LENTIL GERMINATION AS A SOURCE OF PREBIOTIC TO STUDY THEIR EFFECTS IN COMMON CARP PERFORMANCE, PHYSIOBIOLOGICAL, BLOOD, HEALTH AND PROXIMATE ANALYSIS

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ABSTRACT

The experiment was conducted at the University of Duhok's College of Agricultural Sciences. The study used a completely randomized design with four treatment groups, three repetitions, and ten fish per replication in twelve plastic tanks (70 L), each tank receiving proper continuous aeration and cleaning on a daily basis. Positive Control (T2) was higher significantly in each of the Weight Gain, Daily Weight Gain, Relative Growth Rate, Feed Efficiency Ratio, Protein Efficiency Ratio, Fat Efficiency Ratio, Hepatic Index, Kidney Index, Lymphocytes (10^9/dl), Monocytes (10^9/dl), and Granulocytes (10^9/dl). T3 with 25 gm germinated lentil /kg was higher significantly in Intestine Length- Weight Index and MCHC (%). T4 with 50 gm germinated lentil /kg increase significantly Intestine Length- Length Index, WBC (10^9/dl), PLT (10^ 9/L), Cholesterol mg/dl, and LDL mg/dl. Negative Control (T1) and 25 gm germinated lentil /kg (T3) were higher significantly in Feed Conversion Ratio, Triglyceride mg/dl, and HDL mg/dl. T2 and T3 increased significantly RBC (10^ 12/L). All treatments as compared to control enhances Ether extract (Oil) (%), Crude protein (%), Weight without viscera and Head, and Spleen Index.

Keywords: germinated lentil, common carp, performance, physiobiological, blood, health, proximate composition

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استنبات العدس كمصدر للسابق الحيوى ودراسة تاثيره فى اداء نمو سمكة الكارب العادى وبعض الصفات الفيسيوبايولوجى و الدمية و الصحة و التحليل الكيميائى

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المستخلص

اجريت الدراسة الحالية فى كلية هندسة العلوم الزراعية، جامعة دهوك، دهوك، العراق. تم استخدام تصميم العشوائى الكامل لاربعة معاملات/ ثلاث مكررات/ عشرة اسماك لكل مكرر فى 15 حوض بلاستيكى سعة 70 لتر وجهز كل حوض بتصميم تهوية مع تنظيم يومى. كانت معاملة السيطرة الايجابية (المعاملة الثانية) اعلى معنويا فى كل من الزيادة الوزنية و الزيادة الوزنية و معدل النمو النوعى و كفاءة تحويل الغذاء و كفاءة تحويل البروتين و الدهن و دليلى الكبد و الكلية و وتعداد كريات الدم اللمفاوية والوحيدة و الحبيبية. كانت المعاملة الثالثة بنسبة اضافة 25 غم من العدس المستنبت اعلى معنويا فى دليل طول الامعاء نسبة الى الوزن و نسبة MCHC. ان المعاملة الرابعة بنسبة اضافة 50 غم من العدس المستنبت زادت معنويا طول الامعاء نسبة الى الى الطول وتعداد كريات الدم البيض والصفيحات الدمية والكولسترول و LDL. كانت معاملة السيطرة مع المعاملة الثالثة بنسبة اضافة 25 غم من العدس المستنبت اعلى معنويا فى معامل التحويل الغذائى و الكلسريدات الثلاثية وHDL. زادت معنويا معاملة السيطرة الايجابية (المعاملة الثانية) والمعاملة الثالثة بنسبة اضافة 25 غم من العدس المستنبت تعداد كريات الدم الحمر. اختلفت جميع المعاملات بالمقارنة مع معاملة السيطرة السلبية فى نسبة مستخلص الايثر والبروتين ووزن الجسم بدون الاحشاء والراس ودليل الطحال. ارتفعت معنويا جميع المعاملات مقارنة بالمعاملة الثالثة فى وزن الجسم بدون الاحشاء. لم يلاحظ فروقات معنوية فى كل من معدل النمو النسبى ومعامل الحالة ودليل وزن الامعاء وكمية الهيموغلوبين ونسبة .PCV

الكلمات المفتاحية: مستنبت العدس، الكارب العادى، الفيسيوبايولوجى، الدم، الصحة، التحليل الكيميائى

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INTRODUCTION

Annually predicting available quantities for fish consumption was depending for 2012 – 2022 in Iraq. The rates of available quantities growth for fish consumption per capita decreased depending on the annual data of 1990 –2012, and were compatible with predicted quantities which indicated the decreasing but slowly. To keep on the current levels of fish consumption. It must be working on increasing the development of these sections of 1 1.6%, to face the predicted negative development rate of -1.6% (3).Because of the growing interest in the health benefits connected with antioxidants, legumes, a staple food in many parts of the world, have recently been researched for their antioxidant properties. Changes in antioxidant activity during processes like germination, on the other hand, have piqued researchers' curiosity (15). Antioxidants found in the diet may help protect cells from free radical damage. Antioxidant-rich foods may help to prevent various diseases, hence determining their antioxidant capacity is crucial for estimating the impact on oxidative stress in living organisms (14). Preparation procedures such as germination have been devised to dramatically increase the bioavailability of legume components, with the goal of boosting their nutritional value (23). When the dried seed begins to absorb water, germination occurs, and the embryonic axis elongates, it is complete. The seed's stores in the storage tissues are activated at this phase to assist seedling growth. Protective reactions arise from the moment the seed breaks dormancy, thanks to the synthesis of phenolics and other chemicals. Preparation procedures such as germination have been devised to dramatically increase the bioavailability of legume components, with the goal of boosting their nutritional value. Also, it has been reported that the increases in phenolic content on a dry base from dormant seed to 7 days sprout for lentil was about 185% (16). Because the germination process alters phenolic chemicals and their antioxidant activity, lentil sprout flour or extract can be employed as a natural source of antioxidants in functional foods (16). On a dry basis, lentils contain 60%–67% carbohydrate, 20%–36% protein, 4% fat, and 2%–3% ash, earning it the nickname "poor man's meat." (22). The addition of garlic powder has led to an improvement in blood and biochemical characteristics, but the addition of garlic has had some negative effects on the histological characteristics of the intestine and liver, possibly as a result of the long-term feeding of fish to these proportions of garlic (20). These results of using Synbiotic (combination of probiotic and prebiotic) as feed additive can be considered as a beneficial dietary for improving the growth rate and immune response in common carp (10). Abdulrahman et al., (7) the partial replacement of the fishmeal in diets of *C. carpio* by crude lentil seeds was safe at the 5% and 10% levels as indicated by various levels of adverse histopathological effects on the kidneys, liver, gills, and intestine. The purpose of this research was to evaluate the effect of feed containing various ratios of germination lentil as a natural source of prebiotic on the growth of common carp and to evaluate their effect on growth performance, blood and immunity indices, health and biological parameters, and proximate composition in common carp.

MATERIALS AND METHODS

Lentil (*L. culinaris*) seeds were obtained from local market in Duhok city. Seeds were sorted, cleaned and stored in darkness in polyethylene containers at 4°C. Adding about 5 liters of warm water with antifungal in a plastic bucket with seeds for 4 hrs. it helps minimize fungus production on the sprouted seed. Washing seeds with clean water in order to remove antifungal residues. seeds soak in water for about 12 hours. Drain the water and then wash the seeds with clean water. Transfer seeds from gunny bags to trays and evenly spread them. Every day gives 4 times spray the sprouted seeds with clean water. During the 5 days of germination, the sprouted seeds were removed and dry by sun light then grind and used for further as fish feed additive. The experiment carried out at the Fish Laboratory, College of Agricultural engineering sciences, University of Duhok, Iraq. A total of 240 common carp C. carpio L. used in this study. Fish were purchased from a commercial fish farm. Average fish weight ranged from 25-35 gm. The fish acclimatized in the laboratory conditions at about 27 days before the actual

feeding experiment and feed with commercial pellets (their chemical composition shown in Table 1). The experiment lasted for 10 weeks.

Table 1. Dietary ingredients and chemical composition of fish diets Calculated according to NRC (21).

Ingredients	Control diet
Soybean meal	35
Yellow Corn	13
Barley	15
Wheat	20
Fish meal	15
Vitamin + Mineral mix.	2
Total	100
Crude protein (%)	28.46
Crude fat (%)	2.1
Dry Matter	87.4
Crude Fiber %	4.8

Experimental System: Twenty-four plastic tanks (70 L) used in this trial representing eight treatments with three replicates each. Each tank provided with a proper continuous aeration by Chinese's air compressors, Hailea ACO-318 and eleven small aquarium air pumps, Luckiness 828 (power: 5-watt, air flow: 3.5L /min). Each tank stocked with ten fish. The tanks (replicates) randomly allocated to minimize differences among treatments. The continuous water flow discharged non-consumed feed and feces particles from the tank. In addition, a daily cleaning by pumping method applied to remove remained particles from the system.Experimental Design: The experiment used a completely randomized design consisting of eight treatment groups and three replicates with ten fish per replicate. Treatments were as follows:

Negative Control (T1) group: without any addition,

Positive Control (T2) group: Adding Inulin as a source of prebiotic,

T3 group: adding 25 gm germinated lentil /kg,

T4 group: adding 50 gm germinated lentil /kg,

The parameters of the study involve four main fields: growth performance, biological parameters, blood parameters and microbial load in intestine and muscles.

Diet Formulation: Experimental diets contained standard ingredients found in the city markets of Duhok, enriched with the used seed germinated levels. Preparing eight different diets each contain the desired seeds germinated level. Kenwood Multi-processors processed the pellet and dried at room temperature for 4 days, and then crushed to obtain fine particles. In the first week the feeding provided regularly with 3% 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 recalculated based on the new weights. The feeding trial was lasted for 10 weeks.

Growth and feed utilization parameters: For calculating these parameters, the fish weigh every 2 weeks for all replicates. Feed consumption of each treatment recorded and readjusted according to the obtained biomass at every two weeks.

- Weight gain and daily weight gain will be calculated using the following equations:

1- Weight gain (gm. /fish) = W2–W1

Where W_1: Fish weight (gm.) at the beginning of the experimental period and W_2: Fish weight (gm) at the end of the experimental period.

2- Daily weight gain (DWG) (gm. /day) = Weight gain/ Experimental period, = $W2–W_1$/ T

Where T: time between W2 and W1 (70 days).

- Relative growth rate was calculated according to the method described by Brown, (12) as follows:

Relative growth rate (RGR %) = Weight gain/Initial weight x 100 = $W2–W_1$/ W_1 x 100

- Specific growth rate was calculated according to the method described by Uten, (24) as follows:

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 will be calculated as follows:

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

- Feed efficiency ratio was calculated as previously described by Uten (24) as follows:

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

- Protein efficiency ratio was calculated as follows:

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

Biological indices: Five fish randomly be selected from each tank at the end of the experimental period and anaesthetized with clove powder. Weight and length of each fish

determined and after that, the fish dissected and the liver, spleen, gills, viscera, kidney, and intestine weighed. The organs-somatic indices of fish calculated as follows:

- Fulton condition factor (K) = 100 (fish weight; gm.) / (fish length; cm) ^{3}. (18)
- Hepatic somatic index (HSI, %) = 100 (liver weight (gm.) / fish weight (gm.). (18)
- Gills somatic index (GSI, %) = 100 (gills weight (gm.) / fish weight (gm.) (6)
- Kidney somatic index (KSI, %) = 100 (kidney weight (gm.) / fish weight (gm.) (6)
- Spleen somatic index (SSI, %) = 100 (spleen weight (gm.) / fish weight (gm.) (6)
- Intestine Length index (ISI, %) = 100 (Intestine length (cm.) / fish weight (gm.) (6)
- Intestine Length index (ISI, %) = 100 (Intestine length (cm.) / fish length (cm.) (6)
- Intestine weight index (IWI, %) = 100 (Intestine weight (gm.) / fish weight (gm.) (6).

Blood examination: At the end of the feeding period, three fishes removed from each treatment and anesthetized using clove powder for few min and then cut the caudal peduncle for blood obtaining. Blood indices: The blood samples from each fish of the different groups collected by suction of the caudal peduncle. Whole blood samples collected in heparinized vials for determination of some blood indices which were determined by using the hematology analyzer BC-2800 is a compact, fully automatic hematology analyzer USA origin for complete blood count (CBC) test.

Complete Blood Count: Erythrocyte count (RBCs: 10^{12} cells/l), mean corpuscular hemoglobin (MCH; pg), mean corpuscular hemoglobin concentration (MCHC, g/dl), mean corpuscular volume (MCV, fL), hemoglobin (Hb, g/l) and platelets (PLT, 10^9 cells/l). Leukocyte count (10^9 cells/l), and % granulocytes, lymphocytes and monocytes.

Biochemical Parameters: Alanine aminotransferase activity (ALT), aspartate aminotransferase activity (AST), Cytokines (CKI)total proteins, globulin (g/dl), albumin (g/dl) and albumin/ globulin (A/ G) ratio.

Proximate Composition: All fish samples used for the chemical analysis of the muscle (percentage of moisture, crude protein, ether extract and ash) according to A.O.A.C. (1) analytical methods. At the end of the experimental period, three fishes randomly take from each experimental group. All fish samples weighed and their length will be measured individually. Blood samples from each fish of the different groups collected by cutting the caudal vein. Whole blood samples collected in small plastic vials containing heparin and stored under cooling condition at refrigerator temperature (11).Microbial load: After fish dissection the intestine and muscle taken and washed with peptone water carefully, then make the microbial counts as below, Aseptically, 1 ± 0.1 g of the sample weighed, transferred into a sterile blender jar, then 9 ml sterile phosphate buffer were added and blended at high speed for two minutes. This became the 1:10 dilution. The foam was permitted to settle, and then 10 ml of the blended 1:10 dilution pipetted into a 90 ml dilution blank to make 1:100 dilutions. The procedure repeated to prepare serial dilutions of 10^{-3}, 10^{-4}, etc. All dilutions shaken 25 times in a one-foot arc. About 1ml from the 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} etc., then the melted plate count agar used (Biolife, Italy). After culturing, the plates incubated at 35 ºC (Memmert, Germany) for 48 hrs. Statistical analysis: All data generated subjected to one-way analysis of variance (ANOVA), using the General Linear Model procedure of XLSTAT 2016 Version.02.28451. Differences between treatments means were compared by Duncan's multiple range test and were tested for significance at $P<0.05$.

RESULTS AND DISCUSSION

Weight Gain, Daily Weight Gain, and Relative Growth Rate were all considerably greater in Positive Control T2. As seen in the table (2), there are no significant changes in Specific Growth Rate.

Table 2. Means and S.E. for effect of germination of lentil in common carp *Cyprinus carpio* performance

Treatments	Negative Control (T1)	Positive Control T2	25 gm germinated lentil /kg (T3)	50 gm germinated lentil /kg (T4)
Weight Gain	**6.701±1.225 [c]**	**11.700±2.591 [a]**	**6.928±0.972 [c]**	**7.702±0.869 [bc]**
Daily Weight Gain	**0.093±0.016 [b]**	**0.162±0.036 [a]**	**0.096±0.013[b]**	**0.106±0.012 [a]**
Specific Growth Rate	**1.525±0.010 [a]**	**1.573±0.025 [a]**	**1.526±0.003 [a]**	**1.545±0.011 [a]**
Relative Growth Rate	**23.712±4.610 [c]**	**42.479±9.758 [a]**	**24.757±4.054 [c]**	**26.441±2.980 [b]**

Feed Conversion Ratio was considerably greater in the 25 gm germinated lentil/kg (T3) group. As shown in table (3), Positive Control T2 increased greatly in Feed Efficiency Ratio, Protein Efficiency Ratio, and Fat Efficiency Ratio.

Table 3. Means and S.E. for effect of germination of wheat, barley and lentil in common carp *Cyprinus carpio* feed utilization

Treatments	Negative Control (T1)	Positive Control T2	25 gm germinated lentil /kg (T3)	50 gm germinated lentil /kg (T4)
Feed Conversion Ratio	6.583±1.541 [a]	3.777±0.752 [d]	6.194±1.181 [a]	5.482±0.576 [bc]
Feed Efficiency Ratio	16.694±3.229 [c]	29.022±6.504 [a]	17.194±2.755 [c]	18.628±1.841 [b]
Protein Efficiency Ratio	23.638±4.323 [c]	41.787±9.257 [a]	24.474±3.436 [c]	26.743±3.018 [b]
Fat Efficiency Ratio	131.400±24.031 [c]	228.079±50.526 [a]	145.558±20.440 [b]	125.234±14.137 [c]

There were no significant variations in Dry Matter (%), Moisture (%), or Organic Matter (%). In terms of Ether extract (Oil) (%) and Crude protein, all treatments were significantly greater than the negative control (%). Weight without viscera was considerably higher in the different groups than 25 gm germinated lentil /kg (T3). As demonstrated in table (4), weight without viscera and head rose significantly in Positive Control (T2) and 50 gm germinated lentil/kg (T4).

Table 4. Means and S.E. for effect of germination of wheat, barley and lentil in common carp *Cyprinus carpio* meat proximate analyses

Treatments	Negative Control (T1)	Positive Control (T2)	25 gm germinated lentil /kg (T3)	50 gm germinated lentil /kg (T4)
Dry Matter (%)	25.943±0.824 [a]	25.936±0.429 [a]	26.423±0.136 [a]	26.400±0.251 [a]
Moisture (%)	74.056±0.824 [a]	74.063±0.429 [a]	73.576±0.136 [a]	73.600±0.251 [a]
Ether extract (Oil) (%)	14.643±0.697 [b]	15.083±0.187 [ab]	15.796±0.201 [ab]	15.600±0.079 [ab]
Ash (%)	7.230±0.261 [abc]	7.489±0.178 [ab]	7.698±0.181 [a]	6.869±0.431 [bc]
Organic natter (%)	18.713±1.078 [ab]	18.446±0.607 [ab]	18.723±0.218 [ab]	19.530±0.190 [a]
Crude protein (%)	66.543±1.092 [b]	67.409±0.637 [ab]	68.738±0.599 [ab]	67.924±0.275 [ab]
Weight without viscera	82.40±0.201 [a]	81.43±0.843 [ab]	78.53±3.587 [b]	82.16±1.008 [a]
Weight without viscera and Head	61.47±1.193 [b]	63.15±0.355 [a]	59.45±2.718 [bc]	63.62±0.858 [a]

There were no significant variations in Condition Factor and Intestine Weight Index in Table (5). In the Positive Control group, the Hepatic Index was considerably higher (T2). In all groups, the Spleen and Kidney Index was higher than the Negative Control (T1). 25 gm germinated lentil /kg has a higher Intestine Length-Weight Index (T3). Length Index in 50 gm germinated lentil /kg Intestine Length (T4).

Table 5. Means and S.E. for effect of germination of wheat, barley and lentil in common carp *Cyprinus carpio* some physiobiological indices

Treatments	Negative Control (T1)	Positive Control T2	25 gm germinated lentil /kg (T3)	50 gm germinated lentil /kg (T4)
Condition Factor	1.58±0.059 [a]	1.54±0.063 [a]	1.54±0.021 [a]	1.72±0.054 [a]
Hepatic Index	2.45±0.503 [c]	4.16±0.109 [a]	3.56±0.569 [b]	3.18±0.253 [b]
Spleen Index	0.14±0.023 [b]	0.22±0.029 [a]	0.24±0.048 [a]	0.20±0.041 [ab]
Gill Index	3.69±0.462 [a]	3.18±0.342 [a]	3.25±0.307 [a]	3.55±0.370 [a]
Kidney Index	0.40±0.024 [b]	0.72±0.070 [a]	0.61±0.089 [a]	0.57±0.025 [ab]
Intestine Weight Index	4.06±0.162 [a]	4.11±0.290 [a]	4.53±0.755 [a]	4.40±0.069 [a]
Intestine Length- Weight Index	52.16±3.426 [c]	50.93±2.807 [d]	56.58±10.99 [a]	54.99±3.098 [b]
Intestine Length- Length Index	161.27±10.05 [d]	168.21±9.076 [c]	178.40±25.58 [b]	183.30±0.668 [a]

A considerable change in WBC (10^9/dl) counts in 50 gm germinated lentil /kg is shown in table (6). (T4). T2 was considerably greater in Lymphocytes (10^9/dl), Monocytes (109/dl), and Granulocytes (109/dl) in the Positive Control group.

Table 6. Means and S.E. for effect of germination of lentil in common carp *Cyprinus carpio* some differentials WBC counts

Treatments	Negative Control (T1)	Positive Control T2	25 gm germinated lentil /kg (T3)	50 gm germinated lentil /kg (T4)
WBC (10^9/dl)	67.066±9.024 cd	69.966±6.345 c	87.820±29.321 b	93.566±25.765 a
Lymphocytes (10^9/dl)	46.226±2.668 cd	51.020±3.606 a	33.723±10.021 d	47.206±5.476 b
Monocytes (10^9/dl)	2.356±1.106 c	4.283±0.374 a	2.840±0.798 c	3.760±0.635 b
Granulocytes (10^9/dl)	7.223±3.631 c	14.656±2.378 a	8.090±3.471 c	12.860±3.278 b

In each Hb (g/L) and PCV, no significant variations in treatment effects were identified (%). RBC (10 12/L) were significantly affected by Positive Control T2 and 25 gm germinated lentil/kg (T3). T3 (25 gm germinated lentil/kg) has a significant effect on MCHC (%), but T4 (50 gm germinated lentil/kg) has a substantial effect on PLT (10 9/L), as shown in table (7).

Table 7. Means and S.E. for effect of germination of lentil in common carp *Cyprinus carpio* some blood picture indices

Treatments	Negative Control (T1)	Positive Control T2	25 gm germinated lentil /kg (T3)	50 gm germinated lentil /kg (T4)
Hb (g/L)	10.100±0.219 ab	10.490±0.280 ab	10.805±1.260 a	10.456±0.387 ab
RBC (10^ 12/L)	2.788±0.580 b	3.021±0.073 a	3.684±0.138 a	2.634±0.499 b
MCHC (%)	28.333±6.385 b	31.666±0.666 b	39.666±4.409 a	27.333±4.910 b
PLT (10^ 9/L)	2620.330±309.327 b	1906.330±543.985 c	1847.670±529.477 d	3010.000±170.661 a
PCV (%)	30.3000±0.657 ab	31.470±0.840 a	32.416±3.781 a	31.370±1.163 a

Table (8) show the positive effect of 50 gm germinated lentil /kg (T4) on Cholesterol mg/dl and LDL mg/dl but was the lowest significantly in Triglyceride mg/dl and HDL mg/dl.

Table 8. Means and S.E. for effect of germination of lentil in common carp *Cyprinus carpio* some lipid profile indices

Treatments	Negative Control (T1)	Positive Control T2	25 gm germinated lentil /kg (T3)	50 gm germinated lentil /kg (T4)
Cholesterol mg/dl	115.66±2.60 b	97.66±1.85 c	117.00±7.57 b	149.66±2.40 a
Triglyceride mg/dl	308.66±37.35 a	202.00±2.08 c	259.33±15.49 b	259.00±15.09 b
HDL mg/dl	15.33±0.88 a	11.33±1.20 bc	12.33±1.33 bc	9.66±1.20 c
LDL mg/dl	9.66±1.45 b	10.33±4.84 b	5.33±1.33 c	11.00±1.52 a

Positive Control T2 was higher significantly in all microbial counts of each Muscles and intestinal flora as shown in table (9).

Table 9. Means and S.E. for effect of germination of lentil in common carp *Cyprinus carpio* some microflora counts

Treatments	Negative Control (T1)	Positive Control T2	25 gm germinated lentil /kg (T3)	50 gm germinated lentil /kg (T4)
Muscle Bacteria (Nutrient agar D3)	152.000±7.211 d	374.000±14.106 a	173.000±68.000 c	198.666±32.219 b
Muscle Bacteria (Nutrient agar D4)	93.333±6.691 c	192.000±54.243 a	53.000±1.000 d	120.333±12.414 b
Microflora (Nutrient agar D5)	124.333±32.126 c	390.000±50.408 a	181.000±10.000 bc	185.666±14.847 bc
Microflora (Nutrient agar D6)	74.666±9.492 d	272.666±17.676 a	91.000±12.000 c	109.333±16.169 b
Microflora (MacConkey agar D5)	106.500±18.500 d	322.000±15.502 a	117.000±12.000 c	144.666±30.475 b
Microflora (MacConkey agar D6)	78.500±15.500 d	148.333±25.718 a	95.500±9.500 b	87.000±4.000 c

Gharachorloo et al. (16) found that following germination, the number of phenolic compounds increased considerably (p 0.05). Although the rise in phenolic compounds is independent of the solvents used, the quantity of extracted phenolic compounds differed

significantly (p 0.05) between groups. Their findings are consistent with those of Lopez-Amoros et al. (19) who found that germination alters the quantity and quality of phenolic chemicals in legumes. And this could be one of the explanations for the current study's increased health and blood parameters. In addition to phenolics, legumes include other bioactive chemicals such as vitamins and carotenoids in varying amounts, which can influence the antioxidant activity of the samples. These chemicals may have a synergistic effect with phenolic compounds, which could explain the observed differences in antioxidant activity as well as some of the blood markers in this investigation. These increases in phenolic content and antioxidant activity suggest that phenolics may play a significant function during seed germination, as well as that the germination process may boost the nutraceutical value of seeds (13). When fishmeal was replaced by raw lentil seeds powder in the food used in the study of (2), significant variations in weight increase and specific growth rate were noted, and this agrees with the findings of the current investigation. When compared to the control group and other groups that got lower replacements, the T5 demonstrated significantly higher daily and relative weight gain and specific growth rate (p0.05). In compared to the control group, significant variations in feed conversion ratio (FCR) were seen in all treatments. When compared to the other groups, the T5 group had considerably higher FCR, Protein Efficiency Ratio, and Fat Efficiency Ratio. The Hepatosomatic Index, Spleensomatic Index, and Kidneysomatic Index showed no significant changes. The condition Factor was considerably different across the treatment groups, and the intestinal Length Index (based on fish length and weight) also indicated significant differences. In terms of the Intestinal Weight Index, however, no significant differences were seen between the groups. The dietary replacement of soybean meal by differently toasted sunflower seed meal in *Clarias gariepinus* diet can be done up to 30% level using survival, growth, carcass composition, digestibility, haematology and histology of the liver as indices. The physiological condition was comparable with soybean-based control diet. At higher replacement level of 45% for the differently toasted sunflower meal, the fish physiological functioning was becoming compromised though survival was not affected, there was reduced growth and changes to the blood and histopathological parameters of the liver (17). Abdulrahman et al. (5) found that adding germinated barley powder to the diet of common carp had no negative impacts on the muscle ratio of the fish, which is consistent with recent studies. However, Ahmed et al. (8) found that using date palm seed powder in the diet of common carps reduced meat indices. The best performance of common carp feeding on a feed made by replacing 20% of the fish meal diet with plant sources (lentil seed) was attained in the study of Abdulrahman et al. (4) in terms of growth and feed utilization, and (9) found that ammonia and mortality were decreased with increased zeolite level in water, T3 with 10 mg/l can be considered as better treatment which contained zeolite in blood parameters. The germination of lentil seeds was shown to be suitable for use in common carp feed. In addition to improving *C. carpio* growth output, employing germinated lentil would reduce production expenses, increasing net profit by growing it in clay carp ponds. the results of (25) that the polymorphism of the growth hormone receptor gene in common carp fish in the seven discovered sites, there is no significant effect of the difference in the genotypes of the growth hormone receptor gene on the growth characteristics of common carp fish, and the mutations that were discovered in all sites did not affect the studied growth characteristics, whether positive or negative.

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