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USE OF TREATED LENTIL *LENS CULINARIS* L. WITH PHYTASE ENZYME IN DIETS OF COMMON CARP *CYPRINUS CARPIO* L.

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Abstract

This study conducted in Al-Muthanna University, Agriculture College 2020 to evaluate the effect of using of treated lentil *Lens culinaris* L. with phytase enzyme in diets of common carp (*Cyprinus carpio* L.). In this study, two types of phytase enzyme used, commercial and microbial. Fish were fed 3% daily of their weight. The experiment carried out using a floating cage method in the Euphrates River with three replicate for each treatment. The experiment applied with four treatments, the first was for control without lentils (T1) and other diets containing lentils treated with microbial phytase (T2), lentils treated with commercial lentil phytase (T3) and fourth non-treated lentil (T4). Statistical analyses showed that T2 was significantly superior to the other treatments except (the third treatment) in all studied characteristics, outperformed the final weight where recorded (466.33± 4.09), and T1, T3, T4 recorded (388.67±15.8, 440.00±28.9, 405.00±25.9) respectively. The study also concluded that the addition of the phytase enzyme affected the health indicators of fish and a change in the level of some enzymes such as GOT, GPT, where the T2 and T3 recorded the highest level of GOT, GPT value where the index values De-ritis quotient were (9.285, 15.4, 11.31, 15.190) respectively . The study concluded a positive effect on growth parameters when lentil treated with phytase enzyme in same condition environment.
***Keywords**:* phytase enzyme, common carp, lentil.

Introduction

Lentil *Lens culinaris* is cultivated as a winter crop, it can be grown on different types of soil and can withstand neutral soils slightly slanted to alkaline or acidic inclined (Adsule, 1996). The lentils bear a wide range of weather fluctuations, as it is resistant to some degree to heat and cold. Lentils contain a high content of oleic, linoleic and palmitic acid, lentils are a good source of iron, zinc, magnesium and an average source of thiamine, riboflavin and niacin. It characterized by its high ash content. Phytic acid (PA) is found in most of the ingredients commonly used in fish feed like barley, rice, sorghum, wheat, maize, gram, groundnut, rapeseed, soybean, cottonseed and sesame (De Silva and Anderson, 1995).

Phytase is a phosphate enzyme that stimulates the hydrolysis of phytic acid (phytoate) and the liberation of organic phosphorous, as the mechanism of action of this enzyme is to isolate the phosphate group from the inositol ring of phytate to produce a free inorganic phosphorus (available) with a lower chain of phosphoric esters (inositol pentphosphate to Inositol Monophosphate (Debnath *et al*., 2005). Single-stomach animals, including fish, cannot secrete the phytase during digestion unlike ruminants. Therefore, most of the phosphorous ingested is excreted into the environment without benefiting from it, which leads to increased water contamination with algae and plants (Forster *et al*., 1999).

Phytase used in feeding fish, where many studies focused on the use of the optimal dose and added to the diet and most studies were conducted on the use of microbial phytase (Furuya *et al*., 2001, Portz and Liebert, 2005; Cao *et al*., 2007). This study aims evaluate the effect of adding microbial and commercial enzyme phytase to lentils and its effect on common carp growth.

Materials and Methods

This experiment conducted in College of Agriculture in University of Al-Muthanna. Used (120) common carp *Cyprinus carpio* L. fish with average weight (250gm), fish distributed on (12) small cage ($1\times1\times1m^2$) in Euphrates river Fig. 1. 10 fish/cage. Each treatment replicated thrice. Fish was fed with 3% daily from weight basis three times. The modified quantities that given to their after weighing their by weekly. To feed the fish, Four different isoprotein diets were used to feed fish, the first was for control without lentils (T1) and other diets containing lentils treated with microbial phytase (T2), lentils treated with commercial lentil phytase (T3) and fourth non-treated lentil (T4), table 1 shown ingredients and chemical composition of experimental diets.

The process of producing and extracting the phytase enzyme carried out in the laboratory of the Center for Biotechnology-Agricultural Research Department - Ministry of Science and Technology, Using a special culture medium, Phytate Solubilizing Medium (PSM). Depending on the methods mentioned by Kwanyuen *et al*. (2005)

Experiment continue for 84 day after adaptation for 7 day. Data analysis by using program (SPSS) statistical package for social sciences version (22) according to complete randomized design and using Duncan test for comparison between means.

Studied parameters

1. Weight gain (W.G) = Final weight-Initial weight.
2. Daily growth rate (D.G.R) Final weight (g/day) - Initial weight (g/day)/experiments period.
3. Relative growth rate (R.G.R)%

$$= \frac{\text{Final weight } (W_2) - \text{Initial weight } (W_1)}{\text{Initial weight } (W_1)} \times 100$$

4. Specific growth ratio (S.G.R)%
$$= \frac{\text{Ln Final weight } (gm) - Ln \text{ Initial weight } (gm)}{\text{Experment period}} \times 100$$
5. Food conversion efficiency (F.C.E)%
$$= \frac{\text{weight gain (gm)}}{\text{Food intake (gm)}} \times 100$$
6. Food conversion ratio (F.C.R)$= \frac{\text{weight intake (gm)}}{\text{Food gain (gm)}}$
7. Protein efficiency ratio (P.E.R) $= \frac{\text{weight gain (gm)}}{\text{protein intake (gm)}}$
8. Saturated level (SL) $= \frac{\text{food intake (gm)}}{\text{final weight (gm)}}$
9. Apparent Protein Digestibility Coefficient
$$= \frac{Cr2O3 \text{ in food \%} \times \text{protein in feces\%} \times 100}{Cr2O3 \text{ in feces\%} \times \text{protein in food\%}}$$

For measured blood tests blood analysis model chem 200 was used.

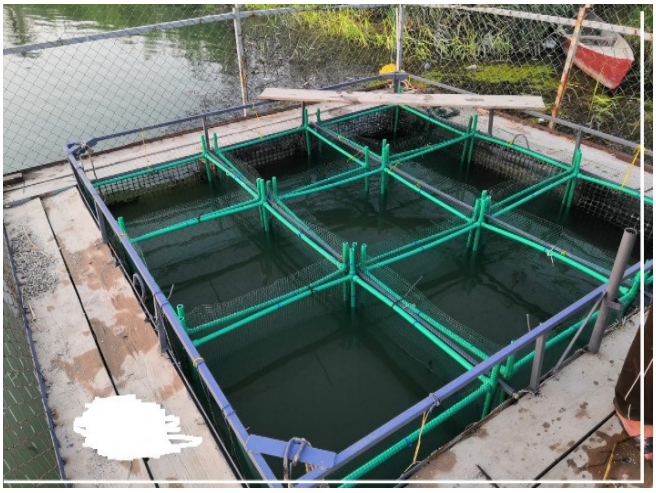

Fig. 1 : Floating cages in which the experiment was performed

Table 1: Ingredient and chemical composition of experimental diets

Ingredient %	**(T1)***	**(T2)****	**(T3)*****	**(T4)******
Fish meal	15	15	15	15
Soybean	35	28	28	28
Lentil	--	15	15	15
Corn	17	17	17	17
Barley	10	10	10	10
Wheat flour	10	7	7	7
Wheat bran	10	5	5	5
Corn oil	1	1	1	1
Salt	1	1	1	1
premix	1	1	1	1
Total	100	100	100	100
Chemical composition				
Protein	29.5	29.29	28.93	29.15
Ether extract	6.02	5.86	5.79	5.84
Fiber	4.21	4.89	5.00	5.11
Ash	3.99	3.67	3.82	4.05
Nitrogen free extract	56.27	56.32	56.46	55.85

*T1 free-lentil control diet. **T2 lentil treated with microbial enzyme. ***T3 lentil treated with commercial enzyme. ****T4 lentil without treated

Results and Discussion

The temperature of the water during the trial period ranged from 24 to 18°C and this degree is suitable for the fish living. Survival of fish 100% in all treatment No losses occurred in all treatments during the trial period. The highest final weight of the second treatment fish as it reached 466.33±4.09 and it significantly outperformed the T1 control 388.67±15.8. While these differences did not appear between the second treatment and the rest of the transactions. The second treatment also outperformed the weight-gain parameters and significantly differ by control treatment, and did not differ significantly from the third and fourth treatment.

No significant differences were observed between experimental treatments on the quantity of feed intake, the food conversion coefficient showed a significant difference in the second treatment, 3.47 ± 0.9 over the first and fourth treatments, while those differences did not appear with the third treatment 3.67±0.39. The same applies to the characteristic of the food conversion efficiency, as it was the least in the fourth treatment 20.5±1.86 and the highest in the second treatment 29.0 ± 0.8. While the first treatment showed significant superiority in the apparent digestibility coefficient, not all treatment were significantly different in the protein digestibility coefficient (Table 2).

Table 2 : Some studied parameters (mean±S.E) for experiment fish which fed on experiment diets.

Parameters	T1*	T2**	T3***	T4****
Initial weight I.W	250 a	250 a	250 a	250 a
Final weight (F.W)	388.67±15.8 b	466.33± 4.09 a	440.00±28.9 ab	405.00±25.9 ab
Weight gain (W.G)	138.7±15.8 b	216.33±4.05 a	190.00±28.9 ab	155.00±25.9 ab
Food intake (F.I)	652.51±19.9 a	745.9±27.87 a	676.6±34.33 a	744.6±59.57 a
Food conversion ratio (F.C.R)	4.78±0.364 bc	3.44±0.097 a	3.67±0.392 ab	4.95±0.466 c
Food conversion efficiency (F.C.E)	21.14±1.72 b	29.05±0.81 a	27.79±2.86 a	20.5±1.86 b
Relative growth rate (R.G.R)	55.47±6.03 b	86.5±1.63 a	76.00±11.54 ab	62.00±10.39 ab
Specific growth rate (S.G.R)	0.59±0.05b	0.84±0.011 a	0.76±0.089 ab	0.64±0.087 ab
Saturated level SL	168.02±1.8 b	159.89±5.14 bc	154.06±2.33 c	183.46±2.95 a
Protein productivity value PPV	34.12±0.31 a	35.12±0.22 a	31.22±0.12 b	31.92± 0.08 b
Apparent Digestibility Coefficient	66.47±0.13 a	64.22±0.26 ab	63.31±0.30 ab	62.43± 0.22 b
Apparent Protein Digestibility Coefficient	72.41± 2.2 a	70.57±3.54 a	70.09±2.98 a	66.27±3.16 a

* Different litters refer to there was a significant differences in probability at level ($p<0.05$) according to multi Duncan multiple test, (Duncan, 1955).
*T1 free-lentil control diet. **T2 lentil treated with microbial enzyme. ***T3 lentil treated with commercial enzyme. ****T4 lentil without treated

As can be notice in Table 2, were no significant differences between the second and third treatments, this means (no significant differences between the two types of enzyme used). As were no significant differences in most of the studied growth traits, including: Initial weight (I.W), Final weight (F.W), Weight gain (WG), Food intake (FI), Food conversion ratio (F.C.R),Food conversion efficiency (FCE), Relative growth rate (R.G.R), Specific growth rate (S.G.R), Saturated level (SL), Apparent Digestibility Coefficient, Apparent Protein Digestibility Coefficient but significant differences appeared in Protein productivity value (PPV). Lawrence and Schwarz (2007) noted addition of phytase significantly increased daily feed intake, but only led to a marginal improvement in the weight gain, SGR and FCR of fish. also Schafer *et al*. (1995) mentioned supplementation with phytase improve utilization of native plant P and reduce the P excretion significantly.

Noted in Table 3 that the percentage of moister in the body composition of fish was close to all treatments where was 77.21%, 76.45%, 76.92%, 78.61% respectively. This means that there is no effect of lentils or phytase on the moisture content of the fish's body. Because lentils are from legumes, which are characterized by a less of moisture content. The protein content was also close to all four coefficients too. They registered 16.28, 16.84, 15.11, 16.15% for the four treatments, respectively. While the T4 in the ether extract was relatively superior to the rest of the transactions registered 6.45 while the other treatments where registered 5.12, 5.14, 6.08%). Excelled T4 on the rest of the treatments for Ash it was (1.11) While was T1 (1.02), T2 (0.92), T3 (0.97).

Table 3: body composition of fish feeding on experimental diets (%)

	T1*	T2**	T3***	T4****
Moister	77.21	76.45	76.92	78.61
Protein	16.28	16.84	15.11	16.15
Ether extract	5.12	5.14	6.08	6.45
Ash	1.02	0.92	0.97	1.11

*T1 free-lentil control diet. **T2 lentil treated with microbial enzyme. ***T3 lentil treated with commercial enzyme. ****T4 lentil without treated

Glutamate-Pyruvic Transaminase (GPT) enzyme found in liver cells cytoplasm with a high concentration, while Glutamic-oxaloacetic Transaminase (GOT) enzyme is found in the liver cells mitochondria, Hammoudi *et al*. (2014) noted there are liver problems, when the value of GOT is greater than GPT, and this indicator was one of the most important biometrics for health.

De-ritis quotient an indicator that determines the extent of liver cell damage. If the index is greater than 1, this indicates liver cell damage and vice versa (De Ritis *et al*., 1957).

From Table 4, the amount of GOT is much greater than the amount of GPT, this led to De-ritis quotient increased in all treatments, the T2 recorded the highest rate for the De-ritis quotient index (15.4), it was the highest compared to all treatments. The rest of the treatments recorded, T1: 9.285, T3: 11.31, this supports the idea that the commercial enzyme is better than the microbial enzyme, because the T3 scored less than the T2.

Table 4: GOT, GPT and Total protein in serum of fish feeding on experimental diets

	T1*	T2**	T3***	T4****
GOT (IU)	97.50	154	90.50	159.50
GPT (IU)	10.50	10	8	10.50
De-ritis quotient	9.285	15.4	11.31	15.190
Total protein (mg/l)	34	31	37	39.5

*T1 free-lentil control diet. **T2 lentil treated with microbial enzyme. ***T3 lentil treated with commercial enzyme. ****T4 lentil without treated

Alterations in total plasma or serum protein concentration relative to a reference interval have been used as a broad clinical indicator of health, stress, and well being of terrestrial and aquatic organisms (Riche 2007). Noted in Table 4 that the amount of total protein in the blood was higher in the T4, It was recorded (39.5) and T2 treatment recorded (31) it was the lowest amount recorded in all treatments, the T1 and T3 recorded (34, 37), respectively. The decrease in protein in the second treatment was the use of the microbial enzyme phytase, which affected the amount of protein in the blood, this confirmed by Reddy and Bashamohideen (1995). That the decrease in amount of

protein in the blood is the result of using some of the compounds for metabolic purposes

Conclusions

Using of phytase, both commercially and microbial, has improved many productive characteristics of fish Like: Final weight (F.W)و Weight gain (W.G), Food intake (F.I), Food conversion ratio (F.C.R), Food conversion efficiency (F.C.E ,)Relative growth rate (R.G.R), Specific growth rate (S.G.R) and Saturated level (SL). While reducing the use of the enzyme from some other characteristics, such as Protein productivity value PPV, Apparent Digestibility Coefficient and Apparent Protein Digestibility Coefficient. We can said that the addition of the phytase enzyme affects the health of fish because of the direct effect on the ratios of some liver enzymes like GOT, GPT which means that some of the additions, even if they improve some of the productive traits, but maybe they negatively effect on some other traits.

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