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Original Article

Effect of poultry waste powder supplemented with papain, phytase, and pepsin enzymes on digestion and growth of common carp, *Cyprinus carpio* L.

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Abstract
This study was conducted to investigate the feasibility of using poultry waste as feed powder in the common carp diet. For this purpose, the poultry waste powder was mixed with 2.5 and 3.5% of enzymes of papain, phytase, and pepsin, and their effects on the digestion and growth parameters of the fish were investigated. A total of 84 fish were divided into 14, 250L tanks at a density of 1.52g/l or 6 fish, with two replicates per treatment. Based on the results, the highest weight gain was found in T6 (99.17g), and the lowest rates in T2 (74.17g). T3 had a 57.02% increase in relative growth, T5 showed specific growth of 0.29g/day, and T4 had a decrease in food conversion efficiency (276.9%). The results revealed that enzymes improve metabolism, fish's utilization of diets, and increase growth rates and weight gain.

Keywords: Aquaculture, Phytase, Pepsin, Digestion, Growth rate.

INTRODUCTION

One of Iraq's most valuable fish species for aquaculture is the common carp, *Cyprinus Carpio* L. (Khazali 2017). It has a rapid growth rate, adapts well to many environments, and can be easily farmed in Iraqi fish farms; in addition, the average Iraqi customer enjoys consuming it (Abd 2014; Khazali 2017). Protein powders derived from animals are a popular and useful option because they include many healthy nutrients and are simple to absorb. They produce from leftovers of the fish and poultry industries (Khan et al. 2013).

To better digest and absorb proteins, carbs, and fats in the gut, enzymes play an important role in fish diets (Kumar et al. 2012). Exogenous enzymes have been shown in the majority of fish-feeding trials to speed up growth and digestion (Liang et al. 2022). In this regard, this work aimed to examine the effects of diets supplemented with different concentrations of enzymes (Papain, phytase, and pepsin) on common carp growth performance, digestion, and levels of essential amino acids.

MATERIALS AND METHODS

Preparation of powder and diets: Poultry waste was minced, and 2.5 and 3.5% of the diet its weight, the enzymes of the papain, phytase, and pepsin were added for 24 hours. Then, the feed was dried at a temperature of 60°C in an electric oven to obtain the powder. The basic experimental diet was prepared (poultry waste powder 10%, maize 25%, wheat 35%, barley 20%, bran 9%, and vitamins and minerals 1%). Based on this basic diet, the treatments were prepared as follows: T1 control diet containing raw poultry powder, T2 experimental diet containing poultry powder with papain enzyme 2.5%, T3 experimental diet containing poultry powder with papain enzyme 3.5%, T4 experimental diet containing poultry powder with phytase enzyme 2.5%, T5 experimental diet containing poultry powder with phytase enzyme 3.5%, T6 experimental diet containing poultry powder with pepsin enzyme 2.5% and T7 experimental diet containing poultry powder with pepsin 3.5%.

Water parameters: The water temperature, oxygen, pH, and salinity were measured every ten days in experimental ponds using a water quality meter (Chinese) (Table 1).

Chemical analyses: Chemical analyzes were carried out according to AOAC (2000). Humidity or

Table 1. Physical and chemical properties of water in rearing tanks during experiment.

Water tests	Period in days						
	0	10	20	30	40	50	60
Temperature	18.30	18.33	18.98	21.22	24.51	26.43	72.31
Dissolved oxygen	8.40	8.40	8.90	8.48	8.48	8.56	8.39
pH	7.52	7.56	7.49	7.52	7.52	7.51	7.61
Salinity	0.45	0.40	0.37	0.33	0.31	0.35	0.36

moisture content was estimated by drying samples of the experimental diets in an electric oven at a temperature of 105°C. The protein of samples was digested to measure nitrogen using the Micro Kjeldahl apparatus with concentrated sulfuric acid, and boric acid as bromocresol green for distillation. The samples were pulverized with HCL acid and a conversion factor of 6.25 was used to estimate the protein of the samples. The percentage of fat was estimated using a Soxhlet apparatus and diethyl ether solvent. The fiber of the samples was sent to the Animal Production Laboratory of the College of Agriculture, University of Baghdad to estimate the percentage of fibers according to the following equation:

The crude fiber percentage % = weight of fiber (g) / weight of the sample (g) x 100

A German-made Muffle Furnace incinerator (550°C/4h) was used to estimate the percentage of ash by burning 1g of each sample for three hours at a temperature of 550°C to obtain the white powder. The percentage of the nitrogen-free extract was calculated by the difference in subtracting the percentages of nutrients (moisture, protein, fat, ash, and fiber) from 100. Digestibility parameter measurements were performed as per Maynard & Loosli (1969) as follows:

Apparent digestibility coefficient of protein (%) = 100 – (Cr_2O^3 in the food% / Cr_2O_3 in the waste% x percentage of protein in the waste% / percentage of protein in the food% x 100)

Apparent digestibility coefficient of fat % = 100 – (Cr_2O_3 in food % / Cr_2O_3 in waste% x Percentage of fat in waste% / Percentage of fat in food% x 100)

Apparent digestion coefficient of carbohydrates (%) = 100 – (Cr_2O_3 in food%/ Cr_2O_3 in waste% x percentage of carbohydrates in waste% / percentage of carbohydrates in food% x 100)

Apparent digestion coefficient of ash % = 100 – (Cr_2O_3 in food% / Cr_2O_3 in waste% x percentage of ash in waste% / percentage of ash in food% x 100)

Growth parameters: The following formulae were used to calculate growth parameters:

Total weight gain (g) = final weight (g) – starting weight (g).

Daily growth rate (g / day) = weight gain (g) / time period of increase (day) (Schmalhausen 1926).

Specific growth rate (g/day) = log final weight – log initial weight/experimental time x 100 (Brown 1957).

Relative growth rate (%) = Final weight (g) – Starting weight (g) / Starting weight (g) x 100 (Utne 1978).

Feed conversion efficiency (%) = fresh weight gain of fish (g) / weight of feed intake (g) x 100 (Utne 1978).

Statistical analysis: Using a completely randomized design (CRD), we compared the efficacy of treatments using the statistical tool Statistical Analysis System (SAS) (2012). Duncan's (1955) test was used to examine statistically significant differences at the 5% significance level.

RESULTS AND DISCUSSION

There are no significant differences (P>0.05) in the moisture between T1, T3, T4, T6, and T7, whereas the moisture decreased in T2 and T5 by 8.11 and 8.12%, respectively (Table 2). The fat content increased in T1, T3, and T6, and these results were consistent with the findings of Al-Bassam et al. (2020) that had mixed chicken powder and digestive enzymes in the diet of common carp. Protein and

Table 2. Chemical analysis of the experimental diet.

Diet groups	Humidity%	Protein%	Fats%	Fiber%	Ash%	Carbohydrates%
T1	8.56±0.23^{a}	36.55±0.26^{a}	8.71±0.28^{a}	4.87±0.33^{a}	5.87±0.33^{a}	34.44±0.29^{b}
T2	8.11±0.28^{b}	36.19±0.19^{a}	8.51±0.42^{b}	4.91±0.48^{a}	5.32±0.28^{b}	35.67±0.37^{a}
T3	8.58±0.61^{a}	36.55±0.22^{a}	8.90±0.29^{a}	4.84±0.34^{a}	5.88±0.22^{a}	35.59±0.23^{a}
T4	8.77±0.32^{a}	36.87±0.87^{a}	8.49±0.49^{b}	4.88±0.39^{a}	5.29±0.41^{b}	35.66±0.18^{a}
T5	8.12±0.54^{b}	36.91±0.29^{a}	8.55±0.22^{b}	4.90±0.18^{a}	5.79±0.48^{a}	35.71±0.33^{a}
T6	8.91±0.33^{a}	36.88±0.43^{a}	8.88±0.45^{a}	4.89±0.65^{a}	5.90±0.36^{a}	35.60±0.49^{a}
T7	8.70±0.14^{a}	36.71±0.29^{a}	8.42±0.11^{b}	4.82±0.36^{a}	5.78±0.11^{a}	35.51±0.38^{a}

Table 3. The results of digestion coefficient of experience factors.

Diet groups	Protein digestion coefficient	Fats digestion coefficient	Carbohydrates digestion coefficient	Ash digestion coefficient
T1	73.55±0.22^{b}	77.39±0.78^{b}	42.73±0.52^{c}	58.99±0.31^{c}
T2	84.47±0.31^{a}	85.71±0.66^{a}	72.61±0.77^{a}	90.33±0.85^{a}
T3	86.51±0.60^{a}	85.76±0.69^{a}	77.39±0.62^{a}	83.85±0.41^{a}
T4	74.37±0.66^{b}	88.53±0.54^{a}	50.32±0.74^{b}	69.75±0.28^{b}
T5	71.99±0.80^{b}	90.44±0.61^{a}	50.09±0.81^{b}	71.42±0.59^{b}
T6	83.31±0.55^{a}	94.75±0.59^{a}	78.31±0.44^{a}	90.11±0.28^{a}
T7	8.11±0.41^{a}	93.45±0.48^{a}	75.44±0.20^{a}	89.88±0.33^{a}

fiber showed no significant differences (P>0.05) between treatments. By adding the chicken powder to common carp diets, the ash was between 5.29 and 5.90% that is lower than those reported by Al-Hasoon (2009) which was 9.39-9.95%.

Table 3 shows the digestion coefficient of the control and the experimental groups. The digestibility coefficient of protein, fat, carbohydrates, and ash in T2, T3, T6, and T7 had increased significantly (P≤0.05) compared to T1, T4, and T5. In T1, T4, and T5, the protein digestibility did not exceed 73.55, 74.37, and 71.99, respectively. This may be due to the supplementing of organic acids, including the pepsin, which has enhanced the deposition of nitrogen in the body and increased the ability to digest nutrients and absorb minerals. As a result, there was an increase in its utilization and the total digestibility coefficient of the protein of the diet (Lückstädt 2008). These results agree with results of Muhammad & Al-Safu (2013) reported that the addition of some probiotics is led to an improvement in growth parameters by improving the digestion parameters in the diet of common carp. These results also agree with the findings of Al-Bassam (2022) that using poultry powders fortified with bromelain and papain in the diets of common carp leads to increasing the digestion rates of all components of the diet.

Weight gain rates for the fish during the experiment are shown in Table 4; there were significant differences between the treatments (P≤0.05). The weight increase varied significantly between treatments, with T3 and T6 (93.52 and 99.17g) showing the highest. The results were consistent with those reported by Hassaan et al. (2019) and Al-Bassam et al. (2022) that used protease in tilapia fish diet. Using papain at a rate of 3.5% in poultry powder in common carp diet, the rate of weight gain reached 97.86g. T3, T5, and T6 also showed excelled in relative growth rates (57.02, 51.67, and 52.88%, respectively), and in specific growth rates and feed conversion efficiency as well. Shakir et al. (2021) were used two enzymes in common carp diets, and reported similar results. Enzymes improve metabolism, fish's utilization of

Table 4. Growth characteristics of common carp fish feed on experience diets.

Diet groups	Initial weight g	Final weight	Wieght increase g	Daily growth rate g/day	Relative rowth rate %	Qualitative growth rate Gm/day	Food transfer competence %
T1	239. 88±0.32^{b}	3.22.32±1.39^{b}	82.44±1.65^{b}	1.39±0.01^{c}	28.51±0.33^{c}	0.18±0.01^{c}	38.26±0.56^{c}
T2	249.99±0.33^{a}	324.16±1.55^{c}	74.17±1.43^{c}	1.17±0.03^{d}	21.49±0.30^{d}	0.11±0.002^{d}	35.32±0.48^{d}
T3	240.11±1.08^{b}	333.36±1.51^{b}	93.521±1.5^{a}	1.92±0.01^{a}	57.02±0.47^{a}	0.27±0.001^{a}	56.27±0.51^{a}
T4	241.99±1.09^{b}	327.12±1.63^{b}	85.13±81.4^{b}	1.19±0.01^{d}	26.55±0.2^{c}	0.17±0.003^{c}	27.69±0.55^{e}
T5	251.67±0.89^{b}	349.39±1.21^{b}	97.72±1.10^{a}	1.89±0.03^{a}	51.67±0.3^{a}	0.29±0.002^{a}	61.09±0.37^{a}
T6	256.97±1.08^{b}	356.14±1.47^{a}	99.171±1.10^{a}	1.90±0.01^{a}	52.88±0.9^{a}	0.28±0.001^{a}	58.31±0.71^{a}
T7	250.01±1.32^{a}	346.21±1.31^{b}	96.20±1.375^{a}	1.65±0.03^{b}	46.28±0.16^{b}	0.23±0.002^{b}	48.03±0.31^{b}

diets, and increase growth rates and weight gain (Ganji et al. 2003).

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مقاله کامل

تأثیر پودر ضایعات طیور همراه با آنزیم‌های پاپائین، فیتاز و پپسین بر روی هضم و رشد ماهی کپور معمولی *Cyprinus carpio* L.

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چکیده: این مطالعه به‌منظور بررسی امکان‌سنجی استفاده از ضایعات طیور به‌عنوان پودر خوراک در جیره ماهی کپور معمولی به‌اجرا درآمد. بدین‌منظور، پودر ضایعات طیور با ۲/۵ و ۳/۵ درصد آنزیم‌های پاپائین، فیتاز و پپسین ترکیب و تأثیرات آن‌ها بر پارامترهای هضم و رشد ماهی مور بررسی قرار گرفت. در مجموع ۸۴ ماهی به ۱۴ تانک ۲۵۰ لیتری با تراکم ۱/۵۲ گرم در لیتر یا ۶ ماهی، با دو تکرار در هر تیمار تقسیم شدند. براساس نتایج، بیشترین افزایش وزن در T6 (۹۹/۱۷ گرم) و کمترین میزان نرخ رشد T2 (۷۴/۱۷ گرم) مشاهده شد. T3 افزایش ۵۷/۰۲ درصدی در رشد نسبی داشت، T5 رشد ویژه ۰/۲۹ گرم در روز و T4 کاهش کارایی تبدیل غذا (۲۷۶/۹ درصد) را نشان داد. نتایج نشان داد که آنزیم‌ها، متابولیسم و استفاده ماهی از رژیم غذایی را بهبود می‌بخشد و سرعت رشد و وزن را افزایش می‌دهد.

کلمات‌کلیدی: آبزی‌پروری، فیتاز، پپسین، هضم، نرخ رشد.