Original Article

Effect of Some Probiotics and Synbiotic Dietary Supplementation on Growth Performance and Some Health Parameters of Common Carp, Cyprinus Carpio

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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±.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 themophilus* 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, condition factor of 1.69 and ration evaluation criteria that the amount of feed included 167.48 g, the feed conversion ratio 2.28. The best protein ratio was 18.28% and the lowest fat percentage was 4.22% in the chemical analysis of body components. Increasing the intestinal content of the total bacterial count and the counts of lactic acid bacteria (63.33×10^6 and 11×10^6) cfu/g, respectively, and improving the lipid profile in the blood serum, which included total cholesterol, triglycerides and both high- and low-density lipoproteins (117.81, 74.63, 56.96 and 45.92) mg/dL respectively.

Keywords: Common Carp, Growth Performance, Health Parameters, Probiotics and Snybiotic.

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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 intake of food containing the probiotic is a good alternative to influence the level of lipids in the blood plasma (Siripun 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, commercial imported probiotic, commercially imported synbiotic and the synbiotic prepared, after adding, it to the cultured carp diets on growth performance and some health parameters (Total cholesterol - a measure of the total amount of cholesterol in blood. It includes both low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol and triglycerides) 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 (5-amp inverter device with 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 m^2/m^3 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 m^2/m^3 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 and 4 pm). The experiment lasted for 70 days, starting from 5/03/2022.

Experience diets

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 Xiao et al. (2020). Then, lactobacillus bacteria (*Streptococcus themophilus, 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). 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 subtilus*, *saccharomyces cervisia*) 1×10^{8} CFU/g. 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 metabolitc) 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°.

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)

(LN Final Weight (FW) (g)- LN Initial Weight (IW) (g))/ (number for days)

4- Feed Conversion Rate (FCR) (Uten, 1978)

(Weight Gain (g))/ (Food Intake (g)

5- The condition factor (K)

((Weight)/ (body length) ^3)×100

Microbiological examinations

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

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).

Serum profile lipid

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 Total Cholesterol (T.CHO), Triglycerides (TG) and high-density lipoproteins (HDL) are measured in serum by a ready-made laboratory kit from Mindray of China and by means of a (BS-230 mindray) device at a wavelength of 510 nm. Low-density lipoproteins in the blood serum depending on what was mentioned (Kerkhofs., 2007)

(LDL) concentration (mg/dL) = Total Cholesterol - (HDL - triglycerides/5)

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).

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

Temperature (C°)	Dissolved oxygen mg/l		NH_3 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

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	0.1	0.1	0.1	0.1

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).

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 count (TBC) and lactic acid bacteria (LAB) prepared and added in the food ration provided to fish in the fifth treatment (T5) showed table (3).

Table 3. Count of bacteria in the prepared concentration of the symbiotic in treatment (T5).

TBC 10^7 cfu/g	LAB 10^7 cfu/g
6.17	0.87

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, Specific growth rate 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 and 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 (4) 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, (167.48 and 2.28) 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 (5) 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 and 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 and Diet assessment criteria for experimental treatments over a period of 70 days.

Transactions	Starting weight g	Final weight (g)	WG (g)	DGR (g)	SGR (%/ day)	Feed intake	FCR	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	143.55 ±1.09 d	3.54 ±0.10 a	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	156.60 ±0.46 b	2.97 ±0.02 b	1.67 ± 0.01ab
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	144.11 ±0.58 cd	3.56 ±0.19 a	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	147.65 ±1.49 c	3.19 ±0.12 ab	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	167.48 ±.82 a	2.28 ±0.06 c	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 0.1%, T3:- commercial importer probiotic ration 0.1%, T4:- commercial imported synbiotic ration 0.1%, T5:- prepared synbiotic ration 0.1%.

The results of table (6) showed the content of the Total Bacterial Count (TBC) of the intestines of the experimental fish to a significant superiority of the two treatments of the prepared synbiotic (T5) and the Iraqi probiotic (T2) over the rest of the treatments (63.33 and 58.33) $\times 10^6$ cfu/g, respectively, followed by the treatment of The imported commercial synbiotic (T4), while there were no significant differences between the two treatments of the control (T1) and the imported commercial probiotic (T3).

Table (5). 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 0.1%, T3:- commercial importer probiotic ration 0.1%, T4:- commercial imported synbiotic ration 0.1%, T5:- prepared synbiotic ration 0.1%.

The results of table (6) the content of the bacterial count of LBA in the intestines of the experimental fish showed a significant superiority of the treatment of the prepared synbiotic (T5) 11×10^6 cfu/g over the rest of the treatments, and there was no significant difference with the Iraqi probiotic treatment (T2), followed by the treatment of the imported commercial synbiotic (T4), while there were no significant differences between the two treatments of the control (T1) and the imported commercial probiotic (T3). The results showed that the presence of *Escherichia coli* bacteria was not recorded in each of the treatments T5, T4 and T2, while its presence was recorded in the two treatments, the control and the commercial booster, 5.7×10^2 and 1.3×10^2 cfu/g, respectively.

Table 6. The content of bacteria presents in the intestines of the experimental treatment fish during a period of (70) days.

Transactions	TBC 10^6 cfu/g	LAB 10^6 cfu/g	*E. coli* 10^2 cfu/g
Mean± SE			
T1	4.57 ± 0.16 c	0.47±0.03 c	5.67 ± 0.03 a

T2	58.33 ± 3.33 [a]	9.3±0.33 [ab]	1.33 ± 0.00 [b]
T3	5.10 ± 1.00 [c]	1.3±0.07 [c]	0
T4	49.33 ± 0.88 [b]	8 ±1.53 [b]	0
T5	63.33 ± 3.18 [a]	11±1.00 [a]	0

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 0.1%, T3:- commercial importer probiotic ration 0.1%, T4:- commercial imported synbiotic ration 0.1%, T5:- prepared synbiotic ration 0.1%.

The results of table (7) analysis of the profile lipid of the experimental treatments showed the superiority of the treatment of the prepared synbiotic (T5) in the analysis of the profile lipid, followed by the treatment of the Iraqi probiotic (T2) and the imported commercial symbiotic (T4) and the commercial imported propiotic (T3) in both total cholesterol (T.CHO), Triglycerides (TG) and high-density lipoproteins (HDL) and low-density lipoproteins (LDL), while there were no significant differences between the treatment of the imported propiotic (T3) and the control (T1).

Table 7. The profile lipids in the blood serum of the experimental treated fish for a period of (70) days

Transactions	**T.CHO**	**TG**	**HDL**	**LDL**
Mean± SE				
T1	143.43±0.49 [a]	85.69±0.45 [a]	67.77±0.36 [a]	58.52±0.49 [a]
T2	122.34±0.13 [d]	79.82±0.04 [d]	58.19±0.27 [d]	48.18±0.13 [b]
T3	139.98±0.05 [b]	83.33±0.27 [b]	65.26±0.18 [b]	58.05±0.05 [a]
T4	127.81±0.13 [c]	81.09±0.39 [c]	62.44±0.50 [c]	49.14±0.13 [b]
T5	117.81±0.15 [e]	74.63±0.39 [e]	56.96±0.40 [e]	45.92±0.15 [c]

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 0.1%, T3:- commercial importer probiotic ration 0.1%, T4:- commercial imported synbiotic ration 0.1%, T5:- prepared synbiotic ration 0.1%.

DISCUSSION

The 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 count and lactic acid bacteria after addition and fermentation for 24 hours to increase the count 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 Xiao et al. (2020), 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 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, Betaglucans) 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 Al-dubakal et al. (2015) when using 0.02% of the Iraqi Probiotic in increasing the final weight, weight gain, and the specific 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 and 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, the 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 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 x 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., 2022), 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 table (4) 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).

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 dirt’s 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 (5) 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 feed conversion ratio 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 use of lactic acid bacteria (*Streptococcus themophilus, Lactococcus bulgaricus*) and their addition to barley flour and their growth and formation of the prepared synbiotic (T5) through the fermentation process led to an increase in the content of the total bacterial count of the intestines of fish treated table (6) with the synbiotic prepared (T5) 6.3 x 10^7 cfu/g There is also an increase in the treatment of the Iraqi probiotic (T2) and the prepared synbiotic (T3) Figure (1), This leads to an increase of lactic acid bacteria in the intestines of the experimental fish, and the highest increase was in the treatment of the prepared synbiotic(T5)1.1 x10^7cfu/g. The fatty acids that result from fermentation by bacteria increase the activity of beneficial bacteria in the intestine (Asaduzzaman et al., 2018). Which reduces the pH in the intestine, which leads to the diversity of the intestinal microflora through the activation of beneficial microflora and the exclusion of harmful microflora (Qi et al., 2019). Lactic Acid Bacteria (LAB) work during the fermentation process of carbohydrates in the digestive system to produce Short-Chain Fatty Acids (SCFA), and these acids have a positive effect on the growth rate and feed consumption by improving the function of the intestine, if it works to provide cells with energy (Magouz et al., 2020).

The study Sahoo et al. (2015) showed that the autoaggregation increases with the increase in the incubation time in lactic acid bacteria taken from the intestines of *Labeo rohita* and *Catla catla* fish, and there is a direct relationship between the incubation period and the auto aggregation, which helps the probiotics to prolong their stay inside Digestive system, which leads to a positive effect on the intestinal microflora of the host. While a study showed the ability to aggregate high lactic acid bacteria with E. coli and a medium capacity with other types of pathogenic bacteria and that the possibility of aggregation is a stress-dependent phenomenon, which is a function of the surface properties of probiotics and pathogens (Bhat and Bajaj., 2020). Differences in the coaggregation potential may be due to differences in the surface-associated protein, and part of the carbohydrate, and is determined by cell surface proteins, whereby removal of these surface proteins leads to a change in aggregation and adhesiveness (Kumar et al., 2020). Cell surface resistance to water indicates the ability of probiotics to adhere to the host intestinal epithelium depends on the cell surface resistance and thus, hydrophobicity shows sufficient adhesion potential of probiotics. Cell aggregation, which causes the aggregates of these cells to stay longer in the Gastrointestinal tract (GI) due to their increased density which enhances the hydrophobic nature of the cell surface enhancing the association of hydrophobic bacteria with the epithelial cells of the (GI) thus imparting more health benefits to the host (Ruiz Sella et al., 2021).

The study Alishahi et al, (2018) when using lactic acid bacteria (*Lactobacillus Plantarum* and *Lactobacillus Bulgaricus*) 5×10^7 cfu/g in the diets of common carp (*Cyprinus carpio*) led to a significant effect on the total counts of bacteria and lactic acid bacteria at The use of *Lactobacillus bulgaricus* bacteria. The study (Al-Niaeem et al, 2019) showed an increase in the numbers of total bacterial count and lactic acid bacteria in the intestines of fish when using the probiotic Biogen by 2% in the diets of common carp fish. As explained by Albadran, (2018), when part of the diet was replaced with vegetable residues at rates (5, 7.5 and 10) % and considered it a prebiotic in the diets of common carp fish, it led to an increase in both the total bacterial count and the counts of lactic acid bacteria in all the replacement ratios, and it was the highest in the percentage 10%. A study showed that *Lactobacillus acidophilus* 10^5 and 10^7 cfu/g can be used in the diets of juvenile cat fish (*Pangasianodon hypophthalmus*) with a feeding rate of 2.5% of the body weight, which led to a positive effect by adding the bacteria to the fish diet, It led to an increase in the total bacterial count and the total counts of lactic acid bacteria in fish intestines compared to the control group (Akter et al., 2019). While the study (Safari et al, (2021) when using a mixture of *Pediococcus acidilactici* with galactooligosaccharides at a concentration of 7.59x10^1 and the amount of addition in the diets 5 and 10 g/kg to feed African Cichlid (*Labidochromis lividus*) led to an increase in both the total bacterial count and the counts of bacteria Lactic acid bacteria and low counts of E. coli bacteria.

The results of table (7) showed that adding the probiotic and the commercially imported synbiotic and the prepared synbiotic to a decrease in the profile lipid in the serum of the experimental fish, and the most decrease was recorded in the prepared synbiotic (T5) in T.CHO, TG, HDL and LDL (117.81,74.63, 56.96 and 45.92) respectively.

Lactic acid bacteria possess the desired functional properties of hypocholesterolemic potential by using the activity of bile salt hydrolase enzymes as an important marker for new probiotic selection for functional traits such as hypocholesterolemic activity (Plaza-Diaz et al., 2019). It is noteworthy that hydrolase are enzymes whose function is to break down chemical bonds and cleavage from each other, as these enzymes, using water, break down and divide large molecules into smaller ones, this process is of great importance for the body, as it digests and transforms large molecules into others that the body can synthesize again, and it is an important source of energy because it is a primary source of carbon needed to manufacture energy (Byakika et al., 2019). Bile salt bio-promoting enzymes help to resist bile salt while transiting the digestive system, especially lactic acid bacteria. It has been observed that lactic acid bacteria prefer glycocholate as a substrate than other conjugated bile salts (Bhat and Bajaj., 2020). The activity of bile salt enzymes causes the breakdown of bile salts, and the resulting free bile acids are excreted through the feces. To compensate for the loss of bile salts, dietary cholesterol is used in the synthesis of bile salts and thus delays the rise in cholesterol (Siripun et al., 2022). It is reported that lactic acid bacteria have the highest ability to disengage bile salt, and significantly lower low lipoproteins

(LDL) in the blood, total cholesterol, and total cholesterol in the liver due to the high activity of bile salt enzymes, which increases the excretion of bile acid in the stool daily and thus accelerates the synthesis of bile acid new cholesterol in the liver (Ma et al. 2019). Cholesterol absorption by a probiotic helps reduce cholesterol available for absorption by intestinal cells, positively modulating fluidity and permeability and also contributes to the regulation of lipid metabolism by reducing the concentration of total cholesterol and triglycerides, Probiotics absorb cholesterol and make it unavailable for absorption by intestinal cells, thus reducing the level of total cholesterol (Vourakis., 2021).

The fatty acids produced by fermentation by bacteria are required to improve the transport of digested nutrients through intestinal epithelial cells (Asaduzzaman et al., 2018). As indicated by Byakika et al. (2019), Short-Chain Fatty Acids (SCFA) produced by lactic acid bacteria can play a key role in biochemical reactions involving cholesterol metabolism, Butyric acid is a powerful inhibitor of 3-hydroxymethyl-3-glutaryl-CoA (HMG-CoA), while acetic acid is converted to acetyl-CoA in the liver and used more in the cholesterol synthesis process. Propionate also inhibits cholesterol and lipid biosynthesis, Propionic acid enhances hepatic bile salt synthesis by enhancing the activity of 7α-hydroxylase, which converts cholesterol into 7α-hydroxycholesterol, Propionate inhibits lipogenesis and cholesterol synthesis by preventing the incorporation of acetate into plasma lipids, which leads to decreased cholesterol synthesis, The ability to produce (SCFA) from probiotics to contribute to the possibility of lowering cholesterol, and there is a relationship between the ability to produce SCFA and the effects of hypocholesterolemia, (SCFA) may reduce serum lipid content by inhibiting hepatic cholesterol synthesis and/or by redirecting plasma cholesterol toward the liver, Lactic acid bacteria ferment indigestible carbohydrates in the intestine to extract energy, and (SCFA) mainly include acetate, propionate, butyrate, etc. (Pushpass et al. 2021). As explained by Albadran (2018), when part of the diet was replaced with vegetable residues (5, 7.5 and 10) % and considered a prebiotic in the diets of common carp fish, it led to a decrease in total cholesterol, triglycerides, HDL and LDL in all the replacement ratios which were low more than 10% replacement rate. A study Dawood et al (2020) confirmed when using the synbiotic consisting of *Aspergillus oryzae* $1x10^8$ cfu/g and weight of 0.5 g/g of bacteria and β-glucan at a weight of 0.5 g / kg in feeding Nile Tilapia fish (*Oreochromis niloticus*), led to a decrease in cholesterol and triglycerides compared to the control treatment. Ajdari (2022) study showed that when common carp fish were fed a diet supplemented with prebiotic, probiotic and synbiotic, it led to an improvement in growth parameters and health status when using the prebiotic, probiotic, and synbiotic, and it was the highest in the treatment of the commercial synbiotic (Biomin Imbo) of *Enterococcus faecium* bacteria and fructooligosaccharide with an added at a weight of 1 g/kg, which reduced both cholesterol and triglycerides in fish serum compared to the control treatment. The use of the synbiotic prepared from barley flour and lactic acid bacteria (*Streptococcus themophilus* and *Lactococcus bulgaricus*) led to the activity of bile salt enzymes and control of cholesterol levels in the serum of experimental fish.

Conclusions

The use of the symbiotic consisting of lactic acid bacteria (*Streptococcus themophilus* and *Lactococcus bulgaricus*) and barley flour added to the fish ration led to stimulating and improving growth and health status by improving the nutritional value of the ration and depositing nutrients in the fish body, as well as increasing both the counts of Total bacteria and lactic acid bacteria, improve the lipid profile in the blood serum of experimental fish, so their use as feed supplements may be important in fish and aquaculture rations.

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