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Effects of Some Dietary Additives on Growth Performance and Feed Efficiency of Young Common Carp *Cyprinus carpio*

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Abstract: Effects of adding three dietary additives and their mixture to control diet (38% protein) on the growth performance and feed efficiency of young common carp *Cyprinus carpio* with an average weight of 16.16±0.09 g were assessed. These additives were probiotic (*Saccharomyces cerevisiae* yeast) (T1), amino acids (lysine and methionine) (T2), a mixture of exogenous enzymes (protease, cellulase, α-amylase, amyloglucosidase, pectinase, beta-glucanase, xylanase, phytase, lipase and beta mannanase) (T3), their mixture (T4) and control (T5). Inclusion of probiotic and exogenous enzymes showed significant ($P \leq 0.05$) improvement in the growth performance and feed efficiency of the fishes compared with control and other additive diets. Otherwise, the inclusion of amino acids and the additive mixture did not show any significant ($P>0.05$) improvement compared with the control diet. Probiotic diet (T1) recorded greatest specific growth rate SGR (0.68%/day) and best feed conversion ratio FCR (2.84), followed by exogenous enzymes diet (0.57%/day and 3.44, respectively), while the SGR and FCR values for T2, T4 and control were decreased significantly ($P \leq 0.05$). Probiotic and enzymes feed additives have proved significant enhancement of fish growth and feed efficiency.

Keywords: Amino acids, Exogenous enzymes, Feed efficiency, Growth, Probiotic

Introduction

The rapid expansion of aquaculture activities led to the increased global demand for fish feed, as well as the need to manufacture nutritionally balanced diets to ensure the best possible growth and feed conversion (Singh et al., 2011; Ghomi & Alizadehnajd, 2012). During the past few decades, intensification of farmed fish production caused the spread of fish diseases and the deterioration of water environmental quality in fish farms, resulting in major economic losses and challenges in controlling and avoiding diseases (Ahmadifar et al., 2011). The use of antibiotics to treat diseases could be responsible for causing microorganisms to develop resistance to these antibiotics, and there is a high risk of these resistant

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microorganisms being transmitted from an aquatic environment to humans. Furthermore, using antibiotics is harmful to aquatic organisms because they kill beneficial microorganisms in their digestive system, so the improvements of non-antibiotic agents are more suitable for health management in aquaculture (Ringø et al., 2014).

Probiotic is defined as a microorganism when used in appropriate amounts conferring a health advantage on the host (FAO/WHO, 2002). Probiotics are products made from beneficial microorganisms, isolated from the intestinal flora of the host's gut, when added to the feed, form colonies, inhabit epithelial cells coating the gastrointestinal tract, prevent pathological impact on the host body, and also contribute to restoring the microbial balance of the intestinal flora when the host is exposed to stressors such as high temperature (Ige, 2013; Nwachi, 2013; Bajagai et al., 2016).

Amino acids are important biomolecules that serve as building blocks for proteins, and are intermediates in numerous metabolic pathways, manufacture a wide range of biologically essential substances and play a major role in protein phosphorylation, cellular signaling, gene expression regulation, transport of nutrients, metabolism in animal cells and mediated immune responses (Wu, 2010; Wang et al., 2013). Amino acids are also considered as essential substances for growth, reproduction, maintenance, feed intake, utilization of nutrients, behavior, larval metamorphosis and resistance to environmental stress and pathogenic organisms in fishes (Namulawa et al., 2012). Amino acids are commonly supplemented with plant-based diets to improve the nutritional profile and palatability and in many compounds feeds as feeding stimulants for carnivorous species (Papatryphon & Soares, 2000). Among those amino acids, lysine, which is particularly important because it contributes the highest concentration among essentioal amino acids in the carcass of many fish species, and methionine, which is usually the first limiting EAA in aquafeed, were normally reported to be insufficient in some plant-based diets (Alam et al., 2005; Luo et al., 2006; Yuan et al., 2011). In addition to its role in protein synthesis, lysine also works in association with methionine as a precursor of carnitine to carry fatty acids through mitochondrial membranes and simplifies their oxidation to produce energy (Deng et al., 2010). The dietary methionine supplementation has been indicated mainly in diets based on soybean protein (Nguyen & Davis, 2009). A methionine addition to the fish feed enhanced the growth performance of numerous species such as common carp *Cyprinus carpio* (Schwarz et al., 1998), juvenile cobia *Rachycentron canadum* (Zhou et al., 2006) and gibel carp *Carassius auratus gibelio* (Hu et al., 2008). Endogenous enzymes are produced by the organisms and microorganisms in the digestive tract, but they are insufficient in the degradation of compounds which are difficult for digestibility, therefore, the studies about the digestibility of the feed with exogenous enzymes have accelerated. Among the main enzymes used in the mixed feed fishery industry are phytase, carbohydrase, protease and lipase (Ogunkoya et al., 2006). Therefore, the current study aimed to evaluate the effect of different diets supplemented with probiotic, amino acids and exogenous enzymes on the growth performance and feed efficiency of young common carp *C. carpio*.

Materials and Methods

Experiment Design

The experiment was conducted by using 15 plastic aquaria with a capacity of 100 l each, filled with 60 l of water. One-third of the water volume of each aquarium was replaced (with aged tap water stored in 2 tons plastic tank to remove chlorine) once daily by siphoning outfeed residual and feces.

The experiment lasted for 75 days, during which the fishes were fed 2% of their body weight. The amount of food given to the fishes was adjusted every 15 days after the fish weighing process was carried out. Some environmental factors of the aquaria water were measured, represented by dissolved oxygen, pH and salinity, while maintaining the water temperature was by using an electric heater with a thermostat.

Fishes

The *C. carpio* used for the experiment were obtained from fish culture ponds in the Al-Hartha Station for Agricultural Researches, north Basrah at Al-Hartha district affiliated to the Aquaculture Unit, College of Agriculture, University of Basrah. A total of 225 fishes was transported by a tank truck to the laboratory. They were placed in a well-aerated 250-liter tank to remove transport stress for 24 hours. The fishes were distributed randomly to the 15 experiment aquaria (15 fishes each) on the following day. Three aquaria have been allocated for each treatment. The aquaria were equipped with electric air pumps. Before the start of the experiment, fishes were acclimated to laboratory conditions and experimental diets for eight days in the experimental system. Fishes were weighed by using a ZH 8257 electronic balance. The average weight of the fishes used in the experiment was 16.16 ± 0.09 g.

Diet Preparation

Basal pelletized fish feed was combined with three types of feed additives and their mixture used in the experiment (Table 1). Five experimental diets were prepared, a control diet (T5) with no additive and four other diets were prepared by supplementing Italian origin Thepax which contains *Saccharomyces cerevisiae* yeast as probiotic (T1), amino acids lysine and methionine (T2), enzymes mixture consisted of protease, cellulase, α-amylase, amyloglucosidase, pectinase, beta-glucanase, xylanase, phytase, lipase and beta mannanase (T3), and a mixture of the above feed additives (T4). The basal diet was formulated in the Fish Feed Manufacturing Plant affiliated to the Marine Sciences Center at the University of Basrah.

Table 1: Ingredients and feed additives of the experimental diets.

	Experimental diets				
	T1	T2	T3	T4	T5 (control)
Ingredients					
Fish meal (%)	30.21	30.21	30.21	30.21	30.21
Soybean meal (%)	30.21	30.21	30.21	30.21	30.21
Corn meal (%)	18.79	18.79	18.79	18.79	18.79
Wheat meal (%)	18.79	18.79	18.79	18.79	18.79
Vitamin-mineral premix (%)	2	2	2	2	2
probiotic (mg/kg)	700	0	0	700	0
Lysine (g/kg)	0	16	0	16	0
Methionine (g/kg)	0	9.76	0	9.76	0
Exogenous enzymes (g/kg)	0	0	1.5	1.5	0

Adding Food Additives to the Basal Diet

The basal diet was divided into five sections, each of which represents one of the five treatments as follows:

Treatment 1 was a probiotic Thepax (700 mg/kg) added to the second section and was well mixed with an electric mixer for three minutes, then shaped in the form of pellets and left to dry at room temperature for 24 hours.

Treatment 2 was amino acids (lysine and methionine) added to the third section according to the levels recommended by Zhou et al. (2007), using the method described by Yuan et al. (2011), represented by mixing amino acids in distilled water and then adding carboxy methylcellulose at a rate of 2% to obtain a dough-like substance, then the mixture was dried using a freeze dryer for three days, grind and sieved with a 250 microns mesh size sieve. The moisture content of the mixture for each amino acid was estimated by drying it in the oven at 105 ºC for 24 hours. The amino acid binder mixture was added to the diet by 16 and 9.76 g/kg of lysine and methionine, respectively. The diet was formed in the form of pellets and left to dry at room temperature for 24 hours, packed and stored at -18 °C.

Treatment 3 was an enzyme mixture added to the fourth section according to Bogut et al. (1995). The enzyme mixture of 1.5 g/kg was dissolved in distilled water at a temperature of 40 °C and added to the diet. The mixture was formed in the form of pellets and left to dry at room temperature for 24 hours. The pellets were frozen at -18 °C.

Treatment 4 was all of the former feed additives added to the fifth section.

Treatment 5 was a basal diet without additive.

Growth Performance and Food Efficiency Measurements

The fishes were weighed at the end of the experiment. Weight gain (WG), the daily growth rate (DGR), the relative growth rate (RGR), the specific growth rate (SGR) and the feed conversion ratio (FCR) were calculated according to the following equations:

WG (g) = FW – IW

DGR (g/day) = (FW – IW) / days

RGR (%) = 100 × [(FW – IW) / IW]
SGR (%/day) = 100 × [(ln FW) – (ln IW) / days]
FCR = feed given (dried weight) / weight gain (wet weight)
Where: FW = Final fish weight (g); IW = Initial fish weight (g).

Results

The results of the proximate analysis of the basal diet is shown in Table 2.

Table 2: Proximate composition of the basal diet.

Nutrients	
Moisture (%)	4.52±0.31
Crude protein (%)	38.00±1.08
Ether extract (%)	6.91±0.47
Carbohydrate (%)	44.10±2.35
Ash (%)	6.47±0.78
Energy (kcal/kg)	4487.90±39.07

The water temperature during the experimental period in the aquaria ranged between 23.32-24.52 °C, pH between 8.12-8.40, dissolved oxygen between 7.89-8.35 mg/l and salinity between 1.22-1.54 PSU (Table 3).

Table 3: Water quality parameters during the experiment.

Water quality	Value
Temperature (ºC)	23.92±0.08
Dissolved oxygen (mg/l)	8.12±0.33
Salinity (PSU)	1.38±0.23
pH	8.26±0.20

Table 4 shows the growth performance and food efficiency parameters of young *C. carpio* during the experiment. The results of the growth parameters and feed conversion ratio indicated that there showed a significant positive effect ($P\leq0.05$) for the diets containing the probiotic and exogenous enzymes compared to the control diet, while the growth and feed conversion ratio did not record any significant improvement ($P>0.05$) for diets containing amino acids or mixtures of additives compared to the control diet.

The WG and the DGR values were converging after 15 days of the start of the experiment, but the differences began to appear clearly after 30 days and continued until the end of the experiment. Treatment T1 recorded the highest WG (3.27 g) and DGR (0.22 g/day) after 60 days, while the remaining treatments T2, T3, T4 and T5 recorded this increase after 75 days concerning the WG (1.12, 2.10, 1.58 and 1.25 g) and DGR (0.07, 0.14, 0.11 and 0.08 g/day), respectively as shown in Figures 1 and 2.

Table 4: Growth performance and feed efficiency of young *C. carpio* during the experiment.

Variables	Treatments				
	T1 (Probiotic)	T2 (Amino acids)	T3 (Exogenous enzymes)	T4 (Mixture of additives)	T5 (Control)
Initial weight IW (g)	16.08^{a}±0.08	16.11^{a}±0.03	16.27^{a}±0.21	16.10^{a}±0.05	16.24^{a}±0.18
Final weight FW (g)	26.84^{a}±2.85	20.87^{b}±0.63	25.05^{a}±2.22	21.51^{b}±1.06	20.92^{b}±0.36
Weight gain WG (g)	10.75^{a}±2.93	4.76^{b}±0.62	8.78^{a}±2.16	5.40^{b}±1.11	4.69^{b}±0.19
Daily growth rate DGR (g/day)	0.14^{a}±0.04	0.06^{b}±0.01	0.12^{a}±0.03	0.07^{b}±0.01	0.06^{b}±0.00
Relative growth rate RGR (%)	66.93^{a}±18.53	29.52^{b}±3.84	53.94^{a}±13.11	33.57^{b}±7.01	28.86^{b}±0.82
Specific growth rate SGR (%/day)	0.68^{a}±0.15	0.34^{b}±0.04	0.57^{a}±0.12	0.38^{b}±0.07	0.34^{b}±0.01
Feed conversion ratio FCR	2.84^{a}±0.77	5.72^{b}±0.67	3.44^{a}±0.86	5.15^{b}±1.06	5.73^{b}±0.15

Means in the same raw with different superscript letters are significantly different ($P \leq 0.05$).

The RGR and SGR were similar in the pattern of changes to each other after 15 days of the experiment, but the differences began to appear clearly after 30 days and continued until the end of the experiment. The treatment T1 recorded the highest RGR (15.64%) and SGR (0.98%/day) after 60 days, while the T2 and T3 recorded this increase after 30 days concerning the RGR (6.18 and 11.62%) and SGR (0.39 and 0.73%/day), respectively and the T4 and T5 recorded this increase after 75 days with respect to the RGR (7.90 and 6.35%) and SGR (0.51 and 0.41), respectively (Figures 3 and 4).

The differences in the FCR began to appear after 30 days. During this period, the treatments T1 and T3 demonstrated the best value compared to the rest of the treatments. This tendency continued until the end of the experiment. The best FCR was recorded by treatment T1 (1.90) and T3 (2.58) after 60 and 30 days from the start of the experiment, respectively (Figure 5).

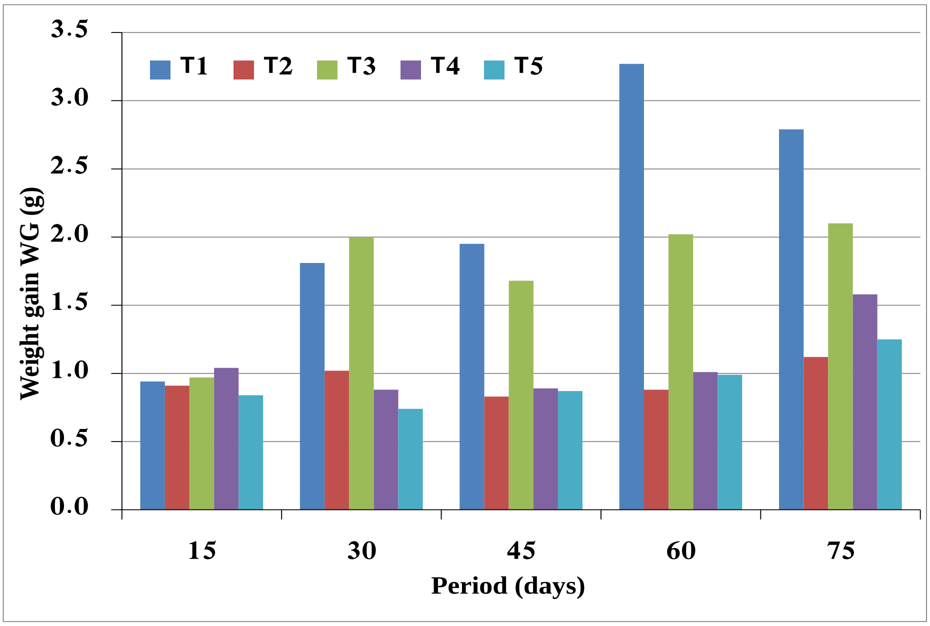

Figure 1: Weight gains of young *C. carpio* during different experimental periods.

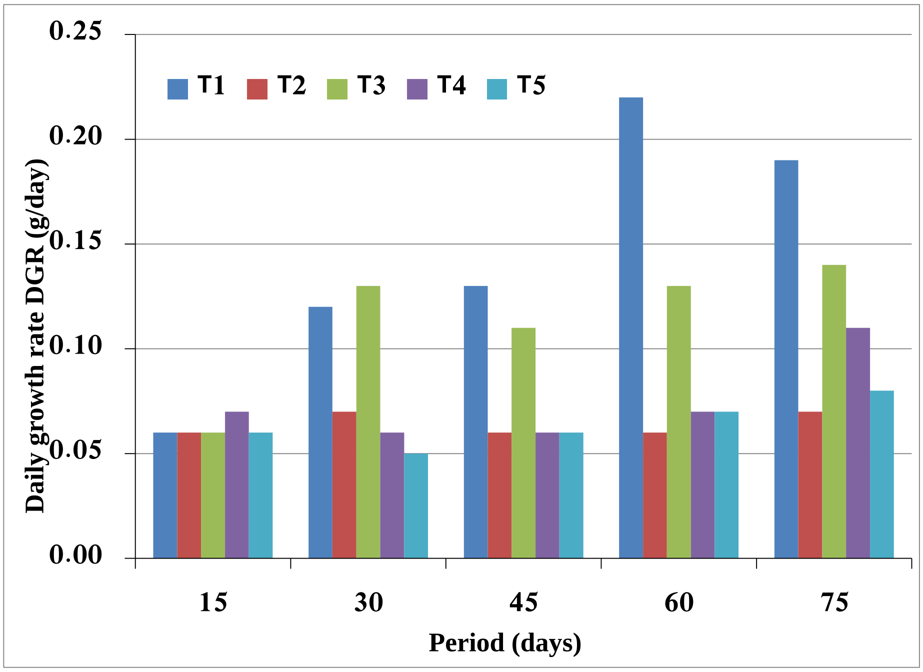

Figure 2: Daily growth rate of young *C. carpio* during different experimental periods.

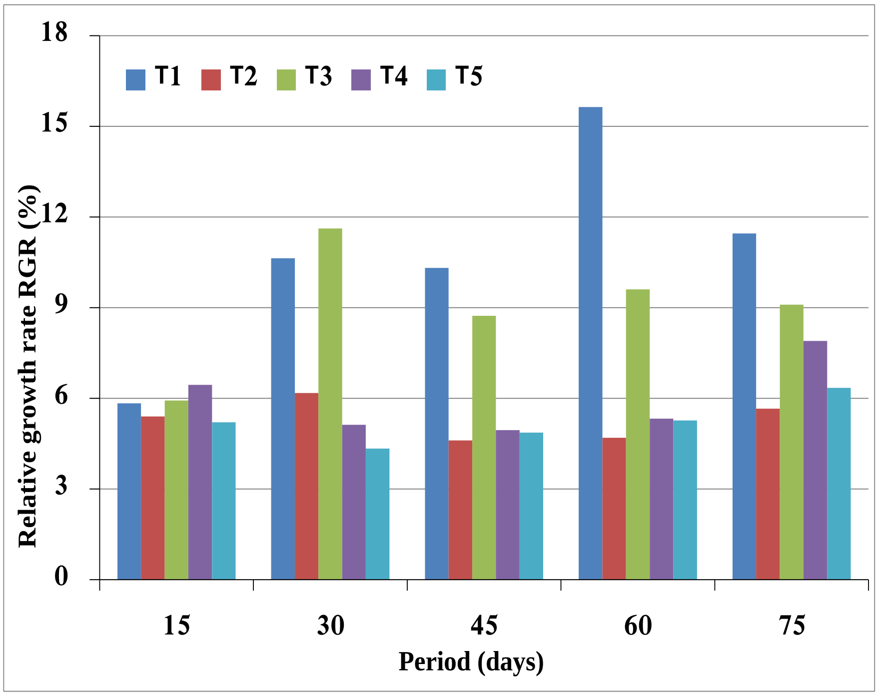

Figure 3: Relative growth rate of young *C. carpio* during different experimental periods.

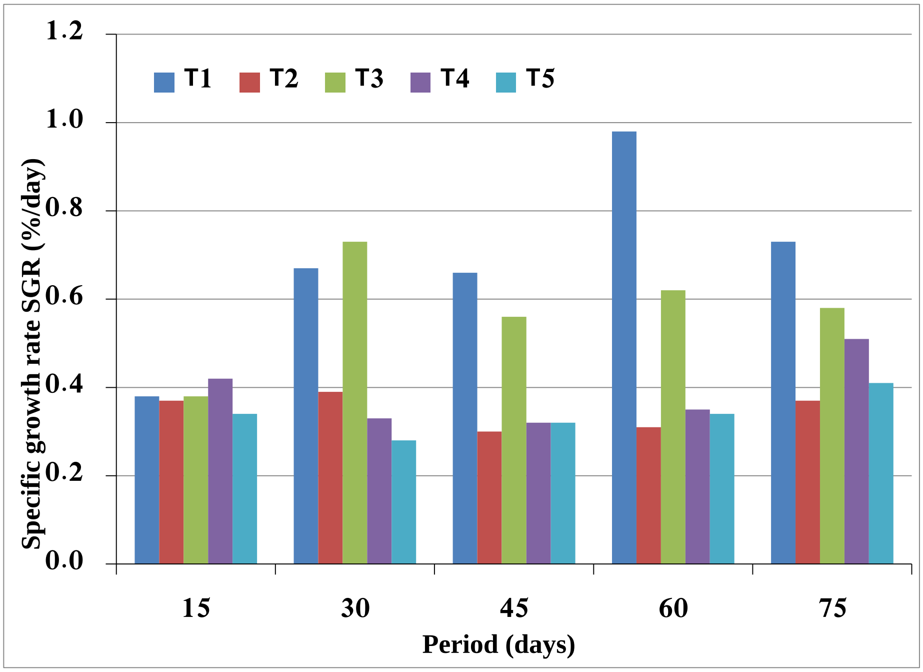

Figure 4: Specific growth rate of young *C. carpio* during different experimental periods.

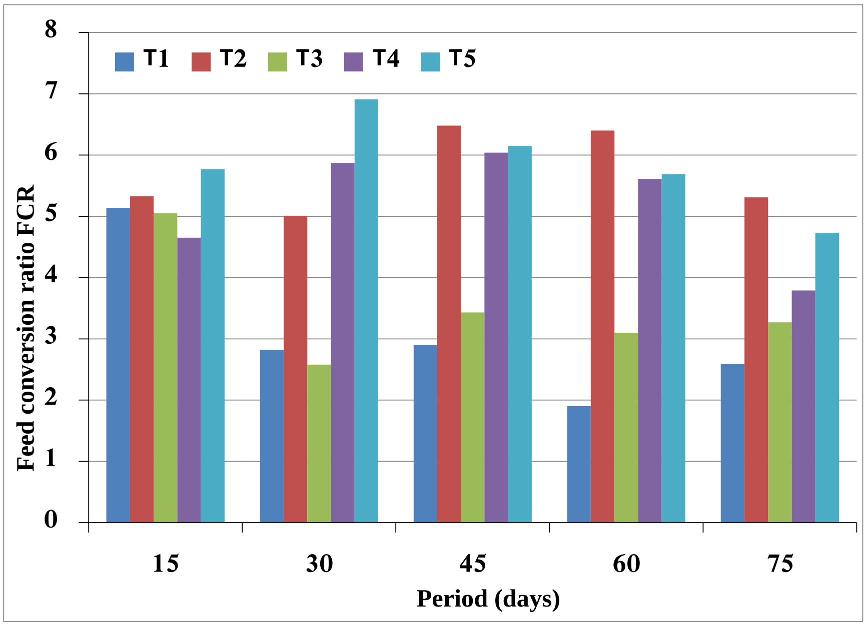

Figure 5: Feed conversion ratio of young *C. carpio* during different experimental periods.

Discussion

Many studies confirm that diet containing probiotic improved growth, survival and health status of fishes (Hai, 2015). The current study indicated that the growth performance of fishes fed on the probiotic-containing diet (T1) were significantly higher ($P \leq 0.05$) compared to all other treatments except the diet containing the enzyme mixture (T3). These results are consistent with the findings of Dhanaraj et al. (2010) in koi carp (*C. carpio*) and El-Ashram et al. (2008), Lara-Flores et al. (2010), Osman et al. (2010), Mohammad et al. (2012) and Ozório et al. (2012) in Nile tilapia (*Oreochromis niloticus*). Many factors could explain this result. Several studies indicated that the inclusion of the probiotic in the diets stimulates fish appetite (Carnevali et al., 2004; Saini et al., 2014; El-feky et al., 2017). This improvement can also be partially attributed to the probiotic itself, as a result of its richness in protein, carbohydrates, nucleic acids and vitamins (Brown et al., 1996). However, the clearest effect may be attributed to their stimulation of the increased activity of digestive enzymes in the fish digestive system (Yang et al., 2014; Wang et al., 2015; Adel et al., 2016).

Fishes fed on the diets containing *S. cerevisiae* showed an improvement in the mucosal surface and the number of mucosal flora of the gastrointestinal tract, which enhanced digestion and absorption of feed. Such diets also accelerate the maturation of the digestive system in small fishes and increased growth and metabolism in large fishes (Li et al., 2007; Abu-Elala et al., 2013). *S. cerevisiae* also improves the digestion of complex sugars, phytic acid and fibers (Tewary & Patra, 2011). The results of the current study indicated that the efficiency of the food conversion in fishes fed on yeast has improved significantly. This result is in agreement with some studies that used this yeast in fish feeds such as Mohammad et al. (2012) who indicated that the improvement of the feed conversion may be due to the increased absorption of nutrients by the fishes. Ai et al. (2011) also showed that the abundance of intestinal flora and its effective contribution to the decomposition of nutrients leads to enhanced protein utilization efficiency. Nayak (2010) indicated that it is due to the abundance of bacteria, including *Aeromonas* spp., which are known to produce the protease enzyme in the gastrointestinal tract of fishes fed with *S. cerevisiae*. Also, the ability of yeast to enhance the digestion of complex proteins and carbohydrates in the diet through its excretion of a wide range of exogenous enzymes such as protease, amylase and lipase that complement endogenous enzyme activities (Banu et al., 2020).

The results of the growth performance of fishes fed on a diet containing enzymes mixture (T3) in the current study indicated the positive effect of adding exogenous enzymes to the diets, which led to an improvement in the digestibility of feedstuffs and thus to growth. This may be due to the direct effect of the added enzymes or to the indirect effect by stimulating the secretion of self-enzymes in fishes or by increasing the number of beneficial bacteria in the gastrointestinal tract (Ng & Chen, 2002; Lin et al., 2007). Ramos et al. (2017) found that adding enzymes break down polysaccharides, such as hemicellulase, pectin and phytates in soybeans, caused increased food viscosity and slow gastric emptying. Castillo & Gatlin III (2015) also noted an improvement in secretion and increased activity of trypsin, chymotrypsin, lipase and amylase enzymes, as well as an increase in the number of *Lactobacillus* bacteria.

The diets of common carp, with a large proportion of difficult to digest plant ingredients, may often contain many anti-nutrients, especially phytate, which reduces its nutritional value as a result of the low ability of fishes to digest them efficiently, especially with the lack of many cultivated fishes, including the common carp, for certain important enzymes such as phytase and cellulose. So enhancing diets with external enzymes can improve the ability of fishes to utilize them. This is consistent with some studies that have obtained similar results. Monier (2020) found that adding exogenous enzymes to diets helped improve feed utilization and inhibited the action of anti-nutrients, which had a positive impact on growth rates and dietary conversion in common carp. The phytate in soybean meal, which is widely used in the fish diet, associated with some important nutrients such as amino acids, phosphorous, calcium, iron, magnesium and zinc, led to reducing their bioavailability, and as a result, the use of exogenous enzymes containing phytase enhances the ability to benefit from these nutrients, especially phosphorous (Dalsgaard et al., 2012; Hlophe-Ginindza et al., 2016). Studies also clearly demonstrated that the number of bacteria in the fish gastrointestinal tract is positively affected by the use of exogenous enzymes in the diets. Bedford & Cowieson (2012) indicated that exogenous enzymes not only affect the efficiency of feed digestion, but also affect the production of nutrients necessary for the flourishing of communities of certain types of bacteria. This is consistent with what Zhou et al. (2013) found with feeds fortified with cellulase fed to grass carp *Ctenopharyngodon idella*.

Many reasons may have led fishes in the current study not to be benefited from the amino acids added to their diet (T2), including those related to the percentage of addition. Murai et al. (1989) mentioned that the added level of methionine and lysine to the common carp diet may be very limited and any increase in the addition level may adversely affect growth. Methionine is important as a source of sulfur as well as the methyl group and plays a role in lipid metabolism (Brosnan & Brosnan, 2006; Espe et al., 2008). However, its positive effect on fish growth is within appropriate limits, and increasing its level above the appropriate level in the diet may cause a negative effect on growth and feed efficiency (Murai et al., 1989; Cheng et al., 2003; Luo et al., 2006). Also, adding amino acids to the diets may lead to opposite results in some cases, including those associated with reducing the fish appetite. This is in agreement with Kasumyan (2004) who found that most amino acids often form non-tasteless substances rather than feed stimuli for fishes. Furthermore, added amino acids tend to leach into the environment before consuming. Besides, it has been proven that the absorption of these amino acids by the digestive system is much faster than those associated with protein, causing an imbalance in absorbed amino acids, which lead to converting them to the process of catabolism instead of anabolism, in addition to the fact that part of them is excreted directly through the urine (Murai et al., 1989; Luo et al., 2006; Nwanna et al., 2012).

The present study concluded that probiotic and enzymes feed additives have proved significant enhancement of growth performance and feed efficiency of young common carp *C. carpio*. It means that, a positive outcome would be an expected if these additives have been added to the diets.

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