EFFECT OF *Chlorella* MICROALGAE AND GERMINATED BARLEY POWDER ON PERFORMANCE, SOME HEALTH INDICES, AND MEAT HYGIENE PARAMETERS OF COMMON CARP (*Cyprinus carpio*)

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ABSTRACT

This experiment was done to assess the effect of *Chlorella*, as a source of protein, and germinated barley powder, as a source of natural prebiotic, on the performance of common carp. The experiment was conducted by using one-way ANOVA (analysis of variance) with a completely randomized design (CRD). Higher significantly in T2 with 5% *Chlorella* and T5 (20% germinated barley). FCR recorded significant differences among treatment as compared to T5 (20% germinated barley), and the opposite was observed in FER. T5 with 20% germinated barley was differing significantly in each of the spleen somatic and kidney somatic indices. Adding germinated barley leads to enhanced intestine weight index in T4 and T5. The parameters were hepatosomatic and gill somatic indices, condition factor, fish weight without viscera, fish weight without viscera and head, lipids, ash and moisture, organoleptic evaluation of mean juiciness, flavor, color, and complete acceptance. We conclude that the use of *Chlorella* and germinated barley potentially enhances the growth performance and fish meat quality. We recommend using both plants in earthen ponds to rely on using natural products in fish feeding.

INTRODUCTION

The fastest rising food-production technology in the world is aquaculture (1,2) stated that Fish and other aquatic products rich in protein, essential fatty acids, vitamins, and minerals play an significant role in international efforts to reduce malnutrition and hunger. (3,4) reported that the microalgae have an excellent nutrient composition and are free of poisons that could pose a threat through transferring up the food chain. In fish farming, the chief microalgae utilization is associated with their use for feed purposes (4).

According to (4,5) around 30 per cent of the world's algae production can be used for feeding stuffs. However, (4,6) demonstrated that microalgae are primarily utilized for larval fish, crustaceans, and mollusks.

When *Chlorella* is cultivated under favorable environmental conditions, the biomass of *Chlorella* might comprise 12–15% lipids and 10–15% carbohydrates. The C16 and C18 fatty acid groups such as C16:0, C16:2, C18:1, C18:2, and C18:3 are the main lipids found in *Chlorella,*Moreover, chlorophyll, in conjunction with a variety of carotenoids such as *β*-carotene, neoxanthin, violaxanthin, lutein, zeaxanthin, and antheraxanthin are also included in *Chlorella*. The amplest carotenoid in *Chlorella* cells is lutein, which may amass around 0.45% of the dry weight of cells (7).

Prebiotics can reduce the risk of some illnesses and improve health. Comprehending the connection between diet and consumer health has increased demand for real knowledge on prebiotics in recent years. Prebiotics has become a rising sector on the world market, boosted by technical advances, new merchandise production and the increasing number of health-conscious consumers (8).

Prebiotics have a long history of safe use and are proven to support human health, including increased mineral bioavailability, immune system control, gastrointestinal (GI) infection prevention, inflammatory conditions, metabolic disorder control and cancer risk reduction(8) Prebiotics are indigestible substances which allow specific changes in the composition or activity of gastrointestinal microbiota or both, which have a positive effect on the nutrition and health status of the host (9) Prebiotics play an important role in host health when developed by beneficial microbiota in the intestine (10,11).

Barley is widely used in both existing and new end-use applications. Much of the barley produced is fed to animals in most countries, in particular cattle and pigs. There is minimal use of the barley for human food. Recent developments in the use of varieties of barley, high in dietary fiber, have however been established. The successful use of high-value products from barley is to manufacture malt as a raw material for the ferm (6). (12) have reported a germinated barley stock rich in arabinoxylan to facilitate the development of bifidobacteria in humans intestines. However, there is still evidence of a consistent clinical advantage of prebiotics in the treatment of irritable bowel disease (IBD) in extensive, placebo-controlled studies (13).

This research aims to compare the utilization of *Chlorella*, a natural source of animal protein, and germinated barley powder, a source of prebiotic, as supplements to commercial feed for common carp.

MATERIALS AND METHODS

Experimental fish: The study lasted 84 days on 90 common carp brought from Peramagrun/Sulaimani/Iraq. Fish weights averaged 53.12 ± 3.34 g. Fish spread in experimental plastic containers, pre-acclimatization laboratory and fed with industrial pellets 30 days before the real feeding trials. Compositions of feed are illustrated in Table 1, and the feeding amount was 3.0% of body weight.

Table 1: Composition of experimental diet

Ingredients	Percentage (%)
Yellow corn	15 %
Wheat bran	15 %
Soya bean meal 48%	35 %
Animal concentrate protein	20 %
Barley	15 %
Total	100
Calculated chemical composition	
Crud protein	28.06
Gross energy (kcal/kg feed)	2242.7

Plastic tanks with a capacity of 70.0 L were used in this trial. Proper continuous aeration by means of air compressors added to each tank (Hailea ACO-318, 45.0 watts power, 70.0 L/min airflow). Each replicate was stocked with six fish. The experimental trial represented five treatments, assigned T1 through T5, with three replicates and six fish per replicate. Group T1 was given a standard diet and served as the control. Groups T2 and T3 fed with 5 g/kg and 10 g/kg *Chlorella*, respectively. Groups T4 and T5 were given a diet supplemented with 10 g/kg and 20 g/kg germinated barley, respectively. Experimental diets included typical ingredients found in the city markets of Sulaimani, enriched with Chlorella, and powdered barley.

Growth and feed utilization parameters: All fish were weighed bimonthly. The feed consumption per replicate was modified every two weeks by the biomass obtained. Weight gained by fish (g/fish) was calculated as fish weight (g) at the end of the experiment divided by fish weight (g) at the beginning of the experiment. Daily weight gain of fish (g/day) was calculated as fish weight gain divided by the duration of the experiment (84 days). The relative growth rate (RGR %) was estimated as weight gain/initial weight × 100. Moreover, the specific growth rate (SGR) was computed according to the formula [(ln final fish weight – Ln initial fish weight)/duration of the experiment] × 100 (14).

Feed conversion ratio (FCR) was estimated by dividing the total ingested feed (g) by the total wet weight gain (g),Feed efficiency ratio (FER) was calculated by dividing total weight gain (g) by total ingested feed (g), Further, the protein efficiency ratio (PER) was computed as total wet weight gain (g/fish)/amount of protein fed (g/fish) (15).

Fish weight index% was estimated by dividing fish weight without viscera (g) by fish weight (g) and multiplying the result by 100. Finally, meat weight index% was calculated as fish weight without viscera and head (g) / fish weight (g) × 100.

Proximate composition: All parts of fish were used to analyze the muscles chemically, including percentages of moisture, protein, ether extract, and ash contents (16).

Moisture was measured by putting fish samples in an oven at 105 °C for 24 hours. Then, the samples were weighed and put again in the oven for two hours and weighed till a stable weight obtained. Fat extraction was made by Soxhlet using organic hexane CH_3 $(CH_2)_4CH_3$ by heating for 16 hours to determine the crude fat content.

The crude protein content of fish determined by Micro-Kjeldahl equipment, which was done by measuring the total nitrogen content in the samples after digestion with H_2SO_4 and Perchloric acid ($HCIO_4$). Later, one part of each of potassium sulfate, copper sulfate, and titanium dioxide, were added and the mixer was left for two hours. Distillation was conducted by boric acid and titration with HCl, and the number obtained was multiplied by 6.25 for nitrogen extraction of each sample. The percentage of ash content was calculated by burning the samples in a muffle furnace at ٥٩٥ °C, till a stable weight was obtained, and weighing the remainder (16).

Sensory evaluation: Seven experienced evaluators tasted the fish fillets and each member filled the sensory evaluation Table as 5 = extremely like; 4 = like; 3 = neither like nor dislike; 2 = dislike; 1 = extremely dislike. The fish fillet specimens were put in aluminum containers and cooked for 15 min in a preheated oven at 200°C.

Statistical analysis: a completely randomized design (CRD) was used, and a comparison between the groups was made using a one-way analysis of variance (ANOVA), followed by Duncan's post hoc. A probability level lower than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Table (2) explain the effect of adding *Chlorella* and germinated barley powder on the performance of common carp, which was significantly higher in T2, with 5% *Chlorella*, and T5, 20% germinated barley. There were no variations in the overall consumed diet in all treatments. FCR recorded significant differences among treatments as compared to T5, and the opposite was noticed in FER.

Table 2: Effect of microalgae *Chlorella* and germinated barely powder on common carp *Cyprinus carpio* performance after a rearing experiment for 84 days

Treatments	Initial Wt.	Final wt.	Wt. Gain	DGR	% SGR	RWG	FCR	FER
T1 Control	55.75 a	60.45 c	4.699 c	0.098 c	174.485 b	7.712 d	7.954 a	0.159 b
T2 5 % *Chlorella*	53.25 a	71.46 a	18.210 a	0.379 a	181.791 a	25.422 a	2.247 b	0.451 a
T3 10 % *Chlorella*	53.49 a	60.16 c	6.671 c	0.139 c	174.303 b	10.994 cd	6.471 ab	0.169 b
T4 20 % germinated barely	54.17 a	64.71 b	10.536 bc	0.220 bc	177.469 ab	16.224 bc	3.690 ab	0.278 ab
T5 20 % germinated barely	55.53 a	71.55 a	16.015 ab	0.334 ab	181.666 a	21.880 ab	2.786 b	0.465 a
Pr > F	0.002	0.052	0.004	0.004	0.007	0.002	0.055	0.048

The addition of germinated barley to fish feed has led to increased weight index of the intestine in T4 and T5, as recorded in Table (3). The results of the fish condition factor did not show significant differences.

Table 3: Effect of microalgae *Chlorella* and germinated barely powder on common carp *Cyprinus carpio* biological indices after a rearing experiment for 84 days

Treatments	Intestine weight index	Intestine length index	Fish Weight without Viscera	Fish Weight without Viscera & Head	Condition factor
T1 Control	2.193 ab	43.764 a	85.842 a	55.382 a	1.443 a
T2 5 % *Chlorella*	1.863 b	31.705 c	84.355 a	55.814 a	1.489 a
T3 10 % *Chlorella*	2.228 ab	41.491 ab	83.681 a	54.320 a	1.444 a
T4 20 % germinated barely	2.517 a	40.318 ab	83.469 a	53.867 a	1.530 a
T5 20 % germinated barely	2.514 a	33.057 bc	82.258 a	55.044 a	1.600 a
Pr > F	0.158	0.016	0.310	0.934	0.258

No significant differences were seen in each of the fish's weight without viscera and fish’s weight without viscera and head. Group T3, which administered 10 g/kg *Chlorella,* had more protein, while no significant differences were seen in the percentages of lipids, ash, and moisture (Table 4).

Table 4: Effect of microalgae *Chlorella* and germinated barely powder on common carp *Cyprinus carpio* proximate analyses after a rearing experiment for 84 days

Treatments	Protein %	Lipids %	Ash %	Moisture %
T1 Control	19.312	6.351	1.443	71.434
T2 5 % *Chlorella*	19.954	6.388	1.764	71.642
T3 10 % *Chlorella*	20.043	6.569	1.783	71.876
T4 20 % germinated barely	19.573	6.669	1.891	71.998
T5 20 % germinated barely	19.826	6.760	1.921	72.032
Pr > F	0.004	0.004	0.002	0.007

No significant differences were observed in the organoleptic evaluation of juiciness, flavor, color, and complete acceptance (Table 5).

Table 5: Effect of microalgae *Chlorella* and germinated barely powder on common carp *Cyprinus carpio* organoleptic evaluation after a rearing experiment for 84 days

Treatments	Juiciness	Flavor	Color	Complete acceptable
T1 Control	4.000 a	3.876a	4.600 a	3.780 a
T2 5 % *Chlorella*	4.000 a	4.544 a	4.560 a	3.980 a
T3 10 % *Chlorella*	3.855 a	3.766 a	3.955 a	3.655 a
T4 20 % germinated barely	3.766 a	3.765 a	3.860 a	3.455 a
T5 20 % germinated barely	3.456 a	3.555 a	3.650 a	3.655 a

Plant sources of proteins are considered a better replacement of fishmeal in aquafeed but are usually missing amino and fatty acids. The microalga is rich in amino acids including methionine, lysine and alanine, *C. vulgaris* is a perfect source of protein in catfish that may replace fishmeal in catfish diets, and *C. vulgaris* meal was entirely agreeable as a feed item and ensured that fish can utilize it well. Diet approval with high SGR lowers the cost of aquafeeds as the planting of the algae is cheap, and it can be cost-saving compared to fishmeal (17). Algae may be a potential source of protein in a number of fish species, for instance when the Spirulina algae replaced fishmeal (18).

(19) observed that adding algae in fish diets enhances growth. The supplementation of a high level of *C. vulgaris* (25%) may lead to a preferable growth rate of *Clarias gariepinus* as compared to fishmeal, and 10.0% *Spirulina* had a potential increase in most studied traits (20).

(21) observed that 50% addition of *Chlorella* to *Macrobrachium rosenbergii* enhances SGR, weight gain, survival rate, and feed efficiency ratio. (22) concluded that mixing of *Chlorella* with rapeseed powder could fully replace fishmeal in diets of crussian carp. *Chlorella* is rich in amino acids like alanine, leucine, aspartic acid, serine, and glycine, and these may be responsible for more than 50% of the total content of the *Chlorella* spp. (23).

As PCR increased, the FCR reduced with the addition of algal meal. This outcome may be due to the collective effects of both amino and fatty acids on the nutrient digestibility. (19) observed that adding algae meal improved FCR, while (20) noted that FCR was lower when the fish had been fed on artificial diets with various algal meal rates. (24) concluded that feeding on a diet supplemented with 20% *S. platensis* enhanced performance, proximate analyses, and body weight gain of Nile tilapia. A linear weight enhancement was noted when *Chlorella* was added, which may be related to lipids enhanced in fish. Moreover, the diets in the present study had high addition levels of carbohydrate, well utilized by carp, but they converted them to fat and stored in the fish.

Using algae in the diet of *C. gariepinus* improved the utilization of carbohydrates. The addition of carbohydrate in the diets of catfish usually leads to an increased HIS, and this had been associated with high carbohydrate diets (20, 25). These findings agree with the results of (22), who noted that *Chlorella* and rapeseed meal could completely replace fishmeal. The reason may be organoleptic as the fish were grazing on the algae on their own before the experiment. It is a normal behavior in fish to graze on algae while carnivores may reject the algae powder inclusion.

The addition of algae, especially *Chlorella*, may be nutritionally favorable, leading to an increased total content of fats, fatty acids, and polyunsaturated fatty acids (PUFAs) in groups of fish fed the green algae. In some length / weight and nutritional values, the inclusion of 10 percent algal powder in the complete carp diet formulas showed significant variances. Adding toxic cyanobacteria reported an impairment of the fish's physiological state. Most of the monitored values were better in the green algae groups compared to the control fish group. Algae from a sewage water treatment lagoon were used as an additive to fish feed to increase the levels of polyunsaturated fatty acids (PUFAs) of lipids in fish (26).

(27) showed that the average weight of carcass, crude protein, fat, and ash content were significantly higher ($P < 0.05$) when fishmeal was supplemented with 20.0% *Spirulina* in the diet of common carp. The results of (28) indicated that a significant increase ($P < 0.05$) was observed in total weight gain, daily weight gain, relative growth rate, and specific growth rate after the supplementation of feed with 7.5 g/kg. The significant differences ($P < 0.05$) were an elevated feed conversion ratio, feed efficiency ratio, and protein efficiency ratio.

The addition of 5.0 g/kg germinated barley to aquafeed caused a significant increase ($P < 0.05$) in growth performance and some blood parameters (29). However, hydroponic germination (5.0 g) and germinated barley (2.5 g) significantly increased on protein efficiency ratio and the intestine length index, which is following the present results.

The results in the present study agree with those reported by (30). When the diet was supplemented with 5.0 g/kg *Spirulina* powder to feed common carp (*C. carpio*) fingerlings for 42 days, the results showed increased weight gain, and the daily, relative, and specific growth rates. Furthermore, the feed efficiency ratio was significantly higher with adding 5.0 g and 3.0 g *Spirulina* powder, compared to the control group. The food conversion ratio in the control group was significantly higher than other treatments.

The study of (31) showed that feeding algae as a feed additive to fish remarkably increased the fish weight without viscera, and weight without viscera and head, and all these agree with the recent results.

Adding 7.5 g/kg *Chlorella* powder in the common carp fingerlings' diet resulted in a better weight gain (35.1 g) and daily growth rate (0.4 g) than other treatments. Moreover, the relative growth rate (88.9) and specific growth rate (0.4) were higher than other treatments (32). The food conversion ratio (0.6) was significantly higher when the diet was supplemented with 2.5g/kg *Chlorella* powder to common carp fingerlings' feed for 105 days. The food efficiency ratio (2.4) and protein efficiency ratio (829.3) in the group fed 7.5 g/kg diet of *Chlorella* spp. was significantly higher than other treatments.The inclusion of *Chlorella* and *Daphnia* to fish diet in the study of (33) changed the growth performance in which it increased the daily and final weight gain, and specific and relative growth rates. Utilization of *Chlorella* and germinated barley caused a reduction in feed conversion ratio, which has a substantial role in determining the cost of aquaculture. Dietary *Chlorella* and germinated barley escalated the feed efficiency ratio and reduced feed conversion ratio. Following the findings of chemical composition, we conclude that a high proportion of *Chlorella* and germinated barley significantly affects the protein and fat ratios of fish.

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