



PHYSIOLOGICAL AND GROWTH RESPONSES OF COMMON CARP (*CYPRINUS CARPIO*) TO DIETARY BROWN SEAWEED SUPPLEMENTATION

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Abstract. The nutritional status of *Ascophyllum nodosum* powdered seaweed, as a natural dietary supplement, was evaluated on common carp (*Cyprinus carpio*) in terms of productive performance, FER, blood parameters and selected biochemical parameters in the present study. The fish were randomly divided among 4 treatment groups: non-supplemented (control), 5g/kg, 10 g/kg and 15 g/kg of the powdered form of *A. nodosum*. It was decided to feed the animals for nine months and the results observed after 60 and 90 days of experimental feeding. The response of common carp to seaweed supplementation was determined by the amount and frequency of seaweed supplementation. The growth response was the highest when fed 15 g/kg diet, with the best parameters of final weight, weight gain, relative growth rate, feed efficiency and protein efficiency. The haematological parameters were also enhanced, such as RBC, haemoglobin and haematocrit, which suggests the physiological condition of the fish fed with the highest dose of the supplement. The responses to the treatments were varied for biochemical parameters and the overall trends were that the supplemented diets had lower blood glucose but higher total protein or albumin in some of the supplemented diets than the control. Some enzymes and lipid profile was also affected, indicating the metabolic changes occurring as a result of the supplementation of seaweed. Positive growth response was not maintained consistently after 90 days however, in some supplemented groups weight gain was less than that of the control group. The results show that it can be concluded that the powder of the species *A. nodosum* can be used as a functional feed additive for common carp and it can be used in the common carp aquaculture in future, but the optimum usage period and rate of this species should be tested.

Keywords: *Ascophyllum nodosum*; brown seaweed; common carp; dietary supplementation; growth performance; feed efficiency; hematological profile; biochemical response.

Introduction

Aquaculture is one of the most vital sectors to food security, especially as fish are a good source of high-quality animal protein and essential nutrients for humans. From the cultured freshwater fishes, common carp (*Cyprinus carpio*) is widely produced due to its adaptability, economic value and suitability for intensive/semi-intensive culture system. As aquaculture continues to expand, the need for a safe, sustainable and natural feed additive to help increase fish growth, improve feed efficiency and enhance fish health has increased.

In the past few years, marine algae are being the subject of a great deal of research as functional ingredients in aquafeeds. Seaweeds are highly rich in different nutrients and bioactive compounds like proteins, amino acids, polysaccharides, phenolic compounds and natural growth promoters. These compounds may exhibit positive effects on digestive system, metabolic rate, antioxidant and immune system of aquatic animals. Algal materials and their extracts have been found to have antioxidant, antimicrobial, antiviral, hepatoprotective, and other biological activities, suggesting their potential applications in fish nutrition and health management [1], [2].

BROWN ALGAE are especially significant due to their significant biochemical composition and as a natural dietary supplement. A number of bioactive compounds (phenolics, polysaccharides) have been reported from the brown macroalga *Ascophyllum nodosum* that may influence the physiology or metabolic activity of the organism. These are associated with antioxidant and anti-inflammatory effects, enzyme modulating effects and stress and disease resistance. Besides, brown seaweeds are rich in vitamins including vitamin E and other bioactive metabolites that could be beneficial to the animals' health [3]–[5].

This supplementation with algae has already been tested with different fish species under various algae, such as *Spirulina* and *Chlorella*, with their effect as feed additive. In these studies, the positive effects on growth performance, blood parameters, immune system and general physiological health have been noted [6]–[8]. The amount of information about the diet of brown macroalgae, especially *A. nodosum*, on common carp is quite limited, however. This gap is required because algal supplementation may influence the fish in different ways, depending upon algal species, supplement level, duration of algal feeding, species of fish and environmental conditions.

Previous research [9], [10] suggested that using macroalgae as an aquafeed feed ingredient would be a sustainable alternative due to the nutritional value, functional compounds and availability. Additionally, bioactive compound studies of *A. nodosum* and other brown algae have revealed their potential to modulate physiological processes and boost metabolic responses [11], [12]. Therefore, the incorporation of the seaweed in the fish feed could serve as a useful source of information to be used in developing natural feed additives in fish aquaculture to improve fish productivity and health in aquaculture systems.

The present study was designed to evaluate the growth performance, feed utilization, hematological indices, biochemical parameters, enzyme activity and lipid profile of common carp (*Cyprinus carpio*) fed different doses of *Ascophyllum nodosum* powder for a feeding period of 60 days and 90 days. This objective was to find out if the same brown seaweed was suitable as a functional feed additive to enhance productive and physiological responses in common carp.

Materials and Methods

Fifty six (56) common carp (*Cyprinus carpio* L.) were collected from the local fish ponds in Hella, Iraq for use in the experiment. The initial weight of the fish was about 84.5 g on average. To reduce handling stress and to acclimate the fish to the new environment, all the fish were acclimated to the laboratory for 21 days prior to the feeding trial and acclimated to the experimental feeding condition by offering a commercial pellet diet.

The fish were randomly distributed (completely randomized design) among the four dietary treatments with two replicates per treatment and seven fish per replicate. Eight plastic tanks filled with 70 L of water were used for the experiment, and were constantly aerated with air compressors and aquarium pumps. Siphoning of the tanks was done every day to remove uneaten feed and excretions, and to ensure good rearing conditions.

Four experimental diets were developed to examine the impact of four different powdered *Ascophyllum nodosum* diets. The first one served as a control and received no supplementation of algal diet. The 2nd, 3rd and 4th treatments consisted of 5, 10 and 15 g/kg diet of *A. nodosum* powder, respectively. The treatments were thus divided into T1 (lowest level of seaweed supplementation), T2, T3 and T4 (highest level of seaweed supplementation).

The basal diet was formulated using local ingredients like soybean meal, yellow corn, barley, wheat and fish meal. The *A. nodosum* powder was added to the diets at the necessary rate per treat. The ingredients were combined with water to make a dough and then the dough was minced into pellets with an electric mincer. Dried for three days at room temperature and then broken into proper size for feeding the pellets. The fish were fed twice daily at 3% of their body weight at 1.00 pm and 8.30 am. Fish were weighed to determine the changes in biomass and feed was adjusted every two weeks.

The growth performance was evaluated after 60 and 90 days of the experimental period. The growth parameters measured were final body weight, weight gain, daily weight gain, relative growth rate (RGR) and specific growth rate (SGR). The feed utilization was evaluated based on feed conversion ratio, feed efficiency ratio and protein efficiency ratio. The methods mentioned in the study were used to compute these parameters.

At 60 and 90 days of the feeding trial, blood samples were taken. Three fish were taken from each treatment and were anaesthetised with 100% clove powder before taking the fish. The blood was taken from the caudal peduncle; the whole blood samples were put in heparinized tubes for hematologic examination. The blood indices such as RBC count, hemoglobin concentration, hematocrit, mean corpuscular hemoglobin concentration, platelet count, plateletcrit, mean platelet volume and leukocytic parameters were calculated using a fully automated hematological analyzer.

The common carp was fed *A. nodosum*-supplemented diets and biochemical analysis was carried out to assess the physiological response of common carp fed *A. nodosum*-supplemented diets. The parameters measured were: Glucose, Creatinine, Total Protein, Albumin, Aspartate Aminotransferase, Alkaline Phosphatase, Cholesterol, Triglycerides, Low-density lipoprotein and High-density lipoprotein. The metabolic activity and changes in lipid profile and liver enzyme reaction were evaluated in all the experimental groups.

The data obtained were analyzed with SPSS software 20. One-way analysis of variance (ANOVA) was used to analyze this data under completely randomized design (CRD) in general linear model procedure. Duncan's multiple range test was used to compare the differences in treatments means and $P < 0.05$ were considered significant.

Results

The effects of dietary supplementation with *Ascophyllum nodosum* on growth performance, feed utilization, hematological parameters, biochemical parameters, enzymatic activity and lipid profile of common carp were summarized and presented in six figures. Control diet (T1) and the diets supplemented with 5, 10 and 15 g/kg *A. nodosum* powder (T2, T3 and T4, respectively). All values were expressed as a relative percentage (%) to make comparisons between different parameters with different units possible.

Growth performance

The effects of *A. nodosum* supplementation on common carp growth performance are presented in Figure 1 where it is revealed that the level of supplementation and rearing duration significantly influenced common carp growth performance. The superiority of the final weight, weight gain and relative growth rate over the other treatments resulted in T4 experiment much higher improvements in 60 days. The T2 had the least growth response during the same period. The beneficial effect of *A. nodosum* was more evident when fed up to 60 days; as seen in the reduction in final weight and weight gain of the supplemented groups compared to the control group after 90 days of feeding.

Feed utilization

Figure 2 shows the effect of *A. nodosum* on feed utilization efficiency. The 15 g/kg *A. nodosum* diet showed the best feed efficiency ratio, protein efficiency ratio at 60 d and the feed conversion ratio was improved in all supplemented fish groups than in the control. Based on the results, it can be concluded that the high inclusion rate resulted in better feed and dietary protein utilization in the initial feeding stage. When considering other feed utilization indicators, however, all of the groups had negative values after 90 days of supplementation, which is associated with the negative weight gain response, and the absence of any continued beneficial response with longer supplementation periods.

Hematological indices

The dietary requirement of common carp for response is shown in fig 3. The results showed that supplementation with *A. nodosum* significantly increased some of the blood parameters such as red blood cell count, hemoglobin concentration and hematocrit. At 60 days, their blood oxygen transporting capacity, in terms of both RBC and HGB and HCT, were most improved, meaning that T4's blood physiology was best. The RBC, HCT and platelet counts remained relatively high in T4 after 90 days, with some parameters, like hemoglobin and MCHC, being higher in other groups. Overall, the hematological results indicate that higher concentrations of *A. nodosum* could be beneficial in the blood status of the common carp.

Blood biochemical parameters

The dietary treatment and duration of feeding had a significant influence on blood biochemical parameters as shown in Fig. 4. The glucose concentration increased

slightly in the control group as compared to the supplemented groups particularly after 60 days. The supplementation with treatments resulted in a rise in the level of total protein, with the highest total protein level observed in the T4 experimental group after 60 days, which could be attributed to a better protein metabolism and nutrition status. There was a slight improvement in some groups receiving albumin as well, especially after 90 days. Based on the results obtained, the carbohydrate and protein metabolism of common carp may be modified by feeding with *A. nodosum*.

Blood enzymatic activity

The changes in the enzyme and creatinine in experimental groups are summarized in Figure 5. Creatinine level became lower in T3 and T4 after 60 days of treatment than in the control while T2 showed the highest creatinine level after 90 days of treatment. The activity of ALT, AST and ALP was significantly different between the treatments and periods. At 60 days and 90 days, T4 activity was higher for those treatments that contained AST; T2 was higher for 60 days; and T2 and T4 were higher for 90 days. The activity levels of ALP were increased in control group and decreased in supplemented groups notably T4. The differences suggest that *A. nodosum* supplementation affected the metabolism and enzyme activities of carp.

Lipid profile

The lipid profile reaction is depicted in figure 6. The cholesterol level of the control group was higher at 60 days, with the lowest level for T4. The triglyceride levels were different for the treatments; greater at 60 days for T2 and 90 days for T3. The LDL level was higher in the control group, and lower in the supplemented groups after 60 days. The control group also had higher level of HDL than the supplemented treatments. Based on this result, the effect of *A. nodosum* on lipid metabolism of common carp could be concluded, but the direction of the effect depended on the lipid fraction, the amount of *A. nodosum* supplementation and the duration of the feeding.

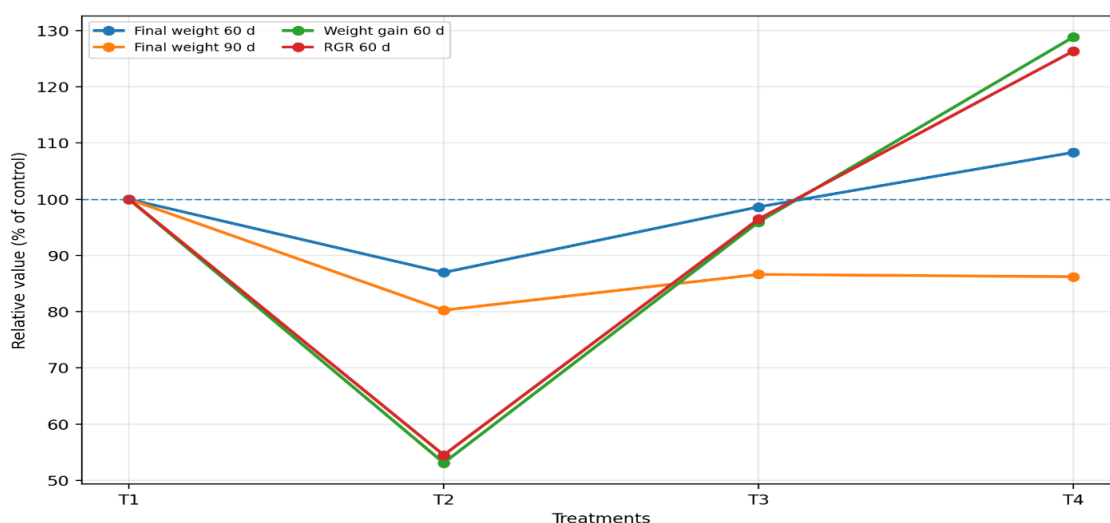


Figure 1. Effect of *A. nodosum* on growth performance of common carp

This figure summarizes final weight, weight gain, and relative growth rate across dietary treatments.

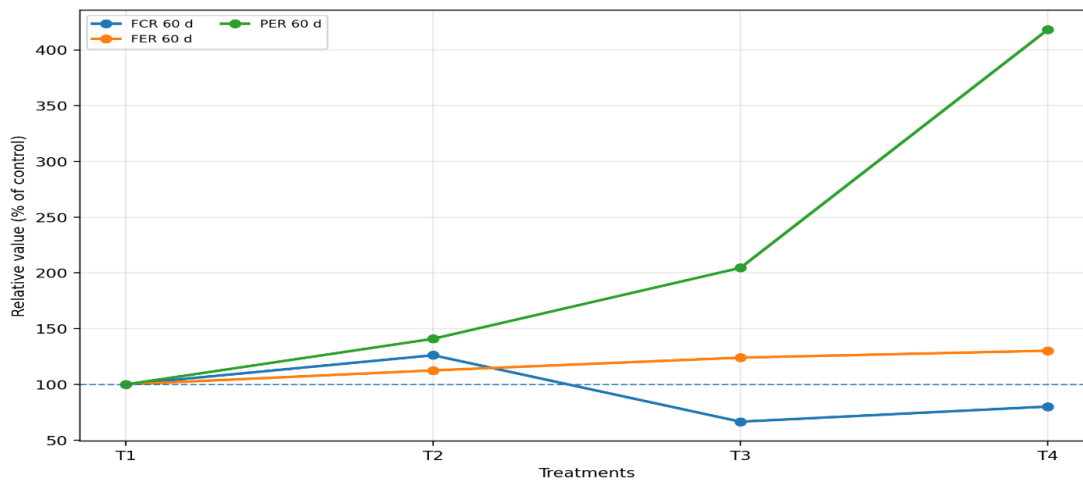


Figure 2. Effect of *A. nodosum* on feed utilization efficiency of common carp
This figure shows changes in FCR, FER, and PER among the experimental groups.

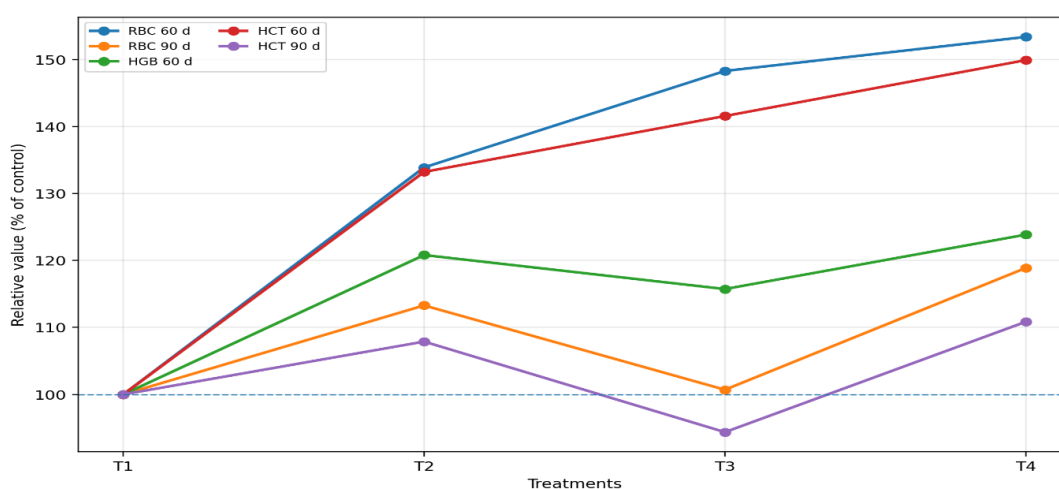


Figure 3. Effect of *A. nodosum* on hematological indices of common carp
This figure presents the main blood indices, including RBC, HGB, and HCT.

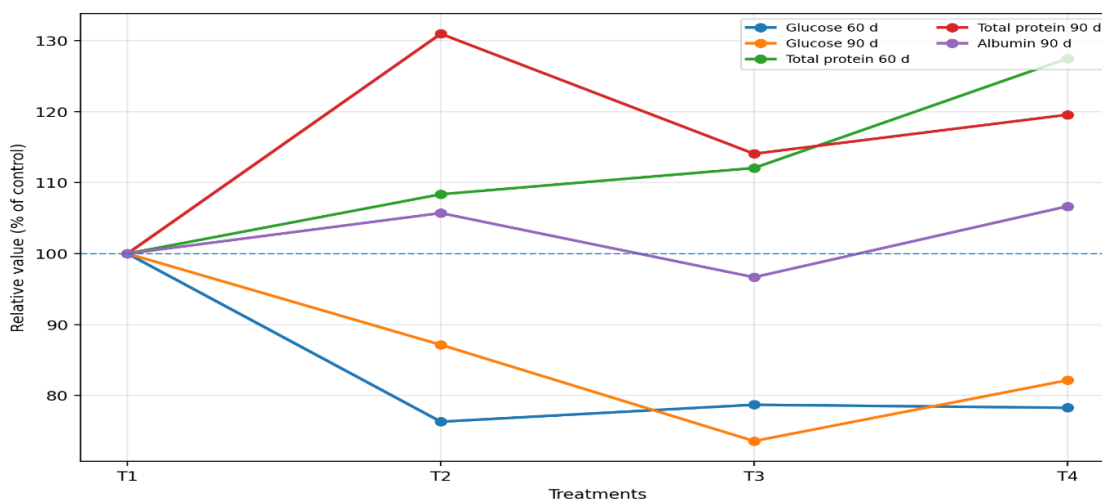


Figure 4. Effect of *A. nodosum* on blood biochemical parameters of common carp
This figure summarizes glucose, total protein, and albumin responses to dietary supplementation.

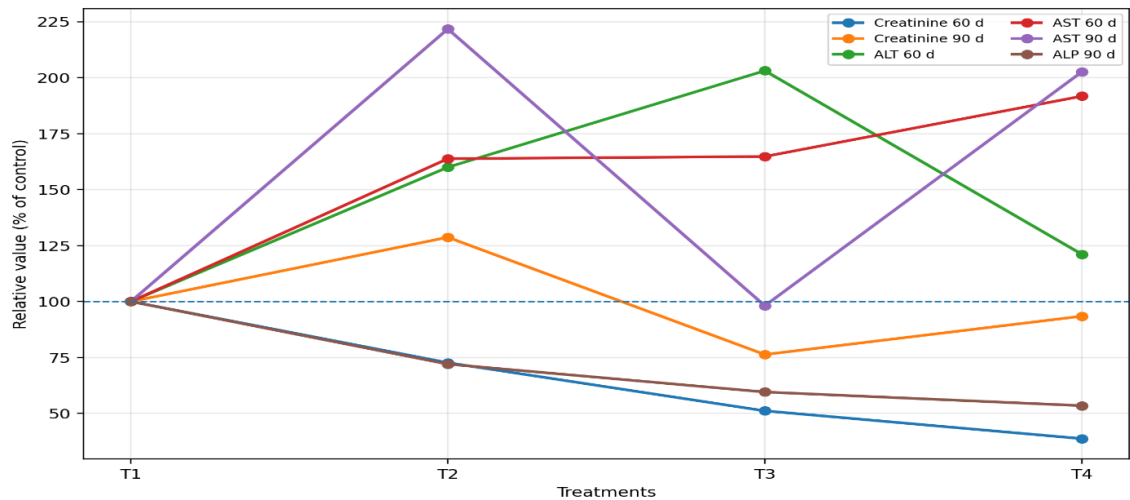


Figure 5. Effect of *A. nodosum* on blood enzymatic activity of common carp
This figure presents changes in creatinine, ALT, AST, and ALP among treatments.

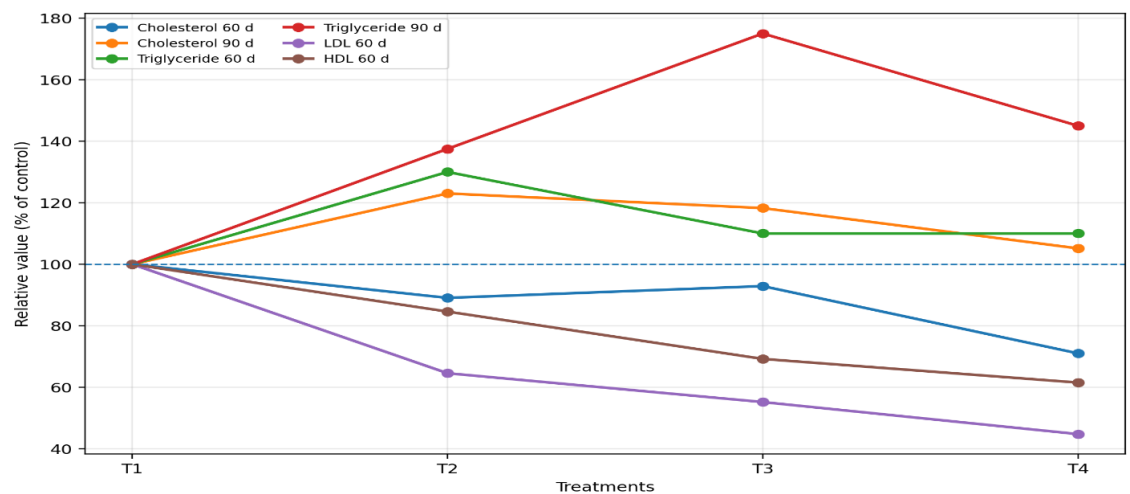


Figure 6. Effect of *A. nodosum* on lipid profile of common carp
This figure summarizes cholesterol, triglyceride, LDL, and HDL levels across treatments.

Discussion

The results of the present research indicated that the feeding of *Ascophyllum nodosum* powder resulted in inconsistent effects on growth performance, feed utilisation, haematological parameters, biochemical parameters, enzymatic activity and lipid profile of common carp. The type of response is clear and is related to both feeding rate of the seaweed, and feeding time. The most positive response was observed when fed after 60 days, particularly for the fish fed the diet with 15g/kg *A. nodosum*, but was not maintained after 90 days.

The nutritional and functional properties of brown seaweed might have contributed to this improvement in the growth rate of T4 after 60 days. The bio-active compounds, mineral, vitamins, and polysaccharide in *A. nodosum*, along with other growth promoting compounds, might assist to improve digestion, absorption of nutrients, and metabolic activity. This is consistent with the previous studies which reported the use of algae and seaweed based supplements at optimal level to enhance growth and health performance of fish [13], [7], [8]. The

sustainable aquafeed ingredients of macroalgae were also mentioned by [10] which reported the potential nutritional value of macroalgae in aquaculture.

The improved feed and protein efficiency of higher supplementation level may be attributed to improved utilization of feed nutrients. Polysaccharides and other bioactive compounds in brown algae can be stimulatory for digestion and can result in better feed conversion ratio. This interpretation is in line with the study carried out by [9] in which they found that compounds present in seaweeds have functional properties having nutritional and biological relevance. The negative growth and feed utilization found in some of the supplemented groups after 90 days however suggest that the positive effect may be cancelled out, or there may be an imbalance in the utilization of the nutrients, if the animals are fed for longer. The effect of *A. nodosum* thus seems to be dose as well as time dependent.

Hematological parameters were positively affected by supplementation with seaweed especially at the higher rate. The increase of red cell count associated with the increase of hemoglobin and hematocrit in T4 might be associated with improvement of the physiological status and oxygen transport capacity. Changes may be linked to anti-oxidant and bioactive properties of algae, which can help maintain stability of blood cells and decrease physiological stress. Algal compounds are reported to possess antioxidant properties, and to also influence cell activation and immune related responses in a beneficial manner [1], [2].

Biochemical results showed that the supplementation of *A. nodosum* had an effect on carbohydrate and protein metabolism. The difference of the glucose level between the supplemented groups and the control groups may represent the action of the seaweed components on the regulation of the glucose level. The result is consistent with Paradis et al. [14] who found that brown seaweed can influence the glucose and insulin response. Furthermore, the rise in total protein and albumin in certain supplemented groups, particularly at 60 days, suggests a better nutritional status and increased metabolic activity. The results show that it is possible to manipulate the nutritional balance of fish with the addition of seaweed.

The changes in the activities of creatinine and enzymes indicate that dietary *A. nodosum* affected metabolic and hepatic related responses. The reduction at 60 days after T3 and T4 confirms that they are more physiologically beneficial at the inclusion level or feeding duration, and the increase in T2 after 90 days is interpreted as being less physiologically beneficial at the inclusion level or feeding duration. The difference in ALT, AST and ALP activity among the treatments is also indicative that enzymatic activity is also changeable through seaweed supplementation. The bioactive substances found in the brown algae, such as polyphenols and polysaccharides, have been suggested to influence physiological and enzymatic processes [12], which could be the reason for such effects.

There was a clear change in the lipid profile with treatment. The lower cholesterol and LDL in supplemented groups at 60 days might indicate that *A. nodosum* may be a useful tool for lipid metabolism regulation. However, this was not observed in all supplemented groups, as in some of these the high level of triglycerides was observed at various intervals. This difference might be due to the metabolic adaptation, dose response and duration of feeding. In previous studies, algal supplementation has also shown inconsistent results depending on the species of fish used, the amount of algae fed to the fish, the experimental conditions, among others [15], [16].

In general, the results indicate that *A. nodosum* powder can be used as a potential natural feed additives for common carp especially used as 15 g/kg feed for 60 days. The positive effects could be associated with better growth, feed utilization, haematological parameters and some biochemical responses. However, the reduction of growth rate after 90 days of supplementation implies that it may not be necessary to supplement for longer periods in all cases. Additional research is warranted to explore the effectiveness of the dose and duration of *A. nodosum* supplementation and elucidate the mechanism by which *A. nodosum* affects metabolism, immunity and physiological performance in common carp.

Conclusion

This study shows that dietary supplementation with *Ascophyllum nodosum* powder has a potential to affect both the productive and the physiological response of common carp (*Cyprinus carpio*). Growth performance, feed utilization, some hematological and biochemical parameters yielded the best results with 15 g/kg of inclusion which exhibited improvement after 60 days feeding. Overall, the results indicate that brown seaweed can be considered a valuable natural feed ingredient to promote fish health and performance when fed appropriately. But a reduction in the growth response following 90 days suggests that it's not a good idea to supplement for too long. Therefore, the optimal dose and time of *A. nodosum* application in the diets of common carp need to be optimized and further studies are needed to confirm the optimal dose and time of application.

References

- [1] A. Q. Hurtado, D. A. Yunque, K. Tibubos, and A. T. Critchley, "Use of Acadian marine plant extract powder from *Ascophyllum nodosum* in tissue culture of *Kappaphycus* varieties," *Journal of Applied Phycology*, vol. 21, no. 6, pp. 633–639, 2009, doi: 10.1007/s10811-008-9395-4.
- [2] E. Rouhani, R. Safari, M. R. Imanpour, S. H. Hoseinifar, M. Yazici, and E. El-Haroun, "Effect of dietary administration of green macroalgae (*Ulva intestinalis*) on mucosal and systemic immune parameters, antioxidant defence, and related gene expression in zebrafish (*Danio rerio*)," *Aquaculture Nutrition*, vol. 2022, Art. no. 7693468, 2022, doi: 10.1155/2022/7693468.
- [3] J. Fleurence and I. A. Levine, *Seaweed in Health and Disease Prevention*, 2016, doi: 10.1016/C2014-0-02206-X.
- [4] O. Babich *et al.*, "Algae: Study of edible and biologically active fractions, their properties and applications," *Plants*, vol. 11, no. 6, Art. no. 780, 2022, doi: 10.3390/plants11060780.
- [5] J. Espinosa-Ramírez, A. C. Mondragón-Portocarrero, J. A. Rodríguez, J. M. Lorenzo, and E. M. Santos, "Algae as a potential source of protein meat alternatives," *Frontiers in Nutrition*, vol. 10, Art. no. 1254300, 2023, doi: 10.3389/fnut.2023.1254300.
- [6] N. Abdulrahman and H. Hamad Ameen, "The effect of replacing fishmeal with *Spirulina* on growth and productivity of common carp *Cyprinus carpio* L.," *American Journal of Scientific Research*, pp. 188–193, 2013.
- [7] N. Abdulrahman, H. Hamad Ameen, S. Hama, B. Hassan Rahim, and P. Jalal, "Effect of microalgae *Spirulina* spp. as food additive on some biological and

- blood parameters of common carp *Cyprinus carpio* L.," *Iraqi Journal of Veterinary Sciences*, vol. 33, pp. 27–31, 2019, doi: 10.33899/ijvs.2019.125527.1049.
- [8] H. Sleman, N. Abdulrahman, N. Hassan, and H. Hama-Salih, "Evaluation of blood, biochemical and biological effects of microalgae *Chlorella* and germinated barley powder as a source of prebiotic on common carp *Cyprinus carpio* L.," *Iraqi Journal of Veterinary Sciences*, vol. 35, pp. 271–277, 2021, doi: 10.33899/ijvs.2020.126788.1378.
- [9] S. L. Holdt and S. Kraan, "Bioactive compounds in seaweed: Functional food applications and legislation," *Journal of Applied Phycology*, vol. 23, no. 3, pp. 543–597, 2011, doi: 10.1007/s10811-010-9632-5.
- [10] A. H. L. Wan, S. J. Davies, A. Soler-Vila, R. Fitzgerald, and M. P. Johnson, "Macroalgae as a sustainable aquafeed ingredient," *Reviews in Aquaculture*, vol. 11, no. 3, pp. 458–492, 2019, doi: 10.1111/raq.12241.
- [11] H. S. Kang, H. R. Kim, D. S. Byun, B. W. Son, T. J. Nam, and J. S. Choi, "Tyrosinase inhibitors isolated from the edible brown alga *Ecklonia stolonifera*," *Archives of Pharmacal Research*, vol. 27, no. 12, pp. 1226–1232, 2004, doi: 10.1007/BF02975886.
- [12] M. Gisbert, D. Franco, J. Sineiro, and R. Moreira, "Antioxidant and antidiabetic properties of phlorotannins from *Ascophyllum nodosum* seaweed extracts," *Molecules*, vol. 28, no. 13, Art. no. 4937, 2023, doi: 10.3390/molecules28134937.
- [13] N. M. Abdulrahman, "Evaluation of *Spirulina* spp. as food supplement and its effect on growth performance of common carp fingerlings," *International Journal of Fisheries and Aquatic Studies*, vol. 2, no. 2, pp. 89–92, 2014.
- [14] M. E. Paradis, P. Couture, and B. Lamarche, "A randomised crossover placebo-controlled trial investigating the effect of brown seaweed (*Ascophyllum nodosum* and *Fucus vesiculosus*) on postchallenge plasma glucose and insulin levels in men and women," *Applied Physiology, Nutrition, and Metabolism*, vol. 36, no. 6, pp. 913–919, 2011, doi: 10.1139/h11-115.
- [15] S. Moutinho, F. Linares, J. L. Rodríguez, V. Sousa, and L. M. P. Valente, "Inclusion of 10% seaweed meal in diets for juvenile and on-growing life stages of Senegalese sole (*Solea senegalensis*)," *Journal of Applied Phycology*, vol. 30, no. 6, pp. 3589–3601, 2018, doi: 10.1007/s10811-018-1482-6.
- [16] G. Sattanathan *et al.*, "Effects of dietary blend of algae extract supplementation on growth, biochemical, haemato-immunological response, and immune gene expression in *Labeo rohita* with *Aeromonas hydrophila* post-challenges," journal details incomplete in the supplied reference, vol. 8, no. 1, Art. no. 7, 2023.