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EFFECT OF RAW LENTIL SEED MEAL IN COMMON CARP *Cyprinus Carpio* L. DIETS AS AN ALTERNATIVE SOURCE OF FISH MEAL PROTEIN

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ABSTRACT

This experiment has been performed to assess the effect of partial substitution of fishmeal with lentil seeds in common carp growth performance, feed utilization and certain biological parameters (Fulton condition, Hepatic somatic index, Gills somatic index, Kidney somatic index, Spleen somatic index, Intestine Length index, Intestine weight index). Fish from a private fish farm were acquired in province of Sulaimaniyah, Iraq. Average fish weight ranged from 95-99 gm. Fish were divided into 5 treatment groups. The treatments were: T1: In fish diet 0% lentil seed was replaced with fishmeal, T2: In fish diet 5% lentil seed was replaced with fishmeal, T3: 10% lentil seed was replaced with fishmeal, T4: 15% lentil seed was replaced with fishmeal, T5: 20% lentil seed was replaced with fishmeal. There had been significant differences in weight gain, daily, relative, and specific growth rate as lentil seed replaced with fishmeal in fish diet. Feed Efficiency Ratio, Protein Efficiency Ratio and Fat Efficiency Ratio was significantly higher in T5 group than that of the other treated groups. No significant differences in the mean values of Hepatosomatic index, Spleensomatic index and Kidneysomatic index were detected. Significant difference in the mean value of Condition Factor was found among treatment groups. Intestine-length index was significant among treatment groups according to fish weight while mean value showed non-significant cording to fish length. The performance and general health of the common carp were significantly improved by raw lentil seed. Due to their low price in comparison to fishmeal and commercially available in all markets, it is suggested to substitute the fishmeal with raw lentil seed in commercial fish diet.

INTRODUCTION

With an average growth rate of 5.8 percent during the period 2001-2016, Aquaculture continues to develop more rapidly than the other major sectors of food production. Aquaculture produces 53 per cent of the fish for human consumption for the first time. In 2018, aquaculture accounted for 96.5 percent of the combined total of wild-collected and cultivated aquatic plants of 31.2 million tons(1).

The average annual increase in global fish intake since 1961 (3.2 percent) has outpaced population growth (1.6 percent) and exceeded that of all terrestrial animals' meat consumption, such as; combined (2.8 percent) and separately (bovine, ovine, pig, other), with the exception of poultry (4.9 percent). Consumption of food fish grew from 9.0 kg in 1961 to 20.2 kg in 2015, at an annual average rate of about 1.5 percent in 2016 and 2017, with estimated estimates suggesting additional increases of approximately 20.3 and 20.5 kg respectively1. The increase in consumption was motivated not only by increased production, but also by a combination of many other factors, including decreased waste, better usage, enhanced distribution networks and higher demand, related to population growth, higher incomes and urbanization(1).

Fisheries and fish products worldwide have an average of just about 34 calories per capita per day. However, the dietary contribution of fish is more significant than as an energy source, in terms of high quality, easily digested animal proteins and, in particular, in the battle against micronutrient deficiencies. A 150 g fish segment contains between 50-60 percent of the daily protein intake of an adult1.

The lentil (*Lens culinaris* Medik.) is a well-known lens-shaped grain legume that is a nutritious meal. It usually grows 20-45 cm tall as an annual bushy leguminous plant and develops many small purse-shaped pods, each containing 1-2 seeds, rich sources of protein, minerals (K, P, Fe, Zn), and dietary vitamins in humans are lentil seeds, the consumption of wheat or rice also provides a balance of essential amino acids for human nutrition due to its high content of lysine and tryptophan2. Lentil plays a significant role in improving the welfare of humans, livestock and soils, which has a special place in the systems of cereal crops. (3, 4).

The project aimed to examine the use of lentil seed powder in realistic diets for common carp production as a partial replacement for fishmeal protein, some biological parameters related to common carp health aspects. To find alternative sources of protein in the fish diet, particularly common carp as it is the main rearing fish in Iraq and Kurdistan, we used raw lentil as a source of protein as a substitute for fishmeal due to its high cost and low local market availability.

MATERIALS AND METHODS

Experimental Fishes: The experiment was performed from 7/9/2019 to 7/1/2020 at the Fish Disease Laboratory, College of Veterinary Medicine, University of Sulaimani, Iraq. This study employed a total of 165 common carps. The fish were bought from a private fish farm in Peramagrun, Sulaimaniya province, Iraq. Average weight of the fish varied from 95 to 99 gm. Approximately 27 days before the actual feeding experiment, the fish acclimatized in the laboratory and were fed commercial pellets (their chemical composition as shown in Table 1). The experiment lasted for eighty-four days.

Experimental design: Fifteen plastic tanks (water 70 L) were used in this trial. For continuous aeration, the Chinese air compressors (Hailea ACO-318) were used. The replicates were selected at random to reduce differences between treatment groups. During the siphoning process remaining feeds and feces were removed daily. The experiment used a completely randomized design consisting of five treatment groups and three replicates with eleven fish per replicate. Treatments were as follows:

T1 group: In fish diet 0% lentil seed was replaced with fishmeal,

T2 group: 5% lentil seed was replaced with fishmeal,

T3 group: 10% lentil seed was replaced with fishmeal,

T4 group: 15% lentil seed was replaced with fishmeal,

T5 group: 20% lentil seed was replaced with fishmeal.

Table 1: Structural formula of the various ingredients in the diet of fish according to (5).

Ingredients	T1 (100/0)	T2 (95/5)	T3 (90/10)	T4 (85/15)	T5 (80/20)
Soya	35	36	37	36	36
Corn	13	12	15	12	15
Barley	15	17	16	17	12
Wheat	20	18	15	18	20
Fish meal	15	14.3	13.5	12.8	12
Lentil	0	0.75	1.5	2.25	3
Vit + min	2	2	2	2	2
Total	100	100	100	100	100
Crude protein (%)	28.46	28.63	28.83	28.30	28.21
Crude fat (%)	3.29	3.30	3.38	3.30	3.30

Diet Formulation: Experimental diets contained traditional ingredients, enriched by lentil amounts, found in the Sulaimani city markets. The five different diets each contain the desired amount of lentils as defined in the experimental design. Kenwood Multi-processors processed the pellet and it was dried for 4 days at room temperature and then crushed to obtain fine particles. Feed was provided regularly with 2% of body weight twice a day at 9:00 a.m. and 2 p.m. Fishes were weight bimonthly at each tank. Then, the feeding levels were recalculated based on the new weights. The feeding trial was lasted for 12 weeks.

Growth and Feed Utilization Parameters: Fishes were weighed (gm) every 2 weeks for all replicates. The feed consumption was then re-adjusted for each replicate.

Weight gain and daily weight gain were calculated using the following equations:

Weight gain (gm/fish) = W2–W1

where W1: Fish weight (gm) at the beginning of the experimental period and W2: Fish weight (gm) at the end of the experimental period.

Daily weight gain (DWG) (gm/day) = Weight gain/ Experimental period, = W2–W1/ T

where T: time between W2 and W1 (84 days).

Relative growth rate was calculated according to the method described by (6).

Relative growth rate (RGR %) = Weight gain/Initial weight x 100 = W2–W1/ W1 x 100

Specific growth rate was calculated according to the method described by (7).

Specific growth rate (SGR) % = (Ln final body weight–Ln initial body weight)/ experimental period) x 100 = ((Ln W2–Ln W1)/ T) x 100

Feed conversion ratio was calculated as previously described by (8).

Feed conversion ratio (FCR) = Total feed fed (gm)/ Total wet weight gain (g) according to

Feed efficiency ratio was calculated as previously described by (7).

Feed efficiency ratio (FER) = Total weight gain (gm)/ Total feed fed (gm)

Protein efficiency ratio was calculated as previously described by (8)

Protein efficiency ratio (PER) = Total wet weight gain (gm/fish)/ Amount of protein fed (gm/fish).

Biological indices: Five fish were randomly selected from each tank at the end of the experimental period and anaesthetized with clove powder (9). Weight and length of the each fish were determined after that. Fish were dissected, and then liver, spleen, gills, viscera, kidney and intestine were weighed. The organs-somatic indices of fish were calculated as follows:

Fulton condition (K) factor = 100 (fish weight; gm) / (fish length; cm) .

Hepatic somatic index (HSI, %) = 100 (liver weight (gm) / fish weight (gm).

Gills somatic index (GSI, %) = 100 (gills weight (gm) / fish weight (gm).

Kidney somatic index (KSI, %) = 100 (kidney weight (gm) / fish weight (gm).

Spleen somatic index (SSI, %) = 100 (spleen weight (gm) / fish weight (gm).

Intestine Length index (ISI, %) = 100 (Intestine length (gm) / fish weight (gm).

Intestine weight index (IWI, %) = 100 (Intestine weight (gm) / fish weight (gm).

Statistical Analysis: All data generated were subjected to one-way analysis of variance (ANOVA), with the help of the General Linear Model procedure of XLSTAT, 2016 Version.02.28451. The Duncan multiple range test compared the variations between the means of treatment. Differences were considered significant at $P<0.05$.

RESULTS

Significant variations in weight gain, daily, relative and real growth rates were observed for fish meal replacement lentils in the fish diet, as shown in Table 2.

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

Treatments	Initial weight	Weight Gain	Daily growth rate	Relative growth rate	Specific growth rate
T1 Control	$98.472^{a} \pm 4.17$	$42.801^{b} \pm 0.65$	$3.567^{b} \pm 0.06$	$43.613^{b} \pm 1.28$	$212.595^{b} \pm 1.90$
T2 5% Lentil	$99.913^{a} \pm 3.66$	$50.480^{b} \pm 2.74$	$4.207^{b} \pm 0.23$	$50.849^{b} \pm 0.30$	$215.341^{ab} \pm 4.47$
T3 10% Lentil	$99.349^{a} \pm 1.25$	$43.288^{b} \pm 3.54$	$3.607^{b} \pm 0.30$	$43.497^{b} \pm 1.46$	$212.997^{b} \pm 3.02$
T4 15% Lentil	$98.679^{a} \pm 2.27$	$47.829^{b} \pm 2.05$	$3.986^{b} \pm 0.18$	$48.426^{b} \pm 1.28$	$214.174^{ab} \pm 1.01$
T5 20% Lentil	$98.968^{a} \pm 3.48$	$60.824^{a} \pm 3.12$	$5.069^{a} \pm 0.26$	$61.459^{a} \pm 1.65$	$217.918^{a} \pm 2.24$

At $P<0.05$, separate letters in one column reflect significant variations.

Feed Efficiency Ratio was significantly higher in T5 group, while, FCR was significant higher in the control group. In all treatment classes, no significant variations were found in protein and fat ratio, the mean values were significantly higher in the T5 group for PER and FER (Table 3).

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

Treatments	FCR	FER	Diet Protein	Protein Efficiency Ratio	Diet fat	Fat Efficiency Ratio
T1 Control	4.237[a]± 0.08	0.236[c] ±0.01	28.460[a] ±0.00	1.504[b] ±0.03	3.290[a] ±0.00	13.009[b] ±0.19
T2 5% Lentil	3.755[bc]±0.12	0.268[b] ±0.02	28.630[a] ±0.00	1.763[b] ±0.09	3.300[a] ±0.00	15.297[b] ±0.89
T3 10% Lentil	4.096[ab]±0.19	0.245[bc]±0.02	28.830[a] ±0.00	1.501[b] ±0.13	3.380[a] ±0.00	12.807[b] ±1.05
T4 15% Lentil	3.648[c] ±0.05	0.274[b] ±0.01	28.300[a] ±0.00	1.690[b] ±0.08	3.300[a]± 0.00	14.494[b] ±0.62
T5 20% Lentil	3.067[d] ±0.11	0.327[a] ±0.02	28.210[a] ±0.00	2.156[a] ±0.12	3.300[a] ±0.00	18.432[a] ±0.95

At $P<0.05$, separate letters in one column reflect significant variations.

Any of the biological parameters, such as the Hepatosomatic Index, Spleensomatic Index, Kidneysomatic Index and Gill-somatic Index, have demonstrated non-significant variations. (Table 4). The condition factor was greatly improved as compared to T4 in all treatments.

Table 4: Effect of five levels of lentil partial replacement with fishmeal on some biological parameters of *C. carpio*.

Treatments	Hepatosomatic Index	Spleensomatic index	Kidneysomatic index	Gillsomatic Index	Condition Factor
T1 Control	1.360[a] ± 0.04	0.191[a] ± 0.01	0.432[a] ± 0.013	2.952[a]± 0.20	1.535[ab]± 0.06
T2 5% Lentil	1.541[a] ± 0.23	0.143[a] ± 0.007	0.372[a] ± 0.063	2.650[a]± 0.22	1.500[ab]± 0.06
T3 10% Lentil	1.839[a] ± 0.21	0.144[a] ± 0.015	0.368[a] ± 0.015	3.202[a]± 0.23	1.466[ab]± 0.02
T4 15% Lentil	1.822[a] ± 0.19	0.160[a] ± 0.008	0.416[a] ± 0.03	3.222[a]± 0.092	1.425[b] ± 0.09
T5 20% Lentil	1.431[a] ± 0.14	0.155[a] ± 0.017	0.427[a] ± 0.048	3.295[a]± 0.33	1.638[a] ± 0.07

At P<0.05, separate letters in one column reflect significant variations.

Significant differences were observed in the mean value of the Intestine-Length Index (depending on the weight of the fish) between treatment groups as shown in Table 5, while non-significant differences were observed in the Intestine-Length index (depending on the length of the fish), but the mean value of the T4 group was numerically higher than that of the others suggesting that the intestine was impaired by lentil feeding. Also, non-significant differences in the mean values of the Intestine Weight Index were observed.

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

Treatments	Intestine Length Index	Intestine Length index	Intestine Weight

	(According to fish weight)	(According to fish length)	Index
T1 Control	$19.085^{ab} \pm 0.51$	$148.164^{a} \pm 0.98$	$2.945^{a} \pm 0.08$
T2 5% Lentil	$15.908^{b} \pm 0.31$	$127.813^{a} \pm 0.618$	$3.168^{a} \pm 0.31$
T3 10% Lentil	$19.514^{ab} \pm 0.56$	$138.494^{a} \pm 0.56$	$2.870^{a} \pm 0.13$
T4 15% Lentil	$22.951^{a} \pm 2.51$	$159.242^{a} \pm 0.14$	$3.221^{a} \pm 0.22$
T5 20% Lentil	$19.934^{ab} \pm 2.09$	$165.580^{a} \pm 0.29$	$3.330^{a} \pm 0.75$

At $P<0.05$, separate letters in one column reflect significant variations.

DISCUSSION

The feasibility of substituting fish meal with protein from plant source differs greatly many of fish species. In rainbow trout, complete soy protein concentrate replacement(10) or combinations of different sources of protein(11) there were no detrimental impacts on growth and feed utilization, and in popular carp diets, fish meal replacement with commercial dry yeast and spirulina (12-14). At the other hand, earlier studies of Mediterranean fish such as European Sea Bass15 and Goldhead Seabream16 found that only 20-30 percent of the substitution of fish meals was feasible.

Partial or complete substitution of fish meal with a mixture of protein from plant sources for a 12-week development trial in juvenile dorado sea bream (corn gluten meal, wheat gluten, extruded peas, rapeseed meal) supplemented with indispensable amino acids (IAA) was investigated (*Sparus aurata*). A fish meal diet was compared to 50%, 75%, and 100% alternative diets as the primary source of protein. Protein retention was improved with more plant protein supply, in fish diets fed with PP50 and PP75, only a small reduction in the final weight gain was observed. However, weight gain in the PP100 category was decreased by up to 30 percent largely due to a substantial drop in feed consumption.

As an alternative plant protein source, the effects on the growth production and amino acid composition of fish were analyzed by replacing fish meal with red lentil meal in juvenile rainbow trout diets. The maximum mean of weight gain was (30.55±0.08 g) of fish in the control group at the end of the 60-day feeding trial (15). The crude level of whole-body / filet protein gradually decreased with an increase in dietary replacement percentages. The results of this analysis revealed that replacement can be combined with 15 percent of the dietary fish meal in juvenile rainbow trout diets without any detrimental effects on growth quality and composition of body amino acids.(16) and The outcomes of the present analysis have been accepted with it. The present study investigated the effects on growth efficiency, feed utilization rate, and body composition of alternative plant protein sources (lentil seeds) from dietary fish meal substitutions. Several other studies have tested the use of sources of plant protein in rainbow trout diets (17, 18, 19-22). However, knowledge is non-existent about the dietary replacement of fish meal by replacement, except the study of(23).

Numerous studies have shown that the use of soybean meal as a supplement for dietary fish meal does not have a detrimental effect on the development of the development of cultivated fish(24). Some research, however, A decrease in fish diet growth production was recorded when fish meal was supplemented by alternate sources of protein compared to soy meal replacement. (25-27). The weight gain decreased when replacing fish meal with carinata (*Brassica carinata*) meal 28. Similar findings were recorded in juvenile rainbow trout by (28-30) when the dietary fish meal was replaced with pods and cotton seeds respectively. Consumption of feed has been reported to decrease with increasing inclusion of rainbow trout diets from plant protein sources (31-32).

An experiment of 12 experiments to substitute animal protein concentrate in typical carp (*Cyprinus carpio* L) diets with the result of different rates of commercial dry yeast *Saccharomyces cerevisiae*, and determines their effect on growth efficiency as well as performance of feed use (feed conversion ratio, feed conversion efficiency) and feed utilization efficiency (protein efficiency ratio and protein intake) and the survival rate of fish. The findings showed that the mean value of the superiority of the T3 group ($P < 0.05$) was contrasted with the mean value of the final weight of the T1 group and the other treatment group ($P < 0.05$), daily weight gain, total weight gain, food conversion efficiency, feed conversion ratio and protein efficiency ratio. In all experimental treatment groups, the survival rate was not affected.

The pea and lentil starch can be used in diets of Nile tilapia up to 300 gm / kg without impacting carp growth efficiency. Furthermore, as a carbohydrate source, fish fed lentil starch had relatively higher growth performance than those fed the other starch sources, although non-significant difference was found(33).

(34) tested five different levels of *Spirulina* spp. for their influence. Algae; *Spirulina* spp. 0 percent (Group T2) in the control T1 group, replacing fishmeal with 5% *Spirulina* spp. (T3 group) replacing fishmeal with 10% *Spirulina spp.,* (T4 group) replacing fishmeal with 15% *Spirulina spp.*, and (T5 group) replacing fishmeal with 20% *Spirulina spp.* The findings in the third treatment group were slightly higher in weight gain, daily growth rate, specific growth rate and relative growth rate than in the other treatment groups. Non-significant ($P<0.05$) variations were observed with respect to the food conversion ratio, while the food efficiency ratio in the third treatment group varied substantially with respect to the protein efficiency ratio in the second treatment group

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