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Effect of Adding some Digestive Enzymes in Diets of Common Carp (*Cyprinus carpio* L.) on the Rates of Digestion, Growth and Hematological Characteristics

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Abstract. The use of exogenous enzymes in fish breeding diets, especially papain, protease, and phytase enzymes, is still not well known, but most studies emphasized their benefits and importance in aquaculture, especially fish. As it had a positive effect on the performance of growth, digestion, high utilization of feed, as well as raising the immunity of fish, so these enzymatic additions were used to demonstrate their effect on the fish under experiment. The use of prepared fish waste powder fortified with digestive enzymes Papain, Protease and Phytase at percentages of 1.5% and 2.5% in the diet of common carp *Cyprinus carpio* L. on digestion rates, growth and blood characteristics. 84 fish, with an average starting weight of 240 ± 1.22 g/fish, were randomly distributed in two repetitions, 6 fish per tank. The fishmeal was prepared, the three enzymes were added to it, and it was added to the experimental diets, and it was analyzed chemically for use in feeding common carp fish. Treatments T4 and T5 recorded the best digestion rates for protein, fat, carbohydrates, and ash, while the lowest digestion rates were in control treatment T1, which were 65.11%, 78.33%, 39.62%, and 62.67%, respectively. At the level ($P \leq 0.05$), there were statistically significant differences across the treatments. Gains in weight (111.05 and 111.92) were largest for Treatments 4 and 5, while gains in daily, relative, and qualitative development as well as in food conversion efficiency were highest for Treatments 4 and 5. The control diet, Treatment 1, resulted in a rise in weight of 75.05 gm. Therapy with T1 resulted in a 34.423 mg/dL decrease in average blood glucose levels, while treatment with T6 resulted in the highest ALT rate of 8.722 IU/L. Total protein levels did not vary noticeably across treatments. When compared to the other therapies, T4 and T5 produced the highest albumin levels (1.109 and 1.335) mg/dL. Treatment T1, the control group, saw a rise of 68.148 mg/dL in cholesterol while treatments T4 and T5, the experimental groups, saw decreases of 53.311 and 49.421 mg/dL, respectively. Fishmeal enhanced with enzymes at 0.5% and 1.5% as a source of animal protein in common carp fish feed resulted in favorable digestion rates, growth, and blood characteristics, according to the results of this study.

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Keywords. Papain enzyme, Protease enzyme, Phytase enzyme, Digestion coefficient, Growth rates, Blood characteristics.

1. Introduction

Fish farming needs feeds with good specifications that contain all the basic nutrients, especially protein and energy, in order to obtain the required growth, high survival rates and reproduction, and thus good profit [1]. Most studies showed that the use of exogenous enzymes in fish feed increased digestion and growth rates [2,3]. The most important statistics indicate that enzymes have become an important component in the food feed industry [4]. Additive enzymes are important as they work with endogenous enzymes to degrade feed proteins, especially the papain enzyme, which is one of the most important exogenous enzymes added to fish feed [5], and the protease enzyme [6]. Exogenous phytase was also widely used in fish feed due to its effectiveness in reducing phosphorus secretion by converting phosphorous phytate into inorganic matter [7]. The common carp is one of the types of freshwater fish found in most countries of the world and is of great importance in Europe and Asia [8]. In order to transform the macronutrients (protein, carbs, and fats) consumed by living organisms into usable energy, digestive enzymes—including exogenous enzymes—are among the most crucial dietary supplements [9]. Fishmeal is produced from the wastes of living organisms, including fish and poultry. Fishmeal is a protein source rich in essential amino acids [10]. This study compared the effects on digestion, growth, and blood characteristics of feeding fishmeal with either 1% or 2% of the enzymes phytase and pepsin, which were generated from fish waste. The purpose of this research is to show how supplementing the diets of common carp fish with fishmeal that has been fortified with papain, protease, and phytase enzymes at concentrations of 1.5% and 2.5% affects digestive rates, growth, and blood characteristics.

2. Materials and Methods

To make the powders and rations, we ground up the fish scraps and added the enzymes papain, protease, and phytase (at rates of 1.5% and 2.5%) for 24 hours before drying the mixture in an electric oven set to 60 degrees Celsius. The diets were prepared by mixing the raw materials well (yellow corn 30% - wheat 30% - barley 20% - fish meal 10% - bran 9% - vitamins and minerals 1%. Seven different relationships were used:

- Control ration containing prepared fish meal free of enzymes.
- A treatment containing fishmeal prepared with papain enzyme 0.5%.
- A treatment containing fishmeal prepared from papain enzyme 1.5%.
- A treatment containing fishmeal prepared with protease enzyme 0.5%.
- A treatment containing fishmeal prepared with protease enzyme 1.5%.
- A treatment containing fishmeal prepared with phytase enzyme 0.5%.
- A treatment containing fishmeal prepared with phytase enzyme 1.5%.

2.1. Chemical Analyses

The following chemical analyses were performed using the procedures described in [11]:

2.1.1. Humidity Rating

Experiment fish, powder samples, and prepared menus were all analyzed for moisture content. We used 5 grams of each powder and dried them at 105 degrees Celsius until their weights were consistent.

2.1.2. Protein Estimate

Nitrogen was quantified using the micro Kjeldahl technique, which entailed first digesting the samples with concentrated sulfuric acid, then distilling them using boric acid and a bromocresol green guide, finally grinding them up with hydrochloric acid, and finally applying a conversion factor (6.25). Try

to calculate the amount of protein in the samples.

2.1.3. Fiber Rating

Fiber content can be determined by boiling a sample in 1.25 sulfuric acid for half an hour, then washing it in distilled water to remove the acid, and finally adding 1.25 nitrogen base sodium hydroxide to the sample for half an hour. The sample should be boiled, the acid washed off with hot distilled water, and then the sample should be washed again in acetone. After determining the mass of the dry lid and blank, the sample is added before the container is baked at 60 degrees Celsius. The above method based on the method mentioned in [11] was carried out in the Animal Production Laboratory of the College of Agriculture - University of Baghdad.

Crude fiber percentage = weight of fiber (g) / weight of sample (g) x 100

2.1.4. Ash Estimate

1 gram of each sample was burned at 550 degrees Celsius for four hours in a muffled oven (MLW electric type LM 212-11, German origin) to obtain a white or gray powder, and the percentage of ash was determined after fixing the weights at 60 degrees Celsius.

2.1.5. Nitrogen Free Extract

By deducting the percentages of hydration, protein, fat, ash, and fiber from 100, the soluble carbohydrates may be determined. Measurement of the coefficient of digestion: The fish are fed daily on diets until satiation in the early morning and the food is left for an hour to give a sufficient period of time for the fish to eat their food, then the uneaten food is withdrawn by the siphon method, and in the morning of the second day the waste is collected by the siphon method as well and this process is repeated until the largest amount of waste is collected, which is dried Aerobic until the digestion coefficient test is carried out according to the method mentioned by [12] and according to the following equation:

$$Y = 0.2089\ X + 0.0032$$

Where (Y) is the absorbance at a wavelength of 450 nm.
(X) chromium oxide concentration mg/100 ml.

2.1.6. Protein Digestibility % [19]

$$= 100 - \left(\frac{Cr_2O_3 \text{ in food\%}}{Cr_2O_3 \text{ in waste \%}} \times \frac{\text{percentage of protein in waste \%}}{\text{percentage of protein in the food \%}} \times 100 \right)$$

2.1.7. Fat Digestibility Coefficient %

$$= 100 - \left(\frac{Cr_2O_3 \text{ in food \%}}{Cr_2O_3 \text{ in waste \%}} \times \frac{\text{percentage of fat in waste \%}}{\text{percentage of fat in the food \%}} \times 100 \right)$$

2.1.8. Carbohydrate Digestive Factor %

$$= 100 - \left(\frac{Cr_2O_3 \text{ in food \%}}{Cr_2O_3 \text{ in waste \%}} \times \frac{\text{percentage of carbohydrates in waste \%}}{\text{percentage of Carbohydrates in the food \%}} \times 100 \right)$$

2.1.9. Ash Digestion Coefficient %

$$= 100 - \left(\frac{Cr_2O_3 \text{ in food \%}}{Cr_2O_3 \text{ in waste \%}} \times \frac{\text{percentage of ash in waste \%}}{\text{percentage of ash in the food \%}} \times 100 \right)$$

2.2. Studied Traits Growth Measurements

2.2.1. Total Weight Gain

The following formula was used to determine the rates of weight gain:

Weight gain (gm) = final weight (gm) - starting weight (gm).

2.2.2. Daily Growth Rate

The following equation describes the exponential daily growth rate [13] :

Daily growth rate g / day = weight gain (g) / time period of increase (day)

2.2.3. Specific Growth Rate

The specific growth rate was estimated according to the following equation [14]:

Specific growth rate g/day = log final weight - log initial weight/experimental time x 100

2.2.4. Relative Growth Rate

Calculate the feed conversion rate according to the following equation [15]:

Relative growth rate % = Final weight (g) – Starting weight (g) / Starting weight (g) x 100

2.2.5. Feed Conversion Efficiency

The feed conversion efficiency was estimated according to the following equation [15]:

Feed conversion efficiency % = fresh weight gain of fish (g) / weight of feed intake (g) x 100

2.3. Blood Serum Biochemical Tests

2.3.1. Determination of Glucose (Glu)

Serum glucose was calculated according to the method recommended by the manufacturer of the work kit (Linear company), according to the following equation [16]:

Glu (mg/dl) = A. sample / A. std x 100

2.3.2. Determination of Total Cholesterol (T.C.)

Total cholesterol was calculated according to the method recommended by the manufacturer of the work kit (Linear company), according to the following equation [17]:

T.C. (mg/dl) = A. sample / A. std x 200

2.3.3. Determination of Triglycerides (T.G.)

TG calculated according to method of [18] as recommended by the manufacturer of the work kit (Linear company), according to the following equation:

T.G. (mg/dl) = A. sample / A. std x 200

2.3.4. Determination of Total Protein (T.P.)

T.P. was calculated using a kit by BIOLABO company, based on the method of [20], according to the following equation:

T.P. (gm/dl) = A. sample / A. std x 6

2.3.5. Determination of Albumin (Alb)

Alb. was calculated by following information of the kit depend on Randox company, according to the following equation [21]:

Alb (gm/dl) = A. sample / A. std x 5

2.3.6. Calculation of Total Globulin (T.Glo.)

The globulin was calculated by subtracting the albumin of the total protein depend on the equation were mentioned of [22].

T.Glo. (gm/dl) = T.P. – Alb.

2.3.7. Estimation of Liver Enzymes Activity (AST, ALT & ALP)

Aspartate amino transferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP) levels in the liver were calculated using a specialized German device called Mindray.

2.4. Statistical Analysis

Complete random designs (CRDs) were utilized for data collection and processing in Statistical Analysis System - [23], and significant differences between means were evaluated using the Duncan multiple range test at the probability level ($P \leq 0.05$).

3. Results and Discussion

Table (1) shows that there were no statistically significant variations ($P > 0.05$) in the percentages of moisture, protein, and fiber between any of the treatments. However, the proportion of fat dropped in treatments T2 and T7, while no other treatments showed statistically significant changes. No significant differences ($P \leq 0.05$) were recorded between the treatments in the percentage of ash T1, T3, and T7, as they reached (5.87, 5.95, and 5.89)%, respectively, which is close to the results of [14] when they used bromelain and phytase enzymes in common carp fish diets.

Table 1. Chemical analysis of experimental diets.

Treatments	Moisture %	Protein %	Fat %	Fiber %	Ash %	Soluble Carbohydrates

						%
Fish feed containing raw fish powder (control) T1	9.50±0.013[a]	36.33±0.711[a]	8.69±0.13[a]	4.61±0.112[a]	5.67.401[a]	35.40±0.134[a]
Fish feed containing fishmeal added to it papain enzyme 0.5 % T2	8.31±0.106[a]	36.19±0.512[a]	8.32±0.250[a]	4.50±0.107[a]	5.24±0.151[b]	35.62±0.123[a]
Fish feed containing fishmeal added to it papain enzyme 1.5 % T3	8.43±0.512[a]	36.73±0.123[a]	8.11±0.128[b]	4.92±0.102[a]	5.95±0.175[a]	35.68±0.116[a]
Fish feed containing fishmeal added to it protease enzyme 0.5 % T4	8.86±0.175[a]	36.17±0.110[a]	8.96±0.246[a]	4.89±0.243[a]	5.37±0.181[b]	34.79±0.519[b]
Fish feed containing fishmeal added to it protease enzyme 1.5 % T5	9.11±0.115[a]	36.99±0.211[a]	8.63±0.145[a]	4.79±0.218[a]	5.29±0.472[b]	34.37±0.361[b]
Treatments	**Moisture %**	**Protein %**	**Fat %**	**Fiber %**	**Ash %**	**Soluble Carbohydrates %**
Fish feed containing fishmeal added to it phytase enzyme 0.5 % T6	8.57±0.11[a]	36.29±0.120[a]	8.95±0.107[a]	4.84±0.228[a]	5.33±0.321[b]	35.90±0.118[a]
Fish feed containing fishmeal added to it phytase enzyme 1.5 % T7	8.77±0.039[a]	36.35±0.622[a]	8.13±0.128[b]	4.89±0.161[a]	5.89±0.712[a]	35.57±0.618[a]

For both the control and experimental groups, the coefficient of digestion is displayed in Table 2. Treatments T4 and T5 outperformed the control treatment, which had a protein digestibility ratio of no more than 65.11 percent and a fat digestibility coefficient of no more than 78.33 percent, statistically ($P \leq 0.05$), suggesting that the protease enzyme was responsible for these improvements, which increased the ability to digest nutrients and absorb minerals and thus increasing the use of them [25], and the results came close to the study of [26], where the apparent digestion coefficient of protein increased when using the protease enzyme with fish meal in Nile tilapia fish diets, [27] found an increase in digestion rates when Use of phytase in common carp diets.

Table 2. Digestion coefficient for experimental diets.

Treatments	Protein digestibility coefficient %	Fat digestibility coefficient %	Carbohydrate digestibility coefficient %	Ash digestibility coefficient %
Fish feed containing raw Fish powder (control) T1	65.11±0.80^{c}	78.33±0.56^{b}	39.62±0.66^{c}	62.67±0.61^{c}
Fish feed containing fishmeal added to it Papain enzyme 0.5 % T2	73.78±0.23^{b}	87.88±0.16^{a}	77.22±0.65^{a}	84.63±0.25^{a}
Fish feed containing fishmeal added to it Papain enzyme 1.5 % T3	72.91±0.82^{b}	88.54±0.81^{a}	76.99±0.73^{a}	86.28±0.21^{a}
Fish feed containing fishmeal added to it Protease enzyme 0.5 % T4	81.10±0.22^{a}	89.22±1.01^{a}	78.36±0.66^{a}	84.42±0.33^{a}
Fish feed containing fishmeal added to it Protease enzyme 1.5 % T5	80.79±0.19^{a}	89.61±0.91^{a}	77.86±0.13^{a}	86.77±0.71^{a}
Treatments	**Protein digestibility coefficient %**	**Fat digestibility coefficient %**	**Carbohydrate digestibility coefficient %**	**Ash digestibility coefficient %**
Fish feed containing fishmeal added to it Phytase enzyme 0.5 % T6	76.23±0.44^{ab}	90.01±1.11^{a}	54.72±0.28^{b}	75.01±0.11^{b}
Fish feed containing fishmeal added to it Phytase enzyme 1.5 % T7	71.81±0.90^{b}	86.92±0.22^{a}	52.11±0.39^{b}	74.78±0.23^{b}

Size requirements for fish given a diet of multiple types of fish meal: Table 3 displays the fish's weight gain rates throughout the experiment.

There is a noticeable difference ($P \leq 0.05$) between the treatments, with the T4 and T5 treatments producing the best results (111.05 and 111.92 g, respectively), suggesting that the enzymes contributed to the fish's enhanced weight gain, and the results were consistent with the findings of [28] when adding feed to some marine fish and studying food and energy requirements Daily growth rate, relative growth rate, qualitative growth rate, and feed conversion efficiency all differed significantly between treatments (T4 and T5 with the protease enzyme were superior, with respective values of 1.85 and 1.86 g/day, 47.85 and 47.80 %, 0.29 and 0.31 gm/day, and 43.67 and 51.55%).

The results of the study are similar to what [29], found when adding protease enzyme to

the powders of some aquatic organisms, where growth rates and feed conversion efficiency recorded the highest results with this addition. [27] supplementing common carp meals with enzyme-fortified powders containing protease enzyme resulted in faster growth. Increases in daily growth rates, specific growth, and relative growth can all be attributed to the addition of additives to the fish's diet, which have a positive effect on the fish's weight gain because of their association with body metabolism and other vital activities, such as enhancing the fish's health and physiological condition [30]. Metabolic enzymes put the meal to good use, which shows in increased growth and body mass [31].

Table 3. Some growth characteristics of common carp fish fed on experimental diets.

Treatments	Starting weight gm	Final weight gm	Weight gain gm	Daily growth rate g/day	Relative growth rat e %	Specific growth rate g/day	Feed conversion efficiency %
Fish feed containing raw fish powder (control) T1	240.32 ±0.355^{a}	311.37 ±0.322^{b}	75.05±1. 135^{c}	1.25±0.02^{c}	31.22±0.227^{d}	0.15±0.0 02^{c}	19.23±0.25^{d}
Fish feed containing fishmeal added to it Papain enzyme 0.5 % T2	231.34 ±0.336^{b}	302.64 ±0.197^{b}	71.30±1. 331^{c}	1.18±0.03^{d}	30.82±0.412 1^{d}	0.17±0.0 04^{c}	27.39±0.66^{c}
Fish feed containing fishmeal added to it Papain enzyme 1.5 % T3	239.65 ±1.205^{a}	327.41 ±1.211^{a}	87.76±1. 375^{b}	1.46±0.03^{b}	36.62±0.471^{b}	0.19±0.0 03^{b}	21.31±0.41^{c}
Fish feed containing fishmeal added to it Protease enzyme 0.5 % T4	232.12 ±1.334^{b}	343.17 ±1.128^{a}	111.05±1 .483^{a}	1.85±0.03^{a}	47.85±0.477^{a}	0.29±0.0 03^{a}	43.67±0.68^{a}
Treatments	**Starting weight gm**	**Final weight gm**	**Weight gain gm**	**Daily growth rate g/day**	**Relative growth rat e %**	**Specific growth rate g/day**	**Feed conversion efficiency %**
Fish feed containing fishmeal added to it Protease enzyme 1.5 % T5	234.14 ±0.612^{b}	346.06 ±1.133^{a}	111.92±1 .3^{a}	1.86±0.04^{a}	47.80±0.554^{a}	0.31±0.0 02^{a}	51.55±0.27^{a}
Fish feed containing fishmeal added to it Phytase enzyme 0.5 % T6	217.55 ±1.335^{c}	303.25 ±0.331^{b}	85.70±1. 391^{b}	1.14±0.03^{d}	39.39±0.26^{b}	0.16±0.0 02^{c}	33.56±0.89^{b}
Fish feed containing fishmeal added to it Phytase enzyme 1.5 % T7	237.67 ±0.236^{b}	321.29 ±0.441^{a}	83.62±1. 301^{b}	1.39±0.02^{b}	35.18±1.02^{b}	0.22±0.0 03^{b}	39.28±0.77^{a}

Table 4. for the blood parameters of the experimental fish indicates that there were significant differences (P≤0.05) in the average blood glucose concentration, as the highest value was 56.574 mg/dL in the T4 treatment compared to the rest of the treatments, and the lowest was in the T1 treatment as it reached 34.423 mg/dL. The results of the study

converged with [27], where the glucose values ranged between (33.143 and 59.737) mg/dL when enzymes were used with powders prepared from fish waste in diets fed common carp fish. ALT was observed to rise in T6 treatment, reaching 18,722 IU/L, while T5 treatment recorded a significant decrease in ALT to 13,283 IU/L, respectively, and the AST value increased in T3 and T7 (61,778 and 59,155) IU/L, respectively, compared to With the rest of the treatments.

The T4 treatment recorded 32.906 IU/L, a significant decrease in the AST rate. The two treatments T4 and T5 (46.268 and 45.265) IU/L gave the lowest value of ALP compared to the rest of the treatments. Indicators of stress include a rise in liver enzyme levels, and changes in the activity of these enzymes, which in turn suggest tissue weakening [32] . The increased percentage of fats in farmed fish feed may be to blame for the rise in liver enzymes. This is because an increase in liver tissue damage and change causes liver enzyme levels to rise in the dead cells, and eventually the blood. Supplementing the diet of common carp with probiotics resulted in minimal pathological damage, according to a study [33]. While there were statistically insignificant changes between treatments for total protein, albumin levels were highest in T4 and T5 (1.109 and 1.335) mg/dL and globulin levels were highest in T1 and T2 (4.889 and 4.810 mg/dL). Total protein in rainbow trout was found to increase when fish silage was used in experimental treatments as opposed to a control meal without silage [34].

[33], obtained an increase in the average total protein of 5.37 mg/dl for common carp fed on diets fortified with probiotics. Treatments T4 and T5 recorded the lowest levels of cholesterol and triglycerides. [35] Cholesterol levels in bream were observed to drop significantly, from 111.31 mg/dL to 85.16 mg/dL, when fishmeal was included in their diets. The absence of cholesterol in the diet and the inability to absorb cholesterol in the intestine contributes to low levels of cholesterol in the body's cells [36]. Which coincides with the decrease in triglycerides, and the decrease in cholesterol in experimental treatments could be the result of an increase in organic acids secreted due to the use of enzymes in diets, and this may inhibit the building of fatty acids [37].

Table 4. Blood parameters of experimental fish.

Treatments	Glucose mg / dl	ALT IU / liter	AST IU / liter	ALP IU / liter	Total protein mg / dl	albums mg / dl	Globulin mg / dl	Chole-sterol mg / dl	Triglyc erides mg / dl
Fish Before Experience	74.332	22.667	96.156	73.122	4.214	0.671	4.446	75.123	54.887
Fish feed containing raw fish powder (control) T1	34.423±0.654[c]	17.221±0.546[b]	51.113±0.287[b]	55.267±0.441[a]	5.512±0.015[a]	0.578±0.021[c]	4.889±0.012[a]	68.148 ±0.609[a]	52.447 ±0.278[a]
Fish feed containing fishmeal added to it Papain enzyme 0.5 % T2	39.324±0.812[b]	16.332±0.489[b]	56.590±0.216[b]	56.766±0.215[a]	5.641±0.027[a]	0.891±0.018[b]	4.810±0.083[a]	55.370 ±0.451[b]	54.782 ±0.331[a]

Fish feed containing fishmeal added to it Papain enzyme 1.5 % T3	38.376±0.112[b]	17.113±0.219[b]	61.779±0.366[a]	54.690±0.266[a]	5.145±0.088[a]	0.689±0.270[c]	4.643±0.065[b]	58.413±0.903[b]	58.809±0.211[a]
Fish feed containing fishmeal added to it Protease enzyme 0.5 % T4	56.574±0.191[a]	15.256±0.551[b]	32.906±0.265[c]	46.268±0.190[b]	5.226±0.078[a]	1.109±0.043[a]	3.996±0.055[c]	49.954±0.266[c]	53.311±0.523[b]
Fish feed containing fishmeal added to it Protease enzyme 1.5 % T5	49.331±1.493[a]	13.283±0.801[c]	28.965±0.438[d]	45.265±0.297[b]	5.209±0.062[a]	1.335±0.109[a]	3.897±0.221[c]	50.916±0.156[c]	49.421±0.532[b]
Fish feed containing fishmeal added to it Phytase enzyme 0.5 % T6	44.366±0.967[b]	18.722±0.133[a]	55.470±0.258[b]	59.690±0.254[a]	5.271±0.052[a]	0.891±0.043[b]	4.655±0.061[b]	58.331±0.478[b]	58.566±0.378[a]
Fish feed containing fishmeal added to it Phytase enzyme 1.5 % T7	42.256±0.112[b]	17.312±0.113[b]	59.155±0.444[a]	57.881±0.322[a]	5.580±0.033[a]	0.921±0.046[b]	4.451±0.088[b]	60.213±0.443[b]	57.241±0.669[a]

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Ethical Approve
We declare that the study does not need ethical approval.

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