Growth Rate (SGR) (%/g) (Jobling and Koskela, 1996)

$$\frac{(\text{LN Final Weight (FW) (g)} - \text{LN Initial Weight (IW) (g)})}{(\text{number for days})}$$
- 4- Relative Growth Rate (RGR) (%) (Utne, 1978)

$$\frac{(\text{Final Weight (FW) (g)} - \text{Initial Weight (IW) (g)})}{(\text{Initial Weight (IW) (g)})}$$
- 5- Thermal-Unit Growth Coefficient (TGC) (Jobling, 2003)

$$\frac{((\text{Final Weight (FW) (g)})^{0.3333} - (\text{Initial Weight (IW) (g)})^{0.3333})}{(\text{temperature } (^{\circ}\text{C}) \times \text{number for days})} \times 1000$$
- 6- Feed Conversion Rate (FCR) (Utne, 1978)

$$\frac{(\text{Weight Gain (g)})}{(\text{Food Intake (g)})}$$
- 7- Food Conversion Efficiency (FCE) (%) (Simple and Roopma, 2010)

$$\frac{(\text{Food Intake (g)})}{(\text{Weight Gain (g)})} \times 100$$
- 8- Protein Intake (Gerking, 1971)

$$\frac{(\text{Food Intake (g)} \times \text{Percentage of feed protein})}{(100)}$$
- 9- Protein Efficiency Ratio (PER) (Gerking, 1971)

$$\frac{(\text{Total weight gain (g)})}{(\text{Amount of protein ingested (g)})}$$
- 10- Productive Protein Value (PPV) (Hepher, 1988)

$$= \frac{(((\text{P2} / 100) \times \text{w2} - (\text{P1} / 100) \times \text{w1}))}{(\text{Amount of protein ingested (g)})} \times 100$$

$$\text{P1} = \text{Percentage body protein at the end of the experiment (g).}$$

$$\text{P2} = \text{Percentage body protein at the start of the experiment (g).}$$

$$\text{w1} = \text{Body weight at the beginning of the experiment (g).}$$

$$\text{w2} = \text{Body weight at the end of the experiment (g).}$$
- 11- The condition factor (K)

$$\frac{((\text{Weight}) / (\text{body length})^3) \times 100}$$

Microbiological Examinations

Total number of bacteria (total number) colony-forming unit (CFU): Estimate the total number of bacteria for the prepared synbiotic, according to Harrigan and McCance (1976). Using the culture medium (Nutrient Agar) by the method of poured plates (Table 3). Estimation of the numbers of lactic acid bacteria (LAB) using the nutrient medium (MRS Agar), by using the dish pouring method (Harrigan and McCance, 1976) and incubated at 37 °C. (Table 3).

Chemical Composition

The chemical composition of feed and fish was analyzed using physicochemical methods according to (A.O.A.C., 1980). Moisture was determined by losing weight in an oven at 105 °C. Ash was obtained by burning a known amount of the sample in an oven at 550°C to a constant weight. The protein determined by the Kjeldahl method was used for total nitrogen determination to convert the result to crude protein. The factor 6.25 was used. Total fat was extracted

using ether and the Soxhlet method. The total caloric value of the standard diet was calculated according to (A.O.A.C., 2000) by multiplying the percentage of proteins, fats and carbohydrates with the values of 4.56, 9.45 and 4.1 kcal/g, respectively. Carbohydrates were estimated by difference according to the following formula: Carbohydrates= 100 - (Moisture % + Protein % + Fat % + Ash %) (Wee and Shu., 1989).

Enzymatic Tests

A total of 2 ml of fish blood was collected for each treatment. Blood was drawn from the myocardium and the blood was placed in a 5 ml tube free of anticoagulant (EDTA). The serum was obtained after centrifugation for 3000 cycles for 15 minutes, and the serum was placed in tubes. Sterile, then, the enzymes α -Amylase and Lipase are measured in the blood serum by laboratory kit from the Chinese Mindray Company and by device (BS-230 mindray) with a wavelength of 570 and 405 nm.

Statistical analysis

The data were statistically analyzed using the program Statistical Analysis System-SAS (2012) to show the effect of adding some probiotics and the prepared synbiotic, and the significant differences between the means were compared using Duncan's test (1955).

RESULTS

The results of the experiment water test showed table (1) providing the optimum environmental conditions for the growth of common carp fish, including temperature, oxygen, ammonia, pH and salinity, which common carp fish need for growth and life.

The results of the examination of the total bacterial number and lactic acid bacteria prepared and added in the food ration provided to fish in the fifth treatment (T5) showed table (3).

Table (3): Number of bacteria in the prepared concentration of the symbiotic in treatment (T5)

Total bacterial count (cfu)	lactic acid bacteria (cfu)
6.1×10^7	0.8×10^7

The results showed table (4) for growth parameters that there were significant ($P < 0.05$) differences for the treatments of the synbiotic (T5), the Iraqi probiotic (T2) and the imported synbiotic (T4) for each of the final weight, weight gain, daily, qualitative and relative growth rate, and thermal growth coefficient. And the condition coefficient of the experimental treatments, if the prepared synbiotic treatment (T5) was significantly ($P < 0.05$) superior to the rest of the treatments and the values were (130.48, 73.65, 1.05, 1.19, 129.55, 0.73, 1.69), respectively, as well as the

absence of significant differences ($P < 0.05$) for the initial weight of the experimental parameters. The results also showed a significant ($P < 0.05$) superiority ($P < 0.05$) of the treatments of each of the Iraqi probiotic (T2) and the imported synbiotic (T4) over each of the treatment of the imported commercial probiotic (T3) and the control treatment (T1). While there were no significant differences between the treatment of the imported commercial probiotic and the control treatment. The survival rate was 100% in the experimental treatments.

The results of table (5) for the criteria for evaluating the diet showed a significant ($P < 0.05$), superiority ($P < 0.05$) of the synbiotic (T5) treatment over the rest of the treatments for the amount of feed intake, the ratio of food conversion, the protein intake, the percentage of protein efficiency, the production value of protein and the retention of nutrients, and the values amounted to (167.48, 2.28, 43.96, 50.76, 1.45, 29.36) respectively. The results also showed a significant ($P < 0.05$) superiority ($P < 0.05$) of the treatments of each of the Iraqi probiotic (T2) and the imported commercial synbiotic (T4) on each of the treatment of the imported commercial probiotic (T3) and the control treatment (T1) and there was no significant difference between them.

The results of table (6) for the analysis of body components showed a significant ($P < 0.05$), superiority ($P < 0.05$) of the synbiotic (T5) with protein percentage over the rest of the experimental treatments, followed by the Iraqi probiotic treatments (T2), the imported commercial synbiotic (T4) and the values reached (18.28, 17.80, 16.66). While there were no significant differences between the treatment of the imported commercial probiotic, the control treatment, the results indicated significant differences ($P < 0.05$) in the percentage of fat, as the treatment (B), control treatment (T1) and the treatment of the commercial probiotic (T3) outperformed. Significant differences between them on the treatments of the Iraqi probiotic (T2), commercially imported synbiotic (T4) and the prepared synbiotic (T5). The results showed a significant superiority ($P < 0.05$) for treatments (B), (T2) over treatments (T1), (T4) and (T5), while there was no significant difference between treatment (T3) and the rest of the treatments in the percentage of ash, and the superiority of significant ($P < 0.05$) in the moisture content of treatment (B) over the rest of the treatments.

Table (4): Growth assessment criteria for experimental treatments over a period of 70 days

Transactions	Starting weight g	Final weight (g)	WG (g)	DGR (g)	SGR (%/ day)	RGR (%)	TGC	K
Mean± SE								
T1	55.18 ±0.90 a	95.78 ±0.02 ^d	40.60 ±0.90 ^d	0.58 ±0.01 ^d	0.79 ±0.02 ^d	73.67 ±2.80 ^d	0.46 ± 0.01 ^d	1.56 ±0.02 ^c
T2	56.42 ±0.19 a	109.25 ±0.50 ^b	52.83 ±.32 ^b	0.75 ±0.01 ^b	0.94 ±0.01 ^b	93.62 ±0.30 ^b	0.56 ± 0.01 ^b	1.67 ± 0.01 ^{ab}
T3	55.56 ±0.49 a	96.22 ±2.45 ^d	40.65 ±2.07 ^d	0.58 ±0.03 ^d	0.78 ±0.03 ^d	73.13 ±3.32 ^d	0.45 ± 0.02 ^d	1.57 ±0.01 ^c
T4	55.72 ±1.05 a	102.03 ±0.28 ^c	46.31 ±1.32 ^c	0.66 ±0.02 ^c	0.86 ±0.03 ^c	83.25 ±3.96 ^c	0.51 ± 0.02 ^c	1.65 ±0.01 ^b
T5	56.83 ±0.74 ^a	130.48 ±3.24 ^a	73.65 ±2.55 ^a	1.05 ±.04 ^a	1.19 ±0.02 ^a	129.55 ±2.96 ^a	0.73 ± 0.02 ^a	1.69 ±0.01 ^a
Means with different letters in the same columns differ significantly from each other at the ($P < 0.05$).								
T1:- control ration free of any additives, T2:- Iraqi probiotic ration 0.01%, T3:- commercial importer probiotic ration 0.01%, T4:- commercial imported synbiotic ration 0.01%, T5:- prepared synbiotic ration 0.01%.								

Table (5): Criteria for evaluating the diet for trial treatments during a period of 70 days

Transactions	Feed intake	FCR	FCE	PI	PER	PPV
Mean± SE						
T1	143.55 ±1.09 ^d	3.54 ±0.10 ^a	28.29 ±0.80 ^c	43.51 ±0.33 ^d	0.93 ±0.03 ^c	16.02 ±0.53 ^d
T2	156.60 ±0.46 ^b	2.97 ±0.02 ^b	33.73 ±0.12 ^b	47.47 ±0.14 ^b	1.11 ±0.01 ^b	22.28 ±0.17 ^b
T3	144.11 ±0.58 ^{cd}	3.56 ±0.19 ^a	28.21 ±1.44 ^c	43.68 ±0.17 ^{cd}	0.93 ±0.05 ^c	15.82 ±0.72 ^d
T4	147.65 ±1.49 ^c	3.19 ±0.12 ^{ab}	1.18 31.39 ± ^{cb}	44.75 ±0.45 ^c	1.04 ±0.04 ^{bc}	18.41 ±0.74 ^c
T5	167.48 ±.82 ^a	2.28 ±0.06 ^c	1.12 43.96 ± ^a	50.76 ± 0.55 ^a	1.45 ±0.04 ^a	29.36 ±0.67 ^a
Means with different letters in the same columns differ significantly from each other at the (P<0.05). T1:- control ration free of any additives, T2:- Iraqi probiotic ration 0.01%, T3:- commercial importer probiotic ration 0.01%, T4:- commercial imported synbiotic ration 0.01%, T5:- prepared synbiotic ration 0.01%.						

The results showed in figure (1) for the digestive enzymes alpha-amylase and lipase that there were significant differences (P<0.05) for the treatments of the prepared synbiotic (T5) and the Iraqi probiotic (T2) and the imported synbiotic (T4) for each of the two enzymes. The synbiotic

prepared (T5) on the rest of the treatments and the values were (61.82 and 17.89) respectively, while there were no significant differences (P>0.05) between the two treatments of the imported commercial probiotic (T3) and the control (T1).

Table (6): Chemical analysis of fish body components for experimental treatments during a period of 70 days

Transactions	Protein	Fat	Ash	Moisture
Mean± SE				
B	15.73 ±0.03 ^e	4.47 ±0.03 ^a	3.15 ±0.02 ^a	75.72 ±0.09 ^a
T1	16.33 ±0.06 ^d	4.38 ±0.01 ^a	3.04 ±0.03 ^b	75.44 ±0.03 ^b
T2	17.80 ±0.02 ^b	4.25 ±0.03 ^b	3.13 ±0.03 ^a	74.15 ±0.03 ^d
T3	16.26 ±0.07 ^d	4.37 ±0.07 ^a	3.06 ±0.03 ^{ab}	75.44 ±0.04 ^b
T4	16.66 ±0.04 ^c	4.24 ±0.01 ^b	3.03 ± 0.04 ^b	75.26 ±0.04 ^c
T5	18.28 ±0.03 ^a	4.22 ±0.01 ^b	3.02 ±0.01 ^b	74.08 ±0.03 ^d

Means with different letters in the same columns differ significantly from each other at the (P<0.05).

B:- Fish body components before the experiment T1:- control ration free of any additives, T2:- Iraqi probiotic ration 0.01%, T3:- commercial importer probiotic ration 0.01%, T4:- commercial imported synbiotic ration 0.01%, T5:- prepared synbiotic ration 0.01%.

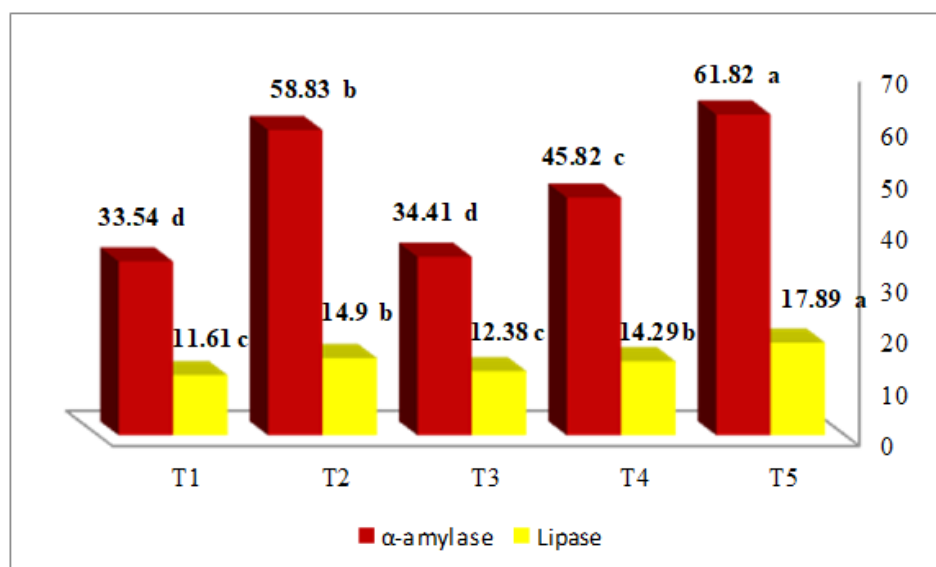


Figure (1): Analysis of digestive enzymes in the blood serum of experimental fish during a period of 70 days

DISCUSSION

Synbiotic, meaning is a combination of two Greek words "συν" and "βίος", meaning "shared or together" and "life," a food supplement that includes both a probiotic and a prebiotic in a synbiotic relationship, helps promote the growth and survival of microflora in the digestive tract of host species by altering the beneficial bacterial community in the gut (Butt et al., 2021).

The results of table (1) show the optimum environmental conditions for the growth of carp fish, and this indicates that the closed system and the biological filter work well, and they are the appropriate environmental factors needed by common carp fish, which were explained by (Rahman, 2015). As these results were suitable for carp fish in the closed system, which among them (Mojer et al., 2021), which did not affect the feeding activity of fish.

Table (3) shows the total bacterial number and lactic acid bacteria after addition and fermentation for 24 hours to increase the number of beneficial bacteria which are eaten by fish after growing on barley flour that contains monosaccharides and polysaccharides, if bacteria dissolve the bonds of sugars, which are food for Lactic acid bacteria promotes growth and reproduction., this was explained by studies, including the study Skrede (2002), the effect of fermentation by *Lactobacillus* bacteria and its growth on wheat and barley flour in the diets of Atlantic salmon. Another study showed the effect of fermentation and the formation of a synbiotic through the fermentation of white soybean meal with lactic acid bacteria *Lactobacillus brevis* 10^9 cfu/g and the study xiang et al. (2019), which leads to the fermentation of nutrients that make up the ration provided to fish, Which has a significant role in improving

the efficiency of the ration of digestion.

The results of table (4) for growth criteria showed that there were significant differences ($P < 0.05$) between the treatment of the prepared synbiotic and the treatments of the Iraqi probiotic, the commercial imported probiotic, the imported synbiotic and the control treatment for the final weight rates, So, the use of the fermentation method with lactic acid bacteria and the formation of the synbiotic added to the diet, which contains 0.8×10^7 of lactic acid bacteria, led to the highest final weight of 130.48 g, followed by the Iraqi probiotic treatment 109.25 g and the commercially imported synbiotic 102.03 g compared to the treatment control and treatment of imported commercial probiotic that were lower, there are significant differences ($P < 0.05$) for the other growth parameters represented by the total weight gain of 73.65 g and the average daily growth of 1.05 g and specific growth of 1.19 g / day and relative growth of 129.55% and thermal growth factor of 0.73 and condition factor of 1.69 for the fifth treatment (T5) Compared with the control treatment and the imported commercial probiotic treatment, which was lower in growth parameters.

Several studies showed the use of lactic acid bacteria as a probiotic and prebiotic and a synbiotic to have positive effects on fish growth, including the study (Eleraky and Reda, 2014) when adding the prebiotic (Mannan, Beta-glucans) at 0.15% and at rates of 0.25% and the addition of the probiotic (Biogreen, which consisted of 10^{10} CFU/g, at 0.05% and 0.1%, led to an improvement in growth parameters, condition coefficient and survival rates. As well as a study Adel et al. (2015) when using 0.02% of the Iraqi Probiotic in increasing the final weight, weight gain, and the specific and relative growth rate. The study Ali and Amal (2016) showed a significant increase in growth parameters

when using the Chinese and Iraqi probiotics in the diet of common carp fish cultured in the closed rotating system when using the Chinese and Iraqi probiotics by 0.2%.

The study Taher et al. (2018) showed the effect of the prebiotic extracted from the leaves of the laurel plant *Laurus nobilis* at three concentrations (1, 2, 3)% on growth, nutritional conversion and survival of the cultured common carp, which gave a weight gain of 7.63 g and a specific growth rate. 0.975% / day due to the use of 2% of the extracted precursor, A study Akter et al., (2019) when using lactic acid bacteria (*Lactobacillus acidophilus*) 10^5 and 10^7 CFU/gm as a probiotic in the diets of catfish (*Pangasianodon hypophthalmus*) confirmed that the growth parameters were increased when the two concentrations of lactic acid bacteria were used. It acts as a catalyst for fish growth, As well as the study (Hoseinifar et al., 2019) when using *Pediococcus acidilactici* bacteria as a prebiotic and prebiotic and mixing them as a synbiotic mixture and adding it to the diets of juvenile carp fish led to a significant improvement in the final weight, weight gain and daily growth rate The treatment of the synbiotic was higher than the use of the probiotic and the prebiotic alone, While the effect of the use of the prebiotic (Thepax) and the synbiotic (Labazyme) on young carp fish and these food additives had a significant effect on weight gain and the specific growth rate, and the best results were when adding 1 g/kg fodder of the prebiotic Thepax to promote growth in fish (Al-Mhanawi et al., 2021). The study (Sutriana et al., 2021) when adding the probiotic and prebiotic individually or jointly with yeast, galacto oligosaccharide, mannan oligosaccharide and β -glucan on growth performance of catfish showed that 1% GOS treatment and 1% yeast + 0.1% treatment) The use of these treatments led to an improvement in growth performance, as the weight gain was (31.57 and 30.90) g, and the specific growth rate was (1.30) %/day for both treatments, and the food conversion rate was (1.44 and 1.42) and the survival rate was 100%., Also when using *Lactococcus* spp. 5×10^8 cfu/g In the diet of common carp fish, it led to an increase in growth parameters such as final weight, total weight gain, specific growth rate and condition factor (Feng et al., 2021), The enhancer, the prebiotic, and the synbiotic act as stimulators of fish growth (Kazuń and Kazuń, 2019; Mugwanya et al., 2021 and Huynh et al., 2021).

The results of the thermal growth coefficient (TGC) ranged between 0.46 to 0.73 and the synbiotic treatment (T5) was significantly ($P < 0.05$) superior to the rest of the experimental treatments, The purpose of using the thermal growth factor is to collect growth data for fish of a specific size at one temperature to predict the growth of fish of different size when kept at other temperatures. It is stable over a wide range of temperatures for many species, and is less affected by fish size. And the interval between weights compared to other growth rate estimates such as daily growth rate, and thus provides a simple model for growth

rate comparisons and use it with modeling techniques (Jobling, 2003 and Reid et al., 2021). A study Sahandi et al. (2012) showed when using bacteria (*Bacillus circulans*, *Bacillus. Licheniformis*) 10^6 cfu/ml with *Artemia urmiana* in carp diets to improve the thermal growth coefficient of fish, while the study of using the fermentation method of fish silage with date fruit residues, the diets of common carp fish showed an increase in the thermal growth coefficient of carp fish, which ranged from 0.47 to 0.51 (Al-Kanaani, 2014). In another study, the use of the probiotic bacteria (*Lactobacillus plantarum*) at three levels of addition ($1.5, 3, 4.5 \times 10^6$ cfu/mg in carp fish diets, which contributed to an improvement in the thermal growth coefficient (Valiollahi et al., 2018).

The results of table (5) for the feed evaluation criteria showed a significant ($P < 0.05$) for the fifth treatment (T5) over the rest of the treatments, followed by two treatments of the Iraqi probiotic (T2) and the treatment of the imported commercial synbiotic (T4), adding that the addition of the prepared synbiotic led to Increasing the amount of feed intake and its palatability by the experimental fish, improving the rate of food conversion, increasing the efficiency of the diet, the protein intake and the productive value of the protein, this indicates an improvement in the digestion of the ration, the availability and balance of amino acids, and the utilization of essential nutrients, which led to an increase in the growth parameters of fish, Several studies showed an improvement in the parameters of the diet eaten by fish, including the study (Skrede, 2002) of the effect of fermentation by *Lactobacillus* bacteria and its growth on wheat and barley flour in Atlantic salmon diets, Bacteria were added to wheat and barley flour and fermented at 30 C° for 16 hours before mixing with the components of the diets, This process led to an improvement in the digestibility of the diets through a decrease in total starch contents and polysaccharide bonds, and an improvement in the digestion of protein, carbohydrates and fats in fermented diets with the synbiotic over non-fermented diets. It was also shown in another study the effect of fermentation and the formation of a synbiotic on the effect of fermentation of white soybean meal with lactic acid bacteria *Lactobacillus brevis* 10^9 cfu/g at a temperature of 30 C° for 36 hours before mixing with the feed ingredients and forming the ration added to the white soybean meal when fed on Atlantic salmon (*Salmo salar*), improved digestibility of the diets led to the elimination of sucrose, a decrease in the level of raffinose, a decrease in the trypsin inhibitor activity and an increase in the ability to digest fats and energy when fed on the synbiotic of fermented diets (Refstie, 2005).

Several studies have shown an improvement in the criteria for evaluating the diet, represented by the ratio of the feed conversion factor, the ratio of feed efficiency, the protein intake, the protein efficiency ratio, and the productive value of the consumed protein when using enhancers, biological precedents, and the synbiotic to an improvement in the

nutritional value of the feed eaten and provided to fish (Eleraky and Reda, 2014; Adel et al., 2015, Taher et al., 2018; Akter et al., 2019; Hoseinifar et al., 2019; Al-Mhanawi et al., 2021, Sutriana et al., 2021 and Feng et al., 2021).

Enhancing the diet with food additives such as the probiotic and the synbiotic led to an increase in the retention and utilization of nutrients, especially the treatment of the synbiotic (T5), this indicates an improvement in the nutritional value of the diets through the availability of nutrients, the most important of which are the important amino acids in growth and tissue building, and this was shown by a study (Yang et al., 2020) when using the synbiotic (Bioture) at rates of 2, 4 and 6 g/kg in largemouth bass rations (*Micropterus salmoides*) led to an increase in fish growth, an improvement in the nutritional value of the ration, and the retention of nutrients, proteins and fats.

The results of table (6) for analyzing the body components of fish showed a significant superiority of the fifth treatment (T5) in the percentage of protein ($P < 0.05$) over the rest of the treatments and a decrease in the percentage of fat. This shows that the use of the synbiotic, which led to improving the nutritional value of the diets, eating fish feed in a larger amount, increasing the values and efficiency of protein and preserving nutrients, led to an improvement in the sedimentation of components in the fish body (Eleraky and Reda, 2014; Akter et al., 2019 and Sutriana et al., 2021).

The results of figure (1) showed the level of digestive enzymes in the blood serum to a significant superiority of the fifth treatment (T5) in the enzyme alpha-amylase 61.81 IU/L and lipase 17.89 IU/L over the rest of the treatments, followed by the two treatments of the Iraqi probiotic (T2) and (T4). Dealing with the synbiotic mixture and making it a part of the diets by adding 0.8×10^7 of lactic acid bacteria led to an increase in the level of secretion of digestive enzymes and an improvement in the ability to digest nutrients in the diets. Studies have shown the activity of enzymes when using the synbiotic, probiotic and prebiotic, including a study (Goran et al., 2017) in which yeast (*Saccharomyces cerevisiae*) was used at rates of (0.5, 1, 1.5) % in the diets of common carp fish and cultured in the (RAS), which led to an increase in the level of amylase enzyme (0.5.1%) and a decrease in the level of amylase enzyme by 1.5%. There is a slight discrepancy in the level of the enzyme lipase, indicating that yeast cells when attached to the alimentary canal release amylase stimuli, which contribute to increasing the intake and benefit of the diets. While the study (Sutriana et al., 2021) confirmed the increase in the level of the activity of digestive enzymes when using the probiotic and prebiotic alone or in combination with yeast, galacto oligosaccharide, mannan oligosaccharide and β -glucan on the growth performance of catfish that 1% (GOS) treatment And the treatment (1% yeast + 0.1% β -glucan).

CONCLUSIONS

The use of the synbiotic mixture consisting of lactic acid bacteria (*Streptococcus thermophilus*, *Lactococcus bulgaricus*) and barley flour added to the fish ration led to stimulating and improving growth by improving the nutritional value of the ration, increasing digestive enzymes and depositing nutrients in the body of experimental fish.

REFERENCES

- Akter, M.N.; Hashim, R.; Sutriana, A.; Siti Azizah, M.N. and Asaduzzaman, M. (2019). Effect of *Lactobacillus acidophilus* supplementation on growth performances, digestive enzyme activities and gut histomorphology of striped catfish (*Pangasianodon hypophthalmus* Sauvage, 1878) juveniles. *Aquaculture Research*, 50(3), 786-797.
- Ali H.S. and Amal T. Al-Kaabi (2016). Effect of using three types of probiotic in the diet of common carp *Cyprinus carpio* L. reared in closed water system. *Journal of Al-Muthanna for Agricultural Sciences*, 4(1).
- Al-Dubakel, A.Y.; Al-Hamadany, Q.H. and Mohamed, A.A. (2015). Effect of local probiotic (Iraqi probiotic) on the growth of common carp *Cyprinus carpio* L. youngs. *Journal of Basrah Researches (Sciences)*, 41, 57-69.
- Al-Kanaani, S.M.N. (2014). Utilization of fish silage fermented with date fruit residues for feeding the common carp *Cyprinus carpio* L. and its physiological and histological effects (Doctoral dissertation, Ph. D. Thesis, Univ. Basrah, 246pp).
- Al-Mhanawi, B.H.; Al-Niaeem, K.S. and Al-Tameemi, R.A. (2021). Effect of food additives, Thapax and Labazyme on growth performance and immune response of young common carp *Cyprinus carpio* L. In IOP Conference Series: Earth and Environmental Science, 779, (1). Doi:10.1088/1755-1315/779/1/012123.
- Ahmed, N. and Turchini, G.M. (2021). Recirculating aquaculture systems (RAS): Environmental solution and climate change adaptation. *Journal of Cleaner Production*, 297, DOI: 10.1016/j.jclepro.2021.126604.
- Butt, U.D.; Lin, N.; Akhter, N.; Siddiqui, T.; Li, S.; and Wu, B. (2021). Overview of the latest developments in the role of probiotics, prebiotics and synbiotics in shrimp aquaculture. *Fish and Shellfish Immunology*, 114, 263-281.
- Cottrell, R.S.; Blanchard, J.L.; Halpern, B.S.; Metian, M. and Froehlich, H. E. (2020). Global adoption of novel aquaculture feeds could substantially reduce forage fish demand by 2030. *Nature Food*, 1(5), 301-308.
- Dawood, M.A. (2021). Nutritional immunity of fish intestines: Important insights for sustainable aquaculture. *Reviews in Aquaculture*, 13(1), 642-663.
- Eleraky, W.; and Reda, R. (2014). Evaluation of prebiotic and probiotic dietary supplementation on growth performance and some blood parameters of *Cyprinus carpio* Fry. *Egyptian Journal of Aquatic Biology and Fisheries*, 18(2), 29-38.
- FAO (2020). The State of World Fisheries and Aquaculture: Sustainability in Action. Food and Agriculture Organization of the United Nations, Rome.
- Feng, J.; Liu, S.; Zhu, C.; Cai, Z.; Cui, W.; Chang, X. and Nie, G. (2022). The effects of dietary *Lactococcus* spp. on growth performance, glucose absorption and metabolism of common carp, *Cyprinus carpio* L. *Aquaculture*, 546. DOI: 10.1016/j.aquaculture.2021.737394.
- Gerking, S.D. (1971). Influence of rate of feeding and body weight on protein metabolism of bluegill sunfish. *Physiological Zoology*, 44(1), 9-19.

- Goran, S.M.A.; Omar, S.S. and Anwer, A.Y. (2017). Assessment of yeast as a dietary additive on haematology and water quality of common carp in a recirculating aquaculture system. In AIP conference proceedings, 1888(1). Doi.org/10.1063/1.5004300.
- Harrigan, W.F. and McCance, M.E. (1976). Laboratory methods in food and dairy microbiology. Academic Press Inc. (London) Ltd.
- Hepher, B. (1988). Nutrition of pond fishes. Cambridge Univ. Press, London, 338 pp.
- Hoseinifar, S.H.; Hosseini, M.; Paknejad, H.; Safari, R.; Jafar, A.; Yousefi, M and Mozanzadeh, M.T. (2019). Enhanced mucosal immune responses, immune related genes and growth performance in common carp (*Cyprinus carpio*) juveniles fed dietary *Pediococcus acidilactici* MA18/5M and raffinose. Developmental and Comparative Immunology, 94, 59-65.
- Hussain, N.; Tariq, M.; Saris, P.E.J. and Zaidi, A. (2021). Evaluation of the probiotic and postbiotic potential of lactic acid bacteria from artisanal dairy products against pathogens. The Journal of Infection in Developing Countries, 15(01), 102-112.
- Huynh, T.G.; Vu, H.H.; Phan, T.C.T.; Pham, T.T. N and Vu, N.U. (2021). Screening utilization of different natural prebiotic extracts by probiotic *Lactobacillus* sp. for development of synbiotic for aquaculture uses. Can Tho University Journal of Science, 13, 96-105.
- Kazuń, B.; and Kazuń, K. (2019). Using probiotics in freshwater larviculture. Fisheries and Aquatic Life, 27(3).
- Jobling, M. (2003). The thermal growth coefficient (TGC) model of fish growth: a cautionary note. Aquaculture Research, 34(7), 581-584.
- Jobling, M. and Koskela, R. (1996). Inter-individual variation in feeding and growth in rainbow trout *Oncorhynchus mykiss* during restricted feeding and in a subsequent period of compensatory growth. Journal Fish Biology, 49, 658 - 667.
- Linares-Morales, J.R.; Gutiérrez-Méndez, N.; Rivera-Chavira, B.E.; Pérez-Vega, S.B. and Nevárez-Moorillón, G. V. (2018). Biocontrol processes in fruits and fresh produce, the use of lactic acid bacteria as a sustainable option. Frontiers in Sustainable Food Systems, 50.
- Mojer, A.M., Taher, M.M.; and Al-Tameemi, R.A. (2021). Comparison of growth for cultivated common carp, *Cyprinus carpio* larvae between earthen ponds and recirculation aquaculture system. Basrah Journal of Agricultural Sciences, 34(1), 192-205.
- Mugwanya, M.; Dawood, M.A.; Kimera, F.; and Sewilam, H. (2021). Updating the role of probiotics, prebiotics, and synbiotics for tilapia aquaculture as leading candidates for food sustainability: A review. Probiotics and Antimicrobial Proteins, 1-28.
- Muhtalief, S.N.; Susilowati, T.; Yuniarti, T.; Harwanto, D and Basuki, F. (2019). Production Performance of Sangkuriang Catfish (*Clarias gariepinus* Burchell-1822) N-2 (Nursery-2) Cultured on Recirculation System with Different Filter Media. In IOP Conference Series: Earth and Environmental Science, 246(1). Doi:10.1088/1755-1315/246/1/012061
- Murray, P.R.; Rosenthal, K.S. and Pfaller, M.A. (2020). Medical microbiology E-book. Elsevier Health Sciences.
- Nie, P.; He, Y.; Liu, F. and Zhang, H. (2021). Livestock and Aquaculture Information Sensing Technology. In Agricultural Internet of Things (pp. 185-218). Springer, Cham.
- Paray, B.A.; El-Basuini, M.F.; Alagawany, M.; Albeshr, M.F.; Farah, M.A. and Dawood, M.A. (2021). *Yucca schidigera* usage for healthy aquatic animals: Potential roles for sustainability. Animals, 11(1), 93.
- Philipose, K.K.; Sharma, S.R.K.; Loka, J.; Divu, D.; Sadhu, N. and Dube, P. (2013). Culture of Asian Seabream (*Lates calcarifer*, Bloch) in open sea floating net cages off karwar, South India. Indian Journal Fish, 60(1), 67-70.
- Pitcher, T.J. and Hart, P.J.B. (1982). Fisheries Ecology. London and Candaerra, Crom Helm Ltd. (American edition): The Avi publi Co., Linc. Westport, Conn. 414pp.
- Rahman, M.M. (2015). Role of common carp (*Cyprinus carpio*) in aquaculture production systems. Frontiers in Life Science, 8(4), 399-410.
- Reid, G.K.; Lefebvre, S.; Filgueira, R.; Robinson, S.M.; Broch, O.J.; Dumas, A. and Chopin, T. B. (2020). Performance measures and models for open-water integrated multi-trophic aquaculture. Reviews in Aquaculture, 12(1), 47-75.
- Sahandi, J.; Jafariyan, H.; Dehghan, M.; Adineh, H.; and Shohreh, P. (2012). Direct inoculation of *Bacillus* to rearing fish tanks effect on growth performance of two carp species fed with *Artemia* sp. World Applied Sciences Journal, 20(5), 687-690.
- SAS (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
- Refstie, S.; Sahlström, S.; Bråthen, E.; Baeverfjord, G. and Krogedal, P. (2005). Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). Aquaculture, 246(1-4), 331-345.
- Taher, M.M.; Al-Niaeem, K.S. and Al-Saad, S.A. (2018). Effect of bay laurel (*Laurus nobilis*) extract as prebiotic on growth and food conversion of common carp (*Cyprinus carpio*). Iraqi Journal of Aquaculture, 15(1), 17-30.
- Uten, F. (1978). Standard methods and terminology in fin fish nutrition from proc. World Sump on Fin Fish nutrition and fish feed technology. Hamburg., 20-23. June 1978. Vol.11 Berlin.
- Valiallahi, J.; Pourabasali, M.; Janalizadeh, E. and Bucio, A. (2018). Use of *Lactobacillus* for improved growth and enhanced biochemical, hematological, and digestive enzyme activity in common carp at Mazandaran, Iran. North American Journal of Aquaculture, 80(2), 206-215.
- Verni, M.; Rizzello, C.G. and Coda, R. (2019). Fermentation biotechnology applied to cereal industry by-products: Nutritional and functional insights. Frontiers in nutrition, 6, 42.
- Wee, K.L. and Shu, S.W. (1989). The nutritive value of boiled full -fat soybean meal In pelleted feed for Nile tilapia, Aquaculture, 81, 303-314.
- Xiao, X.; Tan, C.; Sun, X.; Zhao, Y.; Zhang, J.; Zhu, Y.; and Zhou, X. (2020). Effects of fermentation on structural characteristics and in vitro physiological activities of barley β -glucan. Carbohydrate Polymer Technologies and Applications, 231. DOI: 10.1016/j.carbpol.2019.115685.
- Yamamoto, Y. (2017). Characteristics of closed recirculating systems. In application of recirculating aquaculture systems in Japan (pp. 21-53). Springer, Tokyo.
- Yang, P.; Yang, W.; He, M.; Li, X. and Leng, X.J. (2020). Dietary synbiotics improved the growth, feed utilization and intestinal structure of largemouth bass (*Micropterus salmoides*) juvenile. Aquaculture Nutrition, 26(2), 590-600.
- Yilmaz, S.; Yilmaz, E.; Dawood, M.A.; Ringø, E.; Ahmadifar, E. and Abdel-Latif, H.M. (2022). Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: A review. Aquaculture, 547, 737514.