Comparison the Effect of Bromelain and Papain Enzymes on Fish Food which Prepared from Poultry Waste Powder on Common Carp (Cyprinus Carpio L.) Growth Performance

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Abstract. The study was conducted in the Fish Laboratory / College of Agriculture / University of Tikrit to study the effect of poultry waste powder supplemented with the Bromelain and Papain enzymes at rates of 2% and 3% on growth rates, 60 fish were randomly distributed with two replicates for each treatment, 6 fish for each replicate into 10 aquaria. The fish feed containing poultry droppings powder were prepared and chemical analyzes were conducted and they were used to feed common carp fish Cyprinus Carpio L. Treatment T3 recorded the highest weight gain of 91.49 g, while it decreased in treatment T2 to 69.85 g. The relative growth rate of treatment T3: 54.62% increased, and the specific growth of 0.30 g / day, and the efficiency of food conversion decreased in treatment T4: 28.30% compared with the other treatments. Significant differences were recorded at the level (P≤0.05) between the different experimental treatments, and most of the treatments covered the common carp requirements of essential amino acids.

Keywords. Common carp, Poultry waste powder, Bromelain enzyme, Papain enzyme, Essential amino acids.

I. INTRODUCTION

Common carp Cyprinus Carpio L. is one of the most important types of fish found in many countries of the world [1], and this type of fish is characterized by its tolerance to environmental conditions and its high growth rates in Iraqi fish farms and the ease of cultivation. It provides its nutritional requirements in addition to its palatability by the Iraqi consumer [2], and it is the most important type of fish that is farmed in Iraq [3].

Enzymes play an effective role in fish feeds, as they increase the digestion of proteins, carbohydrates and fats and convert it into easily digestible and absorbable substances in the intestines [4].

[5] explained the importance of containing fish feeds on a protein source rich in amino acids, as there are ten indispensible amino acids that cannot be represented by fish, the most important of which are lysine and methionine, therefore they must be provided in the feeds. Animal butchery waste are a major source of high-value proteins it is nutritious, easy to digest and rich in essential amino acids. It is produced from fish and poultry waste [6].

The current study aims to compare the addition of Bromelain and Papain enzymes at rates of 2% and 3% in fish powder prepared from poultry and added in the diets of common carp fish, and the estimation of the essential amino acids in it and its effect on the growth rates of fish.

II. MATERIALS AND METHODS

- Preparation of Powder and Peeds

The poultry waste was shredded and the enzymes (Bromelain, Papain) were added to it by weight (2 and 3%) of its weight for 24 hours for the purpose of decomposition, after which it was dried in an electric oven at a temperature of 60°C to produce the powder. Five feeds of feed ingredient were prepared (wheat 35% - corn 25% - barley 20% - bran 9% - poultry waste powder 10% - vitamins and minerals 1%), and the feeds were divided into:

- Control T1 fish feed container for raw poultry droppings powder.
- Experimental T2 fish feed containing poultry waste powder prepared with 2% Bromelain enzyme.
- Experimental T3 fish feed containing poultry waste powder prepared with 3% bromelain enzyme.
- Experimental T4 fish feed containing poultry waste powder prepared with 2% Papain enzyme.
- Experimental T5 fish feed containing poultry waste powder prepared with 3% Papain enzyme.

- **Chemical Analytics**
  
  Chemical analyzes were carried out according to what was mentioned by [7] as follows:

- **Moisture**
  
  The moisture content of the experimental feeds was estimated by taking a sample of all kinds of feeds and dried in an electric oven at a temperature of (105) °C.

- **Protein**
  
  Nitrogen was calculated by Micro Kjeldahl method by digesting the experimental samples with sulfuric acid and boric acid was used as a guide for bromocresol green for distillation. It was Titrated with HCL acid and the conversion factor 6.25 was used to estimate the protein of samples.

- **Fat**
  
  Use a soxhlet and diethyl ether to determine the fat content.

- **Fibers**
  
  The estimation of fibers was carried out in the animal production laboratory of the College of Agriculture - University of Baghdad according to the following equation:
  
  \[
  \text{Raw fiber percentage} = \frac{\text{fiber weight (g)}}{\text{sample weight (g)}} \times 100
  \]

- **Ash**
  
  The percentage of ash was estimated using an incinerator of German origin, type Muffle Furnace (550 °C/4 hour), by burning samples weighing 1 g for three hours at a temperature of 550 °C until a white powder was obtained.

- **Nitrogen-Free Extract**
  
  The nitrogen-free extract was calculated as the difference by subtracting the percentages of nutrients (moisture, protein, fat, ash, and fiber) from 100.

- **Essential Amino Acids**
  
  The amino acids of the prepared feeds were estimated using 2 g of each sample, which was placed in a narrow-necked glass digestion tube, 10 ml of 6-N HCl acid was added, the tube was closed tightly by flame and incubated at a temperature of (60-70) °C for 24 hours, then the samples were filtered. It was concentrated by a rotary evaporator to get rid of acid residues and analyzed by an amino acid analyzer located in the Water Resources Department - Ministry of Science and Technology. The experimental samples were read after comparing them with the standard samples of amino acids, [8].

  The following equations were used to calculate growth rates:

  \[
  \text{Total weight gain (gm) = final weight (gm) - initial weight (gm).}
  \]

  \[
  \text{Daily growth rate (g/day) = weight gain (gm) / duration of increase (day) [9].}
  \]

  \[
  \text{Specific growth rate (g/day) = log of final weight - log of initial weight / experiment period x 100 [10].}
  \]

  \[
  \text{Relative growth rate % = \frac{\text{final weight (g)}}{\text{initial weight (g)}} \times 100 [11].}
  \]

  \[
  \text{Feed conversion efficiency % = \frac{\text{wet weight gain of fish (gm)}}{\text{weight of feed intake (gm)}} \times 100 [11].}
  \]

- **Statistical Analysis**

  The statistical program Statistical Analysis System [12] was used to study the effect of various experimental treatments and data analysis according to a completely randomized design (CRD), and the significant differences were compared according to [13] at a probability level of P<0.05.

### III. RESULTS AND DISCUSSION

Table (1) The results showed that there were no significant differences (P≤0.05) for the percentage of moisture between treatments T1, T2, T3 and T4, while the percentage decreased in treatment T5, reaching 8.13%. The percentage of fat in treatments T1, T2 and T4 increased (8.87, 8.56 and 8.57) %, respectively. While it decreased for treatments T3 and T5 and amounted to 8.55% and 8.54%, respectively. These results are compared with the results of [14] when using two types of digestive enzymes in the feeds of common carp. No significant differences (P≤0.05) were recorded between treatments in the percentage of protein, carbohydrates and fiber. The percentage of ash ranged between (5.18 and 5.91%), which is less than what was found by [15] when using it for poultry droppings powder in fish feeds. Common carp ranged between (9.39-9.95) %.
TABLE 1. Chemical analysis of experiment feeds.

| Treatments                                      | Moisture % | Protein % | Fat % | Fiber % | Ash % | Soluble carbohydrates % |
|------------------------------------------------|------------|-----------|-------|---------|-------|-------------------------|
| Fish feed containing raw fish powder (control)  | 8.70±0.22  | 36.68±0.211<sup>a</sup> | 8.87±0.45<sup>a</sup> | 4.96±0.482<sup>a</sup> | 5.74±0.511<sup>a</sup> | 34.83±0.204<sup>a</sup> |
| Fish feed containing fish powder supplemented with the bromelain enzyme 2% | 8.52±0.31<sup>a</sup> | 36.10±0.120<sup>a</sup> | 8.56±0.217<sup>a</sup> | 4.97±0.504<sup>a</sup> | 5.89±0.111<sup>b</sup> | 35.89±0.209<sup>a</sup> |
| Fish feed containing fish powder supplemented with bromelain enzyme 3% | 8.63±0.091<sup>a</sup> | 36.60±0.015<sup>a</sup> | 8.55±0.017<sup>b</sup> | 4.88±0.446<sup>a</sup> | 5.90±0.098<sup>a</sup> | 35.44±0.122<sup>a</sup> |
| Fish feed containing fish powder supplemented with the Papain enzyme 2% | 8.83±0.112<sup>a</sup> | 36.90±0.092<sup>a</sup> | 8.57±0.310<sup>a</sup> | 4.93±0.194<sup>a</sup> | 5.18±0.321<sup>b</sup> | 35.59±0.093<sup>a</sup> |
| Fish feed containing fish powder supplemented with the Papain enzyme 3% | 8.13±0.661<sup>b</sup> | 36.82±0.155<sup>a</sup> | 8.54±0.131<sup>b</sup> | 4.97±0.092<sup>a</sup> | 5.91±0.163<sup>a</sup> | 35.59±0.417<sup>a</sup> |

*Different letters indicate the presence of significant differences within the same column under the level of significance (P≤0.05).

Table 2) The results showed the weight gain rates of fish during the weeks of the experiment, as we notice that there are significant differences between treatments (P≤0.05), and these differences were recorded in the weight gain rates between treatments with the superiority of treatments T3 and T5 (91.49 and 97.86) g, respectively, and the results were close What [16] found when using a protease enzyme in tilapia feeds, and with the results of [13] when using digestive enzymes in the feeds of common carp fish, where the highest rate of weight gain was 96.77. Significant differences were observed between the treatments in the daily growth rates, with treatment T3 significant exceeded (P≤0.5) by recorded 1.93 g/day compared to the rest of the treatments. The T3 treatment was also superior in the relative growth rates of 54.62%, specific growth of 0.30 g/day, and the feed conversion efficiency of 57.18%. and the results converged with the study [17] when using two types of enzymes in the feeds of common carp fish. [18] stated that enzymes improve metabolism and increase fish utilization for feeds, thus improving growth rates and weight gain.
**TABLE 2.** Mean (SD or SE) growth performance and feeding efficiencies of common carp (Cyprinus carpio) different adding rates of bromelain and papain enzyme. Data with different superscripted letters are significant differences (P ≤ 0.05).

| Treatment | Initial weight gm | Final weight gm | Gain weight gm | Daily growth rate g/day | Relative growth rate % | Specific growth Rate g/day | Feed conversion efficiency % |
|-----------|-------------------|-----------------|---------------|-------------------------|------------------------|---------------------------|-----------------------------|
|Fish feed containing fish meal (control) T1 | 273.31 ±0.215<sup>a</sup> | 354.45±1.272<sup>b</sup> | 81.14 ±1.207<sup>b</sup> | 1.40±0.02<sup>c</sup> | 29.41±0.215<sup>c</sup> | 0.17±0.003<sup>c</sup> | 41.33±0.512<sup>c</sup> |
|Fish feed containing poultry waste powder supplement ed with the bromelain enzyme 2% T2 | 241.91 ±1.375<sup>b</sup> | 311.76 ±1.404<sup>c</sup> | 69.85 ±1.391<sup>c</sup> | 1.15±0.04<sup>d</sup> | 22.52±0.252<sup>d</sup> | 0.12±0.002<sup>d</sup> | 36.21±0.322<sup>d</sup> |
|Fish feed containing poultry waste powder supplement ed with bromelain enzyme 3% T3 | 235.76 ±1.090<sup>b</sup> | 327.25±1.332<sup>b</sup> | 91.49±1.101<sup>a</sup> | 1.93±0.02<sup>a</sup> | 54.62±0.462<sup>a</sup> | 0.30±0.001<sup>a</sup> | 57.18±0.466<sup>a</sup> |
|Fish feed containing poultry waste powder supplement ed with the papain enzyme 2% T4 | 243.63 ±1.206<sup>b</sup> | 320.51±1.157<sup>b</sup> | 76.88±1.31<sup>b</sup> | 1.17±0.02<sup>d</sup> | 22.37±0.351<sup>d</sup> | 0.16±0.004<sup>c</sup> | 28.30±0.363<sup>c</sup> |
|Fish feed containing poultry waste powder supplement ed with the papain enzyme 3% T5 | 263.46 ±1.290<sup>b</sup> | 361.32±1.401<sup>b</sup> | 97.86±1.75<sup>c</sup> | 1.62±0.04<sup>b</sup> | 42.82±0.226<sup>b</sup> | 0.24±0.003<sup>b</sup> | 49.41±0.271<sup>b</sup> |

*Different letters indicate the presence of significant differences within the same column under the level of significance (P≤0.05).

Table (3) shows the percentage of amino acids for feeds containing the bromelain and papain enzymes, where the treatment T1 recorded 9.1% the highest percentage of the amino acid arginine, while the treatments T2, T3, T4 and T5 did not cover the fish’s need for arginine, while the same treatments covered the fish's need for histidine (3.9, 4.1, 2.9 and 2.1) %. Treatment T5 recorded the highest percentage of the amino acid isoleucine 29.5%, while the lowest percentage was in the treatment T3 0.5%, and the two amino acids leucine and lysine were found in small percentages in most of the treatments. Treatment T1 outperformed the rest of the treatments in containing the amino acids tyrosine and threonine with percentages...
of 10.1% and 8.4%, respectively. The percentages of acid in valine increased in all treatments T1, T2, T3, T4 and T5 by (11.1, 55.6, 69.4, 30.9 and 7.3) % respectively.

The results of the current study converged with [19] when using poultry droppings powder supplemented with enzymes in common carp fish feed. The reason for the loss of some acids is due to the fact that the powders prepared from the waste contain a high percentage of the connective tissue that consists of collagen and elastin, and thus they are deficient or poor in some essential amino acids [20].

**TABLE 3.** The percentage of amino acids of the prepared feeds.

| Amino acids     | Percentage % | Percentage % | Percentage % | Percentage % | Percentage % | Essential amino acid requirements of common carp% |
|-----------------|--------------|--------------|--------------|--------------|--------------|-----------------------------------------------|
| **Essential amino acids** |              |              |              |              |              |                                               |
| Arginine        | 9.1          | 0.3          | 0.7          | 0.2          | 0.1          | 1.50                                          |
| histidine       | 0.2          | 3.9          | 4.1          | 2.6          | 2.1          | 0.74                                          |
| Isoleucine      | 3.2          | 1.1          | 0.5          | 3.1          | 29.5         | 0.88                                          |
| leucine         | 2.3          | 1.5          | 0.1          | 0.5          | 1.5          | 1.16                                          |
| lysine          | 3.4          | 1.1          | 0.1          | 0.1          | 4.1          | 2.00                                          |
| methionine      | 17.5         | 2.4          | 0.2          | 22.1         | 26.1         | 1.09                                          |
| cysteine        | 8.4          | 0.2          | 1.1          | 21.2         | 1.4          | 1.09                                          |
| phenylalanine   | 0.1          | 11.5         | 7.8          | 3.1          | 0.1          | 2.28                                          |
| glycine         | 7.5          | 0.1          | 0.2          | 0.5          | 0.9          | 2.28                                          |
| tyrosine        | 10.1         | 0.1          | 0.4          | 0.4          | 0.2          | 1.37                                          |
| threonine       | 8.4          | 0.2          | 0.1          | 1.9          | 19.1         | 0.28                                          |
| valine          | 11.1         | 55.6         | 69.4         | 30.9         | 7.3          | 1.26                                          |
| aspartic        | 0.1          | 2.8          | 4.9          | 7.1          | 1.1          | ----                                          |
| glutamic        | 1.3          | 5.1          | 4.9          | 6.9          | 1.9          | ----                                          |
| serine          | 1.6          | 10.1         | 3.1          | 0.8          | 5.5          | ----                                          |
| alanine         | 3.5          | 5.2          | 0.5          | 0.5          | 0.1          | ----                                          |

*Non-essential amino acids* (aspartic, glutamic, serine, alanine).

**CONCLUSIONS**

We conclude from this study that the addition of poultry waste powder prepared with bromelain and papain enzymes, containing high percentages of essential amino acids as a protein source in common carp diet, reduced the production costs of feeds and improved the feeding and growth efficiency of fish.

**REFERENCE**

[1] Davies, S. J. and Gouveia, A. (2006). Comparison of yttrium and chromic oxides as inert dietary markers for the estimation of apparent digestibility coefficients in mirror carp Cyprinus carpio fed on diets containing soybean- maize- and fish-derived proteins. Aquac. Nutr. • 12: 451–458.

[2] Abd- Rahman Shanan (2014). Effect of adding Allium sativum powder and garlic oil on some growth parameters- liver enzymes and blood picture in common carp fish Cyprinus carpio L.. Master's thesis• College of Agriculture• University of Baghdad: 64 p.

[3] Khazali, Baha Kazem (2017). The effect of adding polyethylene glycol to diets on the growth performance of common carp (Cyprinus carpio L.). Master Thesis. College of Agriculture• University of Baghdad: 104 p.
Comparison for Using Two Types of Digestive Enzymes with Fish Powder in the Diets of Common Carp L. Cyprinus Carpio And its Effect on Growth and Blood Parameters. PhD thesis. College of Agriculture University of Mosul: 248 p.

Schmalhausen, I. I. (1926). The problem of death and immortality. Gosizdat, Moscow: 569p.

Brown, M. E. (1957). Experimental studies on growth. In Fish physiology: M. E. Brown (ed) New York: N. Y. Academic Press Vol I: 361-400.

Ume, F. (1978). Standard methods and terminology in fin-fish nutrition from; proc. World Symp. on finfish nutrition and fish feed Technology, Hamburg, (2): 20-23.

SAS Institute. (2012). SAS/OR 9.3 User's Guide: Mathematical Programming Examples. SAS institute.

Duncan, D. B. (1955). Multiple range and multiple F tests. Biometrics 11(1):1-42.

Al-Bassam, N. H.; Shamkhi, I. A.; Al-Douri, M. M. (2021). Comparison for Using Two Types of Digestive Enzymes with Fish Powder in the Diets of Common Carp L. Cyprinus Carpio And Its Effect on Growth and Blood Traits. Annals of R.S.C.B. ISSN:1583-6258; Vol. 25; Issue 6; 2021: 6079 – 6091.

Al-Hassoun, Ahmed Shehab (2009). Effect of different levels of poultry carrot protein concentrate and soybean meal meal on growth rates of larvae of common carp (Cyprinus carpio L.). Iraqi Journal of Aquaculture Volume (6) Issue (2): 95- 103.

Hassan, M. S.; El-Sayed, A. I. M.; Soltan, M. A.; Iraqi; M. M.; Goda, A. M.; Davies, S. J. and Ramadan, H. A. (2019). Partial dietary fish meal replacement with cotton seed meal and supplementation with exogenous protease alters growth; feed performance; hematological indices and associated gene expression markers (GH; IGF-I) for Nile tilapia Oreochromis niloticus. Aquaculture 503: 282-292.

Shakir, H.F.; Al-Bassam, N.H.; Shamkhi, I.A (2021). Effect of adding phytase enzyme and formic acid to diets of common carp Cyprinus carpio L. on coefficient of digestibility and growth rates. Al-Muthanna Journal of Agricultural Sciences Volume (8) Issue (4).

Ganj, S. H.; Kamanna, V. S. and Kashyap, M. L. (2003). Niacin and Cholesterol role in cardiovascular disease (review). J. Nutr. Biochem. 14(6):298-305.

Al-Bassam, Noha Hamid (2020). Manufacture of diets for common carp L. Cyprinus carpio from some protein wastes treated with enzymes and acid on growth profiles and blood parameters. PhD thesis. College of Agriculture, Tikrit University: 168 pp.

Al-Habib, Farouk Mahmoud Kamel (1996). The use of non-traditional feeds in the feeding of ordinary carp fish. PhD thesis, College of Agriculture, Department of Fisheries, University of Basra: 168 p.

N.R.C. (1993). National Research Council. Nutrient Requirements of Fish. Washington:337p.