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Effect of fish meal replacement with lentil seed (*Lens culinaris*) in common carp (*Cyprinus carpio*) L. diet

Nasreen M. Abdulrahman¹, Vian M. Ahmad², Bakhan R. Hassan^{2*}, Zaweta S. Abdulla², Derin O. Muhammed-Ramzi¹. Hevar A. Hama-Salih³. Hemin N. Mohammed²

¹ College of veterinary medicine, university of Sulaimani, Iraq
²Department of Animal Science, College of Agricultural Engineering Sciences, University of Sulaimani, Iraq
³Municipality of Sulaimani, directorate of gardeners, Sulaimani, Iraq

*Corresponding author: bakhan.hassan@univsul.edu.ig

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ABSTRACT

The aim of this study was to see how replacing fish meal with lentil seeds (LS) as an alternative plant protein source in diets for common carp *Cyprinus carpio* affected growth performance, feed consumption, and the composition of the fish's proximate carcass. The fish (100-106 g mean initial weight) were fed 0% (LS0), 5% (LS5), 10% (LS10), 15% (LS15) and 20% (LS20) of Lentil seed protein for 10 weeks. With increasing fishmeal replacement with lentil seed in the fish diet, there was a significant (p<0.05) increase in weight gain, relative and specific growth rate. T5 (20% LS) had a significantly (p<0.05) higher feed efficiency ratio and protein efficiency ratio than the other treated groups. There were no significant (p<0.05) differences in the mean values of proximate composition of fish fillets, such as moisture, crude protein, crude lipid and ash. Finally, no significant differences in muscle ratio (weight without viscera vs. weight without viscera & head) were found. The current study found that lentil seed can replace 20% of dietary fish meal in common carp diets for better growth.

Keywords: lentil seed, fish meal, common carp, growth performance and feed utilization.

ABSTRAK

Tujuan dari penelitian ini adalah untuk melihat bagaimana penggantian tepung ikan dengan biji lentil (LS) sebagai sumber protein nabati alternatif dalam pakan ikan mas *Cyprinus carpio* mempengaruhi kinerja pertumbuhan, konsumsi pakan, dan komposisi karkas proksimat. Ikan (berat awal rata-rata 100-106 g) diberi makan 0% (LS0), 5% (LS5), 10% (LS10), 15% (LS15) dan 20% (LS20) protein biji Lentil selama 10 minggu. Dengan meningkatnya penggantian biji lentil dengan tepung ikan dalam pakan ikan, terjadi peningkatan berat badan, laju pertumbuhan relatif dan spesifik yang signifikan (p<0,05). T5 (20% LS) memiliki rasio efisiensi pakan dan efisiensi protein yang lebih tinggi secara signifikan (p<0,05) dibandingkan dengan kelompok perlakuan lainnya. Tidak ada perbedaan yang signifikan (p<0,05) dalam nilai rata-rata komposisi proksimat fillet ikan, seperti kadar air, protein kasar, lemak kasar dan abu. Akhirnya, tidak ada perbedaan signifikan dalam rasio otot (berat tanpa jeroan vs berat tanpa jeroan & kepala) ditemukan. Studi saat ini menemukan bahwa biji lentil dapat menggantikan 20% makanan ikan dalam makanan ikan mas untuk pertumbuhan yang lebih baik.

Kata kunci: biji lentil, tepung ikan, ikan mas, performa pertumbuhan dan pemanfaatan pakan.

1. Introduction

For several fish species, fishmeal is considered the gold standard nutritional protein source. Its production is dependent on noncommercial wild marine fish (Hertrampf and Piedad-Pascual, 2012). However, it is now considered both economically and ecologically unsustainable, and the aquaculture industry is

under social and economic strain to find substitute proteins (Turchini et al., 2019).

In aquafeeds, plant proteins are the most common fishmeal replacements. They are cost-effective, and they are often preferred due to market perceptions of the use of terrestrial animal by-products for fish feeding (Gajardo *et al.*, 2017; Shepherd *et al.*, 2017). Proteins are highly digestible for fish in most properly

processed feedstuffs, protein digestion coefficients in protein-rich feedstuffs are typically in the range of 75 to 95%, and protein digestibility tends to be depressed as dietary carbohydrate concentration increases (Hemre et al., 2002).

Cyprinus carpio Linnaeus, 1758 which has been a common aquaculture fish for more than 2,000 years, is one of the world's most commercially valuable and widely cultivated freshwater fish, contributing to 11% of the world's total production of freshwater aquaculture (FAO, 2010). More than 90% of this production comes from Asia, where different pond aquaculture systems cultivate common carp. Similarly, in response to changing food resources, common carp can alter its food preference and conduct (Rahman et al., 2008).

Lentil has been referred to colloquially as "Poor man's meat", as it is a rich source of nutrients, composed of 60%–67% carbohydrate, 20%–36% protein, <4% lipid, and 2%–3% ash on a dry basis (Bhatty, 1988). This study aimed to assess the use of lentil seeds as a partial substitute for fish meal protein in common carp diets and evaluate the effect of feed containing different lentil seed ratios on growth, feed utilization and proximate carcass composition in common carp.

2. Materials and Methods

2.1. Experimental Fish

The experiment was performed on 150 common carp. *C. carpio* L. was brought from local fish ponds in Peramagrun/ Sulaimani/ Iraq. The fish weighed between (100-106 g), preacclimation and feeding of commercial pellets were carried out for 30 days before the actual feeding trials.

2.2. Experimental Procedure

In this experiment, fifteen plastic tanks (100 L) were used for five treatments of 3 replicates each; ten fish were stocked with each replica for 70 days. Using Chinese air compressors, Hailea ACO-318, proper continuous aeration was applied to each tank. To decrease the variations between treatments, the replicates were randomly positioned. To extract residual feed and feces from the system, regular cleaning by siphoning process was applied. Treatments were as follows:

T1: 0% lentil seed replaced with fishmeal (control diet).

T2: 5% lentil seed replaced with fishmeal

T3: 10% lentil seed replaced with fishmeal

T4: 15% lentil seed replaced with fishmeal

T5: 20% lentil seed replaced with fishmeal

2.3. Diet Formulation

Experimental diets contained standard ingredients (soya bean meal, yellow corn, barley, wheat bran and lentil seed) found in the city markets of Sulaimani, enriched with the lentil seed levels. Preparing five different diets each contains the desired lentil level as described in table 1. The ingredients items were mixed with water to obtain a dough, then using electrical mincer for pelleting by Kenwood Multiprocessors. Room temperature drying and crushing were used to extract fine particles. Twice daily feeding at 10:00 a.m. and 3:00 p.m. for a bodyweight of 3% was carried out.

2.4. Growth and Feed Utilization Parameters

On all replicates, the fish were weighed together (g) every 2 weeks. Every replicate's feed intake was then recalculated.

• Weight gain (g) = W2–W1

 W_2 : Fish weight at the end of the experiment

 W_1 : Fish weight at the beginning of the experiment

Daily weight gain (DWG) (g) = W2–W₁/T

T: the time between W2 and W1

- Relative growth rate (RGR %) = W2–W₁/W₁ x 100 (Brown, 1957)
- Specific growth rate (SGR) % = (Ln W2

 Ln W1)/ T) x 100 (Lagler, 1956)
- Survival rate (%) = 100 (final fish number / initial fish number)
- Feed conversion ratio (FCR) = Total feed fed (g)/ Total wet weight gain (g) (Uten, 1978)
- Feed efficiency ratio (FER) = Total weight gain (g)/ Total feed fed (g) (Uten, 1978)
- Protein efficiency ratio (PER) = Total wet weight gain (g)/ Amount of protein fed (g) (Uten, 1978)

2.5. Proximate Composition

Moisture content was determined as weight loss after samples were dried in the convection oven at 105°C until the weight was stabilized (Folch *et al.*, 1957). Protein content was determined according to the Association of Official Analytical Chemists AOAC (2005) by using the Micro Kejldahal method and was calculated as follows: Protein% = nitrogen x6.25.

Table 1. Percentage of experimental diet components for fish and chemical composition of the different types of diet by NRC (1993)

Ingredients	Ratio%	Crude Protein %	Crude Fat %	Dry Matter %	Crude Fiber %	Energy Kcal/ kg
Soya bean meal	35	48	1.1	89	7	2230
Yellow corn	13	8.9	3.6	89	2.2	3400
Barley	15	11	1.9	89	5.5	2640
Wheat bran	20	15.7	4	89	11	1300
Fish meal	15	65.4	5.6	90.5	0.00	1290
Vit + min	2					
lentil seed*		25	1	93	11	1480
Total	100					
Crude protein (%)	28.46					
Crude fat (%)	3.29					

^{*} According to the USDA National Nutrient Database

The crude lipid of fish meat was determined according to Folch *et al.* (1957). The percentage of lipids was determined according to the following formula: Lipid $\% = (\text{weigh of extract / weigh of sample}) \times 100$.

Ash content was determined according to the AOAC (2005) method by taking a sample of a known weight of fish flesh and placed in a muffle furnace at 550 °C for 16 hours and then the ash percent was determined as follows: Ash % = (W1/W2) x 100 W1 = weight of ash, and W2 = initial weight.

2.6. Statistical Methods

The outcomes were expressed as mean \pm SE. All data was subject to One-way ANOVA. When significant differences (P<0.05) were found, the Duncan test was used to measure the group means. SPSS V.20 was used to conduct the statistical analyses.

3. Results and Discussion

3.1. Growth performance

Table 2 presents the findings of the feeding experiment. No mortality occurred during the study. All fish grew normally and no specific sign of the disease was observed. The final average weight after a trial of 10 weeks in Cyprinus carpio in control and treated ponds were recorded as 149.78 ±0.45, 151.91 ±0.17, 150.69 ± 0.93 , 151.55 ± 0.82 and 162.59 ± 0.97 g respectively, the highest values of weight gain were observed in treatment five (20% Lentil seed). The daily weight gain, relative growth rate and specific growth rate of the C. carpio were significantly (P<0.05) affected by feeding fish with lentil seed. Treatment five (20% lentil seed) achieved the best results in terms of growth performance, according to the findings of this research, as indicated in table 2.

3.2. Feed utilization

Nutritional indices such as the feed conversion ratio (FCR), feed efficiency ratio (FER), and protein efficiency ratio (PER) was shown in the tables 3. Fish feeding on a diet containing 20% lentil seed (T5) had a better

Table 2. Effect of fishmeal partial replacement with five levels of lentil seed on growth performance of *C. carpio*.

Treatments	Initial Wt. (g)	Final Wt. (g)	Wt. gain (g)	Daily WG (g)	Specific GR %	Relative GR %	Survival %
T1 Control	106.53 ^a ±0.43	149.78° ±0.45	43.24° ±0.14	0.62 ^c ±0.04	0.49° ±0.03	40.59 ^c ±0.44	100
T2 5% Lentil seed	106.86 ^a ±0.25	151.91 ^b ±0.17	45.05° ±0.32	0.64 ^c ±0.03	0.50° ±0.01	42.16 ^c ±0.95	100
T3 10% Lentil seed	101.55 ^a ±0.65	150.69 ^b ±0.93	49.15 ^b ±0.53	0.70 ^b ±0.02	0.57 ^b ±0.05	48.39 ^b ±0.34	100
T4 15% Lentil seed	101.04 ^a ±0.76	151.55 ^b ±0.82	50.51 ^b ±0.33	0.72 ^b ±0.03	0.57 ^b ±0.04	49.99 ^b ±0.17	100
T5 20% Lentil seed	103.02 ^a ±0.13	162.59 ^a ±0.97	59.57 ^a ±0.41	0.85 ^a ±0.05	0.66a ±0.07	57.82 ^a ±0.92	100

Different letters in one column mean significant differences at P<0.05.

Treatments	Feed conversion ratio FCR	Feed efficiency ratio FER	Protein efficiency ratio PER
T1 Control	$4.28^a \pm 0.05$	$0.24^{\circ} \pm 0.04$	$1.52^{\circ} \pm 0.02$
T2 5% Lentil seed	$3.35^{\circ} \pm 0.1$	$0.3^{b} \pm 0.02$	$1.57^{\circ} \pm 0.02$
T3 10% Lentil seed	$4.69^{a} \pm 0.9$	$0.22^{\circ} \pm 0.04$	$1.71^{b} \pm 0.03$
T4 15% Lentil seed	$3.85^{b} \pm 0.02$	$0.26^{\circ} \pm 0.09$	$1.79^{b} \pm 0.03$
T5 20% Lentil seed	$3^{d} \pm 0.09$	$0.34^a \pm 0.02$	$2.11^a \pm 0.05$

Table 3. Effect of fishmeal partial replacement with five levels of lentil seed on feed utilization of *C. carpio*.

Different letters in one column mean significant differences at P<0.05.

feed conversion ratio compared to other treatments. At treatment five (20% lentil seed), the FER and PER were significantly (P<0.05) higher than the other treatments.

3.3. Proximate Composition

Fish fillet proximate composition such as moisture, crude protein, crude lipid and ash was shown in table (4). The highest moisture content of carp meat was in the second treatment (5% lentil seed) (66.61 \pm 1.21) without statistically significant differences (P<0.05) with other treatments. The results showed that the highest ratio of crud protein and lipid was in the fifth (10% lentil seed) and fourth (15% lentil seed) treatment 18.83± 0.72 and 12.33± 0.53 respectively and did not differ statistically with the rest of the treatments. The findings of the study's treatment of ash content in fish meat did not show any significant (P<0.05) differences in the ash content of fish meat.

3.4. Muscle ratio

The amount of flesh weight was calculated using data on muscle ratio (weight without viscera and weight without viscera and head), and the results were shown in table (5). There were no significant differences between treatments.

Different scientists have suggested different cost-effective sources of protein for feed preparation to achieve optimal fish growth (Kaushik and Troell, 2010; Radhakrishnan *et al.*, 2016). In fish feed, plant ingredients have a great potential to replace fishmeal, it is possible to substitute fishmeal fully or partially with plant-based protein without loss of growth efficiency (Middleton *et al.*, 2000; Kaushik *et al.*, 2004).

According to different studies, herbivores require considerably less protein than carnivores. Vechklang *et al.* (2011) noticed that *O. niloticus* performed better growth when 20% of the fish meal was substituted with protein from a mixture of different plant protein meals.

Table 4. Effect of fishmeal partial	replacement with	five levels of lenti	I seed on proximate analyses
of C. carpio.			

Treatments	Moisture %	Crude Protein %	Crude lipid %	Ash %
T1 Control	66.54 ^a ± 0.39	17.10 ^a ± 0.04	12.10 ^a ± 0.06	$1.60^a \pm 0.27$
T2 5% Lentil seed	66.61 ^a ± 1.21	17.67 ^a ± 0.29	$12.05^a \pm 0.52$	$2.83^{a} \pm 0.28$
T3 10% Lentil seed	65.67 ^a ± 0.49	$18.69^a \pm 0.42$	12.32 ^a ± 0.05	$2.83^{a} \pm 0.26$
T4 15% Lentil seed	$66.11^a \pm 0.59$	$18.35^{a} \pm 0.89$	$12.33^a \pm 0.53$	$2.65^{a} \pm 0.24$
T5 20% Lentil seed	65.10 ^a ± 0.24	$18.83^a \pm 0.72$	$12.26^a \pm 0.18$	$2.59^{a} \pm 0.32$

Different letters in one column mean significant differences at P<0.05.

Treatments	Weight of fish meat without head Index (g)	Weight of fish meat without head & viscera Index (g)
T1 Control	84.61 ^a ± 0.59	57.36° ± 2.31
T2 5% Lentil seed	83.83 ^a ± 1.71	53.93° ± 1.31
T3 10% Lentil seed	$87.81^a \pm 0.66$	$54.89^{a} \pm 0.86$
T4 15% Lentil seed	$85.90^{a} \pm 1.62$	51.21 ^a ± 1.41

Table 5. Effect of fishmeal partial replacement with five levels of lentil seed on meat indices of *C. carpio*.

Different letters in one column mean significant differences at P<0.05.

 $84.70^a \pm 1.36$

Guo (2016) found that up to 300 g/kg of pea and lentil starch can be used in Nile tilapia diets without impacting growth efficiency. Also, fish fed with lentil starch as a source of carbohydrates had relatively higher growth performance than those fed from other sources of starch, even though no significant difference was observed. Zhou and Yue (2010) and Mahboob (2014), stated that fish meal could be successfully replaced with other plant protein source in *O. niloticus* diet. Our findings were consistent with these results.

T5

20% Lentil seed

Contrary to our findings, some studies have reported a reduction in the growth output of fish fed diets when a fish meal was supplemented by alternative sources of protein other than soybean (Bullerwell et al., 2016; Anderson et al., 2018) maybe because alternative plant proteins have lower profiles of amino acids than the ingredients they substitute (Reigh, 2008). Kasiga and Brown (2019) were also found to decrease weight gain with increased carinata (Brassica carinata) meal replacement of fish meal. The final weight of the control group at the end of the Yürüten Özdemir and Yıldız (2019) experiment was higher than the other experimental fish groups when they substituted fish meal with red lentil meal (RLM) as an alternative source of plant protein in juvenile rainbow trout diets. The discrepancies between these findings can be linked to the source of protein, the quality and processing, the species and size of fish, the experimental time and the cultivation systems.

The efficiency of feed utilization is well known to be influenced by the content of dietary protein (Sotolu and Faturoti, 2011). Dietary protein intake and its transformation into fish weight gain are related to FCR and PER (Koumi et al., 2009). In the T5 (LS20) in this research, the lowest FCR and the highest PER were obtained and varied significantly (P<0.05) from those of all other groups. Relatively good

(lower) FCR, FER and PER were obtained by Luo et al. (2011) in the case of mitten crab Eriocheir sinensis fed a diet containing 7.5 % soybean meal and 7.5 % rapeseed meal, also Al-Thobaiti et al. (2018) found that replacing fish meal with a mixture of different plant protein meals can observe better feed consumption in Nile tilapia Oreochromis niloticus. Combined, these results suggest that the diet of plant protein caused related effects on feed parameters and nutrient utilization in common carp, mitten crab and Nile tilapia

 $55.04^{a} \pm 3.13$

The results of this finding are contradictory to those found in a recent study by Özdemir and Yıldız (2019), in which replacing fish meal with red lentil meal has no effect on nutritional indices in juvenile rainbow trout. Jiang et al. (2013) stated that PER and FER were steadily reduced and FCR increased by increasing the replacement of fish meal with cottonseed in Chinese mitten crab *Eriocheir sinensis*. Poor palatability, a deficit of one or more essential amino acids and a less apparent coefficient of digestibility has been recorded for plant-based diets, which may have impacts relating to reduced feed intake (Tusche et al., 2012; Gatlin et al., 2007; Bautista-Teruel et al., 2003).

Various and contrary effects have been published in the literature regarding the impact of plant protein sources on body composition in cultured fish. In this experiment, the chemical composition of the whole carcass was not affected by the different dietary treatments, which is in line with the findings of Salman and Alkhafaji (2020), who stated that there was no effect of lentils or phytase on the body composition of common carp. Furthermore, Acar and Türker (2018) noted that when a fish meal was substituted with a peanut meal, no significant differences were observed in terms of fillet proximate compositions of rainbow trout. In other fish species, similar findings have been shown when they were using sources of plant

protein, such as siberian sturgeon *Acipenser baerii* (Yun *et al.* 2014), large yellow croaker *Larimichthys crocea* (Zhang *et al.* 2016), red drum *Sciaenops ocellatus* (Minjarez-Osorio *et al.* 2016), grass carp *Ctenopharyngodon idella* (Köprücü and Sertel, 2012) and turbot *Psetta maxima* (Bonaldo *et al.* 2011).

Contradictory to the results of our, Cabral et al. (2013) found that up to 75% of fish meal replacement by plant protein sources can affect whole-body lipid in Senegalese sole. According to another study conducted by Yürüten Özdemir and Yıldız (2019), with a rise in red lentil meal RLM percentages in diets, crude protein amount of whole-body fillets steadily decreased.

There are several reports in the literature on the assessment of plant proteins as potential dietary feed ingredients for various fishes, but there are only a few reports on their impact on meat indices in fish. Abdulrahman *et al.* (2020) showed that there were no adverse effects on the muscle ratio of fish when adding *chlorella* microalgae and germinated barley powder to the diet of common carp, and this agrees with recent findings. However, Abdulrahman (2014) found an improvement in fish meat indices of *C. carpio* when replacing fishmeal with *Spirulina* spp. Ahmed *et al.* (2017) noted also that the use of date palm seed powder in the common carp diet had a negative effect on meat indices.

4. Conclusions

In this study, the best performance of common carp feeding on a feed prepared by replacing 20 percent of the fish meal diet with plant sources (lentil seed) was achieved in growth and feed utilization. It was concluded that the lentil seed can be used in common carp feed up to 20% replacing it with fish meal. In addition to achieving better growth output in *C. carpio*, the substitution of fish meal with local plant sources would also be advantageous in lowering production costs, thus increasing net profit.

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