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The Impact of Seaweed-Derived Feed Additives (Aschomax) on Growth, Feed Utilization, Immunity, and Biochemical Profiles of Common Carp (*Cyprinus carpio*)

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ABSTRACT

The overuse of antibiotics and synthetic feed additives in aquaculture involves risks to fish health and the environment. Finding natural alternatives to synthetic materials is critical for sustainable aquaculture enterprises. The present study was conducted to investigate the effects of Seaweed-Derived Feed Additives (Aschomax) supplementation with different concentrations (0%, 1%, 2%, and 3%) on growth, feed utilization, immunological parameters, and blood biochemical indices of common carp (*Cyprinus carpio*) over a 60-day feeding period. The results demonstrated thatgrowth performance parameters were significantly enhanced in fish fed with 2% Aschomax supplementation. Moreo-ver, immunological parameters were markedly improved across all Aschomax-supplemented groups, with the 2%inclusion level showing superior results. This was evidenced by significantly elevated levels of Myeloperoxidase(MOP: 0.59%), Nitroblue tetrazolium (NBT) activity (38.5%), and lysozyme activity (37.8 U/mL), indicating en-hanced non-specific immune responses. Furthermore, 2% of Aschomax demonstrated the most favorable lipid profile,characterized by reduced total cholesterol (TC) (140.21 mg/100 ml) and triglycerides (TG) (75.23 mg/100 ml), along-side elevated high-density lipoprotein (HDL) (93.60 mg/100 ml) and reduced low density lipoprotein (LDL) (50.54mg/100 ml) levels. Notably, liver enzyme activities (ALP, ALT, and AST) remained unchanged across all treatments.

These findings suggest that Aschomax supplementation at 2% effectively enhanced growth performance and stimulates the immune system of *C. carpio*. The alteration in blood biochemical parameters suggests improved metabolicefficiency and overall health status.

KEYWORDS: Growth promoter, Brown seaweed, Common carp, Lipid profile, Liver enzymes

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1. Introduction

The global population is expected to grow significantly and may attain 8.6 billion by 2030 (UN, 2017). Consequently, the demand for food will increase, making sustainable food production a priority. Aquaculture is essential for supplying the growing global need for protein-rich dietary sources. The contribution of aquaculture to global aquatic food production is expected to reach 53% in 2030 (FAO, 2020). In addition to providing a reliable source of nutrition, aquaculture contributes significantly to the global economy, especially in developing countries, where it is essential for the livelihood of millions.

Due to increased demand for fish meat in Iraq and its scarcity from natural sources, intensive carp culture has been widespread by earthren ponds and floating cages in rivers (Ahmed, 2020). These enterprises face major challenges, such as the excessive use of antibiotics and synthetic feed additives to fight fish diseases and reduce microbial infections (Amangelsin et al., 2023). The continuous use of antibiotics in aquaculture could lead to various hazards with concern to the health of cultured fish, consumers, and aquaculture environment. This highlights the need to find alternatives to the chemical substances, antibiotics, and veterinary drugs used in fish farming projects to ensure the production of food safe for human consumption while preserving an unpolluted environment (Ahmed et al., 2023; Al-Turaihi et al., 2023; Aulia et al., 2024).

Brown seaweed (Phaeophyceae) are rich sources of bioactive compounds with great nutritional benefits. The bioactive compounds derived from algae and seaweed have unique properties, namely, antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory effects (Pal et al., 2014; Li et al., 2021; Matin et al., 2024). For this reason, pharmaceutical companies pay remarkable attention to extracting the bioactive substances from seaweed for drug development (Pal et al., 2014). Recently, the advancement in extraction techniques has facilitated the integration of the bioactive compounds into drug and food products (Ahmed et al., 2024). In aquaculture studies, the incorporation of 3 and 10% of seaweed meal derived from brown seaweeds *(Laminaria sp., kelp)* improved growth metrics in Atlantic salmon (*Salmo salar*) (Kamunde et al., 2019). More advantages were recorded by Sheikhzadeh et al. **(**2022) who found that dietary brown seaweed (*Padina australis*) supplementation positively affected the growth of *Cyprinus carpio* and enhanced digestive enzyme activity, immunological responses, and disease resistance to bacterial infection. Nazarudin et al., (2020) found that the addition of 1.5 and 3% of brown seaweed (*Sargassum polycystum*) to the feed of *Asian sea bass* fingerlings significantly improved weight gain, feed consumption, and quality of fish carcass.

The present investigation aims to evaluate the impact of locally manufactured prebiotics (Aschomax, Al-Joud Company, Iraq) on the growth performance as well as on the immunological parameters, key liver enzymes, and lipid profile of *C. carpio* with its potential use in aquaculture as sustainable alternative to artificial and imported feed additive.

2. Material and Methods

2.1. Feed preparation

The basic diet contains 22% fish meal, 25% wheat bran, 20% wheat, 30% soybean, 2% vegetable oil, and 1% starch (Table 1).

Table 1. Dietary formulation of experimental diets (g/kg)

Ingredients (g/kg)	Control	T1	T2	T3
Fish meal	220	220	220	220
Wheat bran	250	240	230	220
Wheat	200	200	200	200
Soybean	300	300	300	300
Vegetable oil	20	20	20	20
Starch	10	10	10	10
Aschomax powder	0	10	20	30

Brown seaweeds extract (Aschomax) was provided by Al-Joud Company, Karbala province, Iraq, and the other ingredients were purchased from the local markets. The control diet was free of Aschomax supplementation, while other experimental diets were supplemented with various levels of Aschomax (1%, 2%, and 3%) and were labeled as T1, T2, and T3. The dry ingredients for each diet were blended with tap water (500 mL/ kg) and vegetable oil gradually. Then, the mixture was made into noodles using an electric meat mincer machine. The noodles were dried at room temperature and were crushed manually to produce small pellets. The dry diets were packed in plastic containers and stored in a dry and cold place until required. The chemical analysis of formulated diets was conducted following the Association of Official Analytical Chemists (AOAC) protocol (AOAC, 2002). Table 2 shows proximate composition of experimental diets (%).

Table 2. Proximate composition of experimental diets (%)

Proximate composition (%)				
Moisture	2.88	3.17	3.24	2.96
Protein	32.56	31.80	30.48	31.24
Lipid	9.11	9.25	10.02	9.23
Ash	5.93	6.02	5.59	5.89
Carbohydrate	49.52	49.24	51.49	50.68

2.2. Experimental fish and husbandry

The experimental fish (average initial weight 55.4 ± 0.59 g) were provided from Al-Hartha fish farm, Basrah province. During the two weeks of acclimatization in the stock tanks, fish were fed with control diet of the current experiment (1% of their body weight). The design of the trial was (4 treatments × 3 replicates with 10 fish in each tank) in a recirculating plastic tank (100 liters capacity for each tank). During the experiment, fish were fed 3% of their body weight twice a day. For growth monitoring and feed ratio adjustment, all fish in each tank were weighed collectively every 2 weeks after a 24-hour fasting period. Throughout the experiment, temperature was maintained at 26.5 ±1.03°C, dissolved oxygen 7.10 ±0.45 mg/L, and pH 7.4 ±1.30. The nutrition trial was performed in the aquaculture laboratory, Marine Science Center, University of Basrah, for 8 weeks.

2.3. Growth parameters calculation

The initial body weight (IBW) of fish in each tank was determined at the beginning of the trial, while the final body weight (FBW) was assessed at the end of the feeding trial. Other parameters, weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR), were calculated as follows:

"WG = FBW (g) − IBW (g),
SGR = (ln FBW − ln IBW) /t ×100
where t = time in days
FCR = Feed intake (g) /weight gain"

2.4. Estimation of serum lipid profile

At the end of the feeding trial, the experimental fish were subjected to 24 h starvation period and then, 6 fish from each treatment were collected randomly for blood collection. Fish were anesthetized with Tricaine methanesulfonate (MS-222) from Sigma-Aldrich at 0.1 g/L. The whole blood from each fish was collected by a medical syringe from the caudal vein and placed into duplicates of 1.5 ml Eppendorf tubes. The tubes were centrifuged at 1500 rpm for 3 min and the serum samples were removed for serum biochemistry analysis. The total cholesterol (TC), triglycerides (TG), and high-density lipoproteins (HDL) in the serum were measured according to NCHS (2006). A Mindray laboratory kit (Mindray Medical International Limited, China), and a BS-230 device were used for the measurement at a wavelength of 510 nm. The results (mg/dL) were calculated using the following equation according to NCEP (1994).

LDL = TC – HDL − (TG / 5).

2.5. Non-specific immune parameters

The nitroblue tetrazolium (NBT) assay was carried out according to Siwicki (1987). For plasma collection, the whole blood was collected in tubes provided with EDTA as anticoagulant. 50 µL of plasma from each sample was transferred into micro-calibration tube and incubated at 22 °C for 1 hour. After the incubation, the floater was removed, and the tubes were washed with phosphate buffer saline. Then, 50 µL of NBT (0.2%) was mixed with 1 µL/mL of phorbol myristate acetate and the mixture was incubated for an hour at 22° C. Methanol (100%) was used for cells fixation. Later, the tubes were washed by 70% alcohol. After drying, 60 µL of potassium hydroxide and 70 µL of dimethyl sulfoxide were added to dissolve the produced blue formazan. The optical density of the solution was measured by a spectrophotometer at 450 nm.

The concentration of Myeloperoxidase (MOP) in the serum was measured according to Quade and Roth (1997), while the lysozyme activity was measured according to Siwicki et al. (1994). Briefly, 0.1 of serum was added to 1.9 mL of *Aeromonas hydrophila* suspension (0.2 mg/L) in 0.05 mL of buffer solution (pH 6.2). The solution was incubated at 25°C and the absorbance was measured after 0.5 and 5 minutes by a spectrophotometer at 450 nm.

2.6. Key liver enzymes

The concentration of three critical enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), was measured. Blood samples were obtained from different fish groups via the caudal vein. The whole blood was placed into Eppendorf tubes provided with EDTA and the plasma was separated by the centrifugation of the whole blood for 3 minutes at 1500 rpm. The levels of relevant enzymes were measured following the instructions provided with each kit (Mindray Medical International Limited, China).

2.7. Statistical analysis

Data was analyzed by one-way analysis of variance (ANOVA), SPSS program (version 24), at 0.05 significant levels and expressed as mean ± standard deviation (SD). Least significant differences (LSD) were performed to evaluate statistical differences.

3. Results

3.1. Growth performance

The growth performance of *C. carpio* fed diet supplemented with the Aschomax product over 8 weeks showed significant differences among the experimental groups. Overall, the inclusion of the Aschomax product at the T2 level markedly enhanced growth performance and improved feed conversion ratio (lower FCR) in *C. carpio* (Table 3).

3.2. The activity of the nonspecific immune response enzymes

The supplementation of seaweed-derived additive significantly enhanced immune parameters in *C. carpio* (Table 3). MOP, NBT, and lysozyme activities were highest in the T2 group, indicating improved immune responses compared to the control and other treatments (Table 4).

3.3. Lipid profile parameters

The effects of different treatments on lipid profile parameters are presented in Table 4. The results showed significant variations in TC, TG, HDL, and LDL concentrations across the experimental groups. The control group consistently showed the least favorable lipid profile, while T2 (2%) was the most effective in improving the lipid profile in the lowest TC, TG, and LDL levels and the highest HDL levels.

3.4. Liver enzymes activities

Supplementation with different levels of the algal-derived powder did not significantly affect liver enzyme concentrations in *C. carpio*. The ALP, ALT, and AST were similar across all treatments, with no significant differences observed between the control and experimental group (Table 6).

Table 3. Effects of Aschomax on the growth performance of *C. carpio* after an 8-week feeding trial

Parameters	Control	T1	T2	T3
IBW (g)	55.18±1.58	55.65±0.86	56.83±1.28	55.72±1.82
FBW (g)	95.78±0.04c	96.22±4.24c	130.48±5.61a	102.03±0.48b
WG (g)	40.60±1.55c	40.65±3.59c	73.65±4.41a	46.31±2.28b
SGR	0.79±0.04c	0.78±0.05c	1.19±0.03a	0.86±0.05b
FCR	3.54±0.18a	3.56±0.32b	2.28±0.10b	3.19±0.21b

Different letters indicate significant difference ($p < 0.05$)

Table 4. The activity of the nonspecific immune response enzymes in *C. carpio* at the end of the trial

Parameters	Control	T1	T2	T3
MOP%	0.14±0.03d	0.43±0.04b	0.59±0.05a	0.37±0.03c
NBT%	21.0±2.11d	32.3±1.15b	38.5±2.64a	30.4±2.84b
Lysozyme activity (U/mL)	25.0±1.16c	30.5±1.24b	37.8±1.82a	29.8±1.21b

Different letters in the same row indicate significant difference at $p < 0.05$

Table 5. The effect of different levels of Aschomax on lipid profile parameters after an 8-week feeding trial

Parameters(mg/dL)	Control	T1	T2	T3
TC	234.20±2.24d	190.03±2.18b	140.21±2.42a	200.09±2.33c
TG	122.14±2.12d	86.53±1.20b	75.23±2.10a	120.35±1.14c
HDL	73.01±2.01d	90.02±1.18b	93.60±2.15a	80.42±2.30c
LDL	70.88±2.54d	59.02±1.12b	50.54±2.20a	63.20±2.25c

Values with different letters (a, b, c, d) indicate significant differences ($p < 0.05$) between treatments for each parameter, where "a" represents the most favorable outcome (lowest TC, TG, and LDL, and highest HDL), while subsequent letters indicate less favorable values.

Table 6. Liver enzymes activities (IU/L) of *C. carpio* fed diets supplemented with different levels of Aschomax for 8 weeks

	Treatments			
Enzyme	Control	T1	T2	T3
ALP	61.21±1.32	60.50±1.31	60.33± 1.28	63.51±1.11
ALT	5.45±1.06	5.24±1.43	5.20±1.22	6.00±0.21
AST	37.05±0.94	36.41±1.26	36.30±1.24	38.01±0.11

4. Discussion

In the current study, we tested the potential use of brown seaweed as a natural feed additive to enhance growth performance, immune responses, biochemical parameters, and lipid profile of *C. carpio* fingerlings. The Aschomax product we used (Al-Joud Company, Karbala, Iraq) is a brown seaweed extract, which has been reported to be rich in nutritional substances such as lipids, peptides, amino acids, fatty acids, and minerals (Alloyarova et al., 2024). In this investigation, the Aschomax product at level 2% has improved growth and feed utilization of *C. carpio*, which aligns with previous study of Atlantic salmon (*Salmo salar*) and of largemouth bass (*Micropterus salmoides*), when fish were fed the dietary brown seaweed supplementation (Kamunde et al., 2019; Shen et al., 2025). The positive influence of the Aschomax on the growth of *C. carpio* may be attributed to the high contents of bioactive compounds present in the brown seaweed extract (Alloyarova et al., 2024). It seems that polysaccharides from seaweed play a vital role in modulating the intestinal microbiota, thereby improving growth and fish health (Remya et al., 2022;

de Lima et al., 2024). In addition, Alloyarova et al. (2024) demonstrated that the bioactive substances in brown seaweed can promote digestive enzymes activities and thereby improve nutrient absorption and feed conversion ratio. These findings are essential to understand the mechanism by which the seaweed extract contributes to enhance growth and feed utilization of fish. Increasing the level of Aschomax more than 2% in the current study did not yield further benefit maybe owing to a reduction in feed-palatability as previously reported by Abdel-Warith et al. (2016).

MOP is a peroxidase enzyme mainly found in neutrophils and plays a key role in the immune system by producing hypochlorous acid which is involved in fighting pathogens (Buchan et al., 2019). The results of our investigation provided evidence of a remarkable increase in MOP in the serum of *C. carpio* when fed diets supplemented with seaweed-derived prebiotics. The T2 group (2% supplementation level) exhibited the highest levels of MOP enzyme activity compared to control and other treatments. The notable enhancement in MOP activity observed in the T2 group may result from the presence of bioactive substances, such as fucoidan, which has been documented to possess immune-enhancing properties (Yang et al., 2023). Further, the polysaccharides derived from seaweed can enhance the immune response by activating the production of cytokines and thereby activating immune cells (Leonard et al., 2011).

The NBT assay is a recognized technique for assessing respiratory burst activity in fish immune cells. The respiratory burst, or oxidative burst, is an essential immune mechanism in which immune cells produce reactive oxygen species (ROS) as a result of microbes' exposure (Biller-Takahashi et al., 2013). In our investigation, the elevated NBT observed in the T2 group indicated an improved respiratory burst activity which may result from the presence of sodium alginate in brown algae which is well known as immunostimulant agent (Cheng and Yu, 2013). Our finding supports those reported by Thepot et al. (2021) who tested the effects of eleven seaweed species on the innate-immune response (cellular and humoral immunity) of the rabbitfish (*Siganus fuscescens*) and concluded that dietary seaweed supplements can improve the immune response of the mentioned fish species.

Lysozyme is a β-1,4-glycosidase that breaks down the polysaccharide backbone of bacterial cell walls and thereby contributes to the host's innate immune response to infection (Han et al., 2024). The increased lysozyme activity in the T2 group further highlights the immunomodulatory effects of the Aschomax product. The improvement in the innate immune response observed in this study aligned with findings from studies on other fish species. In this context, Pham et al., (2006) reported that the dietary inclusion of Hizikia, a brown seaweed, enhanced the nonspecific immune responses and disease resistance of juvenile olive flounder (*Paralichthys olivaceus*) due to phenolic compounds existing in brown algae. Similarly, polysaccharides from brown algae improved the nonspecific immune system in shrimp *Litopenaeus vannamei*, *Penaeus monodon*, *Fenneropenaeus indicus*, and tilapia (*Oreochromis niloticus*) (Muahiddah and Diamahesa, 2022). Moreover, Yang et al. (2014) reported that the bioactive substances derived from *S. horneri* significantly increased serum lysozyme and the respiratory burst activity of yellow catfish *P. fulvidraco* phagocytes.

The current investigation revealed a positive effect of the seaweed extract on the lipid profile (a decrease in TC, TG, and LDL

levels and an increase in HDL levels) in *C. carpio*. The beneficial impact of dietary seaweed on lipid profile was previously reported in juvenile Japanese flounder (*Paralichthys olivaceus*) by Ragaza et al., (2015) and in juvenile black sea bream (*Acanthopagrus schlegelii*) by Shi et al., (2019). This enhancement may be linked to the possible impact on cholesterol biosynthesis and lipoprotein metabolism (Ara et al., 2002; Ara et al., 2005). Further, a significant reduction in these parameters may result from the presence of the secondary metabolites which have been found to modulate the expression of the enzymes responsible for cholesterol and triglyceride synthesis in the liver (He et al., 2023). Another study reported that seaweeds are rich in fibers which are well known to cause reduction in TC and TG in serum (Jiménez-Escrig and Sánchez-Muniz, 2000). Moreover, the elevated concentration of HDL in the T2 group reflects an improvement in lipoprotein metabolism. In this respect, Ara et al., (2002) reported that the modulation of lipoprotein metabolism could lead to an increase in HDL levels in the blood.

Some enzymes such as ALP, ALT, and AST are essential for assessing liver health in fish. Elevated concentration of these enzymes in fish blood often indicates liver damage or dysfunction (Samanta et al., 2014; Mohamed et al., 2019; Xie et al., 2022; Ahmed et al., 2023). The current study demonstrated that the levels of liver enzymes in the serum of *C. carpio* remained stable across all treatments suggesting that dietary supplementation of Aschomax does not have a significant impact on liver function.

5. Conclusion

In conclusion, dietary Aschomax supplementation at 2% improved growth indices and immune system parameters. More advantages were obtained from Aschomax supplementation, namely, reduction in TC, TG, and LDL in blood and significant elevation in HDL. Further, the study revealed that the seaweed extract had no effect on ALP, ALT, and AST. These observations can be attributed to the presence of bioactive compounds. Despite the positive effect of dietary Aschomax, long-term studies are needed to evaluate its impact of the general health of cultured fish.

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Compliance with Ethical Standards

Conflict of interest
The authors declare that they have no competing interests.

Ethical approval
All applicable international, national, or institutional guidelines for the care and use of animals were followed. Ethical approval for this study (MSC119-2024), dated 01-September 2024 was obtained from the MSC Ethics Committee, Marine Science Center, University of Basrah, Iraq.

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Data availability
Not applicable.

Consent for publication
Not applicable.

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