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Effects of plant- Ulva lactuca based diet supplementation with exogenous enzymes or yeast on growth performance, feed utilization, and biochemical parameters in Nile tilapia (Oreochromis niloticus)."

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ABSTRACT : **The growing aquaculture sector demands the development of sustainable and cost-effective feed ingredients. A feeding trial was conducted to determine the effect of supplementation of of plant-based diets of Ulva lactuca (PL-Ulva) with exogenous enzymes (mono or multi-enzyme complex) and yeast on growth performance ,feed utilization and biochemical parameters of Nile tilapia (Oreochromis niloticus). Six isonitrogenous and isocaloric diets were formulated to supply 28% protein and 425 kcal/100 g diets. The treatments were: (1) positive control diet (fishmeal-based), (2) negative control diet (PL-Ulva based diet), (3) PL-Ulva based diet + 1.50 g phytase/kg, (4) PL-Ulva based diet + 1.50 g xylanase/kg and (5) PL-Ulva based diet + 3 g yeast /kg (6) PL-Ulva based diet + 1.50 g multi-enzyme complex /kg.The fish that were fed the fishmeal-based diet and the PL-Ulva diets supplemented with different enzymes or yeast had significantly (p d with) better growth performance and survival rate values compared to those fed PL-Ulva alone. The highest values of blood parameters (Hb, RBCs, and Ht) were observed in fish fed the multi-enzyme supplemented diet. There were no significant differences in total protein serum and albumin among the experimental treatments. The results suggest that supplementation of a plant-Ulva based diet with enzymes or yeast can improve the nutrient utilization and growth performance of Nile tilapia while remaining cost-effective.**

KEYWORDS: **Nile tilapia, Ulva, plant diets, exogenous enzymes, yeast, growth performance, biochemical parameters**.

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I. INTRODUCTION

Fishmeal is the primary protein source for aquaculture diets because it has a high protein content, well balanced amino acid and fatty acid composition and high digestibility and palatability (Yildirim et al., 2014). However, the use of fishmeal in aquatic feeds is limited by the high price and decline of fishery resources. Additionally, the excessive use of fishmeal with high phosphorous content in aquatic feeds can lead to

environmental problems.Minimizing the proportion of fishmeal in formula feeds is thus important for reducing phosphorus excretion by farmed fish (Yang et al., 2011).

In recent years, increasing attention has been placed on the search for alternative sources of affordable and high-quality ingredients to replace fishmeal in aquatic feeds.Plant ingredients are often used in lieu of fishmeal to reduce cost, improve sustainability, and provide an alternative high-quality protein.However, plant components such as soybean and rapeseed meal are high in non-starch polysaccharides (NSP) and phytate, which are undesired in fish diets due to their anti-nutritional properties (Sinha et al., 2011).

Macroalgae or seaweed has great potential as an alternative feed material because it is both nutritional and easy-to culture (Wassef et al., 2013). In general, the major components of seaweed are complex polysaccharides, proteins, lipids, ash, and minerals (Wi et al., 2009). Ulva species have a good vitamin and mineral profile and are especially rich in ascorbic acid (Garcı´a-Casal et al., 2007). To expand the use of plant-based protein for fish feed, it is essential to develop adequate processing technologies to sufficiently remove or degrade anti-nutritional factors (ANFs) from plant feed ingredients. The use of natural bioactive agents and exogenous enzymes for this purpose is gaining much attention (Hlophe-Ginindza et al., 2016). The addition of exogenous enzymes to fish diets containing a high proportion of plant protein can specifically degrade certain ANF's; greatly enhancing the nutritional value (Dalsgaard et al., 2012).

Supplementing fish feed with an enzyme or mixture of enzymes that possess a broad spectrum range of activities may improve plant ingredient digestibility resulting in improved growth performance in cultured fish species such as Pangus catfish (Debnath et al., 2005), tilapia (Drew et al., 2005) and salmon (Odetallah et al., 2005). However the digestibility of all nutrients, including carbohydrates, protein, and minerals also seem to be affected by the addition of exogenous enzymes (Felix and Selvaraj, 2004).

Phytase enzymes can be used to improve nutrient utilization and growth rate and to reduce phosphorus pollution in the fish environment (Kumar et al. 2012). phytase catalyzes the hydrolysis of phytic acid and phytate , consequently releasing phosphorus and other minerals or protein associated with the phosphate ions. Thus, the addition of this enzyme to fish diets can remove apportion of the undesired substances, reducing production costs and improving husbandry outcomes (Rachmawati and Samidjan, 2016).

The addition of yeast to fish feed can improve immune response and promote growth in farmed fish. Baker's yeast, Saccharomyces cerevisiae is a particularly important natural bioproduct that contains immune-stimulating compounds such as nucleotides, β-glucan, mannan oligosaccharides, and chitin (Abdel-Tawwab, 2012). Commercial brewer's yeast is inactive yeast (dead yeast cells) that is a byproduct of brewing. The cell wall, which can comprise 200–250 g/kg of the dry weight of the cell, consists of about 85%–90%. The polysaccharide component consists of a mixture of mannan, glucan, and small amounts of chitin (Nguyen et al., 1998).

The present study was conducted to evaluate the effect of Ulva lactuca plant based diets supplemented with mono or multi-enzyme complexes and yeast on growth performance, feed utilization and biochemical parameters of Nile tilapia (Oreochromis niloticus).

II. FIM IN RECORD VALUES AND THEIR CONCOMITANTS FOR HK-FGM

Enzymes and diet formulation and preparation:

The following enzyme supplements were added to the fish feed treatments (Table 1).

Phytase a powdered micro-granulated phytase enzyme preparation that contains 10,000 FTU/g(Danisco Animal Nutrition, Wiltshire, United Kingdom). Xylanase® enzyme and yeast product (Kemin Industries Inc., Des Moines, U.S.A).Natuzyme® a powdered micro-granulated multi-enzyme preparation feed (Bioproton Pty Ltd., Sunnybank, Australia).

The Ulva lactuca seaweed was collected from Alexandria beach and washed well in freshwater to eliminate salts, algae, and outruns. The seaweed was then dried at a temperature of 60–70 °C to avoid releasing the nutrients

of importance to marine fish larvae.The dried seawee was ground in grinder mixer and stored in plastic sacs until added to the diets.

Six isonitrogenous and isocaloric diets were formulated with natural ingredients to provide a 28% protein and 425 kcal/100 g diet according to the known nutritional requirements of tilapia (NRC, 2011) (Table 2 and 3). Treatments were as follows: T1 contained herring fishmeal as the main protein source (FM-based) to serve as a positive control, T2 was a plant-only diet containing Ulva (PL-Ulva) that served as a negative control, T3 (PL-Ulva + Phytase, 2000 IU/kg), T4 (PL-Ulva + Xylanase, 1500 IU/kg), T5 (PL-Ulva + yeast, 1.5 g/kg) and T6 was a multi-enzyme diet (MEM) (PL-Ulva + MEM, 1.5 g/kg).

The dietary ingredients were homogeneously ground to 500 µm and thoroughly mixed. A sufficient amount of water (about 400 ml/kg diet) was added and mixed to obtain stiff dough which was passed through a 1.5 mm diameter mincer. The diet pellets were air dried with an electric fan at room temperature for 24 h. All diets were packed in sealed plastic bags and stored at 4 °C until use.

Fish and husbandry conditions:

This research was conducted according to the local ethical and animal welfare guidelines in Egypt; the official decrees of the Ministry of Agriculture in Egypt relevant to animal welfare are No. 27 (1967) that enforces the humane treatment of animals in general.

The experiment was performed at Abbassa, Abu-Hammad, Sharkiya governorate, Egypt. Nile tilapia (Oreochromis niloticus) fry were obtained from CLAR hatchery ponds. Fish were held in an indoor tank and fed the basal diet (T1) for two weeks for acclimation to the laboratory conditions prior to the trial. Twenty fish with an average initial body weight of (5.14±0.08 g) were weighed and stocked into fifteen100 L glass aquaria (three replicates of five treatments). Half of the water in each aquarium was changed daily to avoid the accumulation of the metabolites. Each aquarium was supplied with an air stone for continuous aeration using an electric air pump to maintain the oxygen level. All fish were fed to apparent satiation, twice per day, six days per week for twelve weeks. During the course of the experiment, all fish were collected from each aquarium every two weeks and collectively weighed.

Sampling, analytical Procedure and measurements:

Fish from each tank were sampled at the beginning and end of the trial, dried and immediately stored at −20 °C pending analyses. Diet and carcass samples were submitted to proximate composition analysis according to the standard methods of AOAC (1990) for moisture, crude protein, total lipids, and ash. Moisture content was estimated by drying the samples at 85°C in a drying oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA) until a fixed weight was achieved. Crude protein was estimated by multiplying the nitrogen content which was determined using a micro-Kjeldahl apparatus (Labconco Corporation, Kansas, Missouri,USA) by 6.25. Lipid content was determined by petroleum ether extraction in a Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois,USA) at 40-60 °C for 16 h. Ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 °C for 6 h. Crude fiber was estimated according to Goering and Van Soest (1970) and the nitrogen free extract (NFE) was calculated as:

NFE (%) =100 − (% crude protein + % crude lipid + % crude fiber + % ash). Gross energy was calculated according to NRC (1993).

Growth performance parameters:

The following equations were used to calculate the weight gain, daily weight gain, specific growth rate and survival rate, respectively.

Weight gain (WG) = W1-W0. Daily weight gain (DWG) = (W1-W0)/T.

Specific growth rate (SGR%/day) = [(Ln W1− Ln W0)/T] × 100.

Where, Ln = natural log, W0 = Initial body weight (g), W1= Final body weight (g) and T= time (day).

Survival rate (%) = 100 × (number of fish at the end/number of fish stocked at the beginning).

Feed utilization parameters:

The following equations were used to calculate the feed intake, feed conversion ratio, protein efficiency ratio, protein productive value and energy retention, respectively.

Feed intake (FI) = total feed consumed over 12 weeks (g)/ number of fish.

Feed conversion ratio (FCR) = feed intake (g)/body weight gain (g).

Protein efficiency ratio (PER) = total weight gain (g)/protein intake (g).

Protein productive value (PPV %) = 100 (protein gain/protein intake).

Energy retention (ER %) = 100 (gross energy gain/gross energy intake).

Water quality analysis:

Water samples were collected biweekly from each aquarium for the duration of the experiment. Water temperature and dissolved oxygen were measured with a YSI model 58 oxygen meter (Yellow Spring Instrument Co., Yellow Spring, Ohio, USA) and pH was measured with a pH–meter (Digital Mini-pH Meter, model 55, Fisher Scientific, USA). Unionized ammonia, total alkalinity and total hardness were determined according to Boyd and Tucker (1992).

Physiological measurements:

At the end of the feeding trial, three fish from each aquarium were taken for physiological investigation. Fish were anaesthetized using buffered tricaine methanesulfonate (20 mg/L), and blood was collected from the caudal vein with a sterile syringe and divided equally among three clean and dry tubes. The first part was centrifuged at 3,000 g for 15 min and the serum was stored at −20◦C for further assays. The second part was mixed with sodium fluoride as an anticoagulant and centrifuged at 3000 g for 15 min for separation of plasma for glucose analysis. The last part was mixed with EDTA solution for measuring hemoglobin (Hb), red blood cell (RBCS), and hematocrite (Hct). Hemoglobin level was determined colorimetrically using a spectrophotometer according to Stopkopf (1983). Hematocrite was determined using the microhaematocrit method (Schalm, 1975). Red blood cells were determined according to the method described by Natt and Herrick, (1952). Total protein content was determined colorimetrically according to Henry, (1964). Colorimetric determination of serum albumin was performed according to (wotton and Freeman, 1982) using a spectrophotometer. Cholesterol is estimated as a colored complex according to the method of (Young, 2001). Creatinine was determined calorimetrically according to Henry, (1974). Glucose was determined colorimetrically according to Trinder (1969). Activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitmanand Frankel (1957).

Amino acids determination

Samples were prepared for hydrolysis according to Blackburn (1978) and Walker (1996) before determination of amino acids. Amino acid analyses were carried out using an LC 3000 Eppendorf/Biotronik amino acid analyzer using an H 125×type column at the Regional Center for Food and Feed, Agricultural Research Center Cairo, Egypt. The values of amino acids profile were presented as percent of the total amino acids content.

Statistical analyses:

A one-way ANOVA was performed and values were expressed as the mean ± SD of the replicates. Differences were considered significant if P was less than 0.05. All statistical analyses were conducted using SAS, (SAS Inc., 2002). Significant differences ($p \leq 0.05$) among means were tested by the method of Duncan (1955).

III. RESULTS AND DISCYSSIONS

The water temperature in the tanks ranged from 28.0 to 28.8°C, while pH ranged from 7.4 to 7.8. Dissolved oxygen level (DO) was higher than 5.85 mg DO/L and unionized ammonia concentration was lower than 0.2 mg NH3/L throughout the study period. The total alkalinity and total hardness values ranged from 125

to 165 mg/L and 165-180 mg/L as CaCO3, respectively. No significant differences in water quality parameter were detected among the treatments.

The growth performance parameters [final body weight (FBW), daily weight gain (DWG) and specific growth rate (SGR %)] of Nile tilapia, O. niloticus, fed at different experimental diets for 12 weeks period are presented in Table 3. FBW, WG and SGR were significantly ($p \leq 0.05$) affected treatment. The fishmeal based diet (FM) and multi-enzyme based diet (MEM) produced the highest growth performance parameters with no significance difference between the two treatments. There were no significant differences in FBW, weight gain (WG), relative body weight gain (RBWG %) and SGR between the phytase based diet (PHY), xylanase based diet (XYL) and yeast based diet treatment. The negative control produced the lowest values of all performance parameters, as well as a significantly lower survival rate (76.67%). There were no significant differences in survival rate among the other treatments.

The results of the feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV %) and energy retention (ER %) of fish fed different experimental diets are presented in Table 4. The FI, results revealed that, fish fed the fishmeal based diet (FM) diet had the highest feed intake, followed by fish fed the (MEM) multi-enzymes based diet. However there were no significant differences in FI between the (FM), (PHY), (XYL), yeast and (MEM) diets.

The mean FCR values were significantly better (lower) in fish that were fed the FM-based diet and the Ulva plant diets supplemented with enzymes or yeast compared to those fed the PL-Ulva diet.The poorest FCR value ($p \leq 0.05$) was 1.81 in the fish fed the negative control(PL-Ulva) . There were no significant differences ($p > 0.05$) observed in PER values among different treatments. Fish fed the FM diet exhibited higher energy utilization while there were no significant differences between the FM, XYL, yeast, and MEM diets. The highest PPV was 35.10%, which was observed in the fish fed the XYL diet. However, there were no significant differences among the FM, PHY, XYL, yeast, and MEM diets. The lowest PPV values were observed in the fish fed the PL-Ulva diet (negative control).

Hematological and biochemical parameters:

There were no significant differences in Hb, RBCs, and Ht among the FM, MEM, PHY, XYL, and yeast diets (table5). The highest blood parameter values (Hb, RBCs, and Ht) were observed in the fish fed the MEM diet (10.80 g/dl, 2.01 cmm, and 24.95%, respectively), while the lowest were recorded in the fish fed the PL-Ulva diet (8.70 g/dl, 1.49 cmm, and 18.32 %, respectively). Nile tilapia fed the FM diet exhibited a higher MCV value, although, the difference was not significant compared to the MEM, PHY, XYL, and yeast diets. There were no significant differences in WBC, platelets, and lymphocytes among the experimental treatments, except for the fish fed the MEM diet, which exhibited higher values for these parameters.

There were no significant differences in total protein serum and albumin among the experimental treatments (Table 6). Fish that were fed fish the FM diet had the highest total protein (2.65 g/dl), followed the MEM diet (2.64 g/dl), while the lowest total protein was recorded in fish fed the PL-Ulva diet (2.1 g/dl).

There were no significant differences in serum glucose and cholesterol between fish fed the FM, MEM, PHY, and XYL diets. There were also no significant differences in creatinine levels; however, the lowest creatinine value was recorded for the MEM diet (0.14 mg/ dl).

The highest value of serum aspartate aminotransferase (AST) (16.03 U/L) was observed in fish fed the PL-Ulva diet and there were no significant differences among the treatments. Serum alanine aminotransferase ALT levels decreased in the other treatments and increased in the PL-Ulva treatment, while the lowest value was recorded with the MEM diet (7.23 U/L).

Amino acid profile of studied fish

The 17 amino acids detected in the amino acid profile of the fish are presented in Table 7. The most abundant amino acids in all fish were lysine, leucine, valine, and therionine. In contrast, for NEAA, the major amino acids in all fish were glutamic acid, glycine, aspartic acid, and alanine.

The data collected from this study demonstrate that growth performance in terms of FBW, WG, relative body weight gain (RBWG %), and SGR of Nile tilapia were significantly affected by the experimental feed treatments. The Ulva plant-based diet (negative control) produced the lowest growth performance parameters. This result may be attributed to the presence of ANFs, high levels of fiber and NSP, which reduce digestion (Hassaan et al., 2017).

The fish that were fed the PL-Ulva diet supplemented with phytase had higher mean WG and SGR than the fish fed PL-Ulva alone (negative control). The improvements in growth performance could be attributed to the hydrolysis of phytic acid and phytate, which releases phosphorus and other minerals or proteins associated with the phosphate ions. Phytases increase the bioavailability of dietary amino acids and dietary energy (Selle et al., 2010 and improve phytate digestibility and bioavailability of phosphorus (P) due to high absorption of calcium (Ca), magnesium (Mg), zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu) divalent cations (Sardar et al., 2007). Moreover, phytase reduces the amount of supplementary organic phosphorus (P) needed to reach high levels of growth, increase bone mineralization (Cao et al., 2008), and improve the utilization of dietary protein and energy (Cheng and Hardy, 2004).

Olusola and Nwanna (2014) also report that phytase inclusion in the diet of O. niloticus is a potential and promising dietary supplement that positively influenced the growth of farmed O. niloticus. Additionally, Amer et al. (2019) reported that the highest significant means ($p \leq 0.05$) of growth performance and survival rate values were achieved by fish fed an FM-based diet and an SBM-based diet supplemented with 1.0 g phytase/kg compared to treatments without phytase.

The growth performance of Nile tilapia also improved with xylanase supplementation to the PL-Ulva diet. These results may be attributed to the ability of xylanase to disrupt plant cell wall integrity, thereby reducing the molecular size of NSPs. Consequently, digestion efficiency is improved by reducing viscosity in the gut (Adeola and Cowieson, 2011). Furthermore, exogenous enzymes may affect substrate availability for specific populations of gut microbes that promote digestion and synthesize nutrients that the fish need (Jiang et al., 2014; Zhou et al., 2013).

Improved growth performance through xylanase supplementation was also reported in juvenile Jian carp (Jiang et al., 2014). However, Dalsgaard et al. (2012) indicated that xylanase supplementation did not affect the growth of rainbow trout (O. mykiss).

The results of the present study revealed that the PL-Ulva diet supplemented with multi-enzymes produced the highest growth performance parameters with no significant difference compared to the FM diet. This result demonstrates that the use of multiple enzymes together is more effective for improving the growth response than using them individually. It has been previously reported that using purified enzymes is less effective for improving performance than mixtures of a number of different enzymes together (i.e., “enzyme cocktails”) (Graham and Inborr, 1993).

Similar results of multi-enzyme mixture enhancement of growth performance were reported by Khalafalla et al. (2010). They demonstrated that the addition of a cocktail of amylease, xylanase, protease, cellulose, lipase, phytase, b-glucanase and alphagalactosidase enzymes (amecozyme®) at levels of 0.5 and 1.0% in the diet improved the growth performance of Nile tilapia, Oreochromis niloticus, fingerlings. Similarly, Amer (2017) reported that supplementation of SBM-based diets with a multi-enzyme complex; Natuzyme® (NZ) maintained a similar growth rate as the FM-based diet.

With regard to yeast, Hoseinifar et al. (2011) reported that dietary supplementation of 2% S. cerevisiae var. ellipsoideus significantly improved FCR compared to the control treatment. Hassaan et al. (2018) reported that yeast extract resulted in the best nutrient utilization compared to other treatments. In this study, the PL-

Ulva diet supplemented with yeast increased the growth rate of Nile tilapia more than the PL-Ulva alone (negative control). This result may due to the immune-stimulating compounds in yeast, such as nucleotides, β-glucan, mannan oligosaccharides, and chitin, which have been proven to influence fish immune response and promote growth (Abdel-Tawwab, 2012).

There were no significant differences in survival rate among those fish fed the FM diet and the PL-Ulva diets supplemented with different enzymes or yeast, while the fish fed PL-Ulva alone (negative control) exhibited significantly lower survival rates. The exogenous enzyme supplementation did not exert any negative effect on the survival rate of tilapia among treatments in the present study. Similarly, Mahmoud et al. (2014) reported that the inclusion of commercially prepared exogenous multi-enzyme preparations (Pan Zyme and Phytase-plus broiler 500) had no effect on the survival rates of Nile tilapia fed SBM-based diets.

In the present study, supplementation of Ulva lactuca plant-based diets with mono-or multi-enzymes or yeast significantly improved FI and FCR of O. niloticus fingerlings. The increased palatability and conversion rate of the diet may be attributed to improved release of nutrients from plant-based diets through the degradation of the majority of ANFs and the bonds between phytate-protein and phytate-mineral complexes, as well as the improvement in protein digestion and amino acid absorption. The exogenous multi-enzymes may enhance the palatability of the plant diets (Deguara et al., 1999).

These results were consistent with those reported by Liebert and Portz (2005) who found significant improvements in FCR and PER due to phytase addition to plant-based diets. Amer et al. (2019) also demonstrated that the addition of phytase to soybean meal-based diets at a rate of 1000 FTU/kg can improve the nutrient utilization (FCR, PER, PPV %, and energy retention %).

For xylanase supplementation, Jiang et al. (2014) indicated that feed efficiency, PER, protein production value improved with increasing levels of xylanase up to a point, and thereafter declined. Similar results were observed by (Ai et al., 2007) where xylanase increased the PPV and PR in Japanese sea bass (L. japonicus). In addition, Cheng et al. (2018) reported that fish that were fed diets containing 1500 and 1950 EPU X-xylanase had significantly better feed efficiency compared to fish fed the commercial diet and other experimental diets.

Similar positive results were obtained by Lin et al. (2007) who reported that supplementation with a commercial enzyme complex (neutral protease, β- glucanase and xylanase) significantly improved feed utilization of juvenile hybrid tilapia (Oreochromis niloticus × O. aureus).

Hematological and biochemical parameters:

The results of this study indicate that the hematological parameters of hemoglobin, red and white blood cell count, and hematocrit were higher in the Nile tilapia fed the FM diet or any of the experimental diets compared to those fed PL-Ulva alone. Similar results were obtained by Xing et al. (2007) who reported that there were significantly higher red blood cell (RBC) numbers, hemoglobin (HGB) levels and mean hemoglobin in red blood corpuscles (MCH) in the fish fed the diets containing 0.10% xylanase than that in the control. Hassaan et al. (2018) reported that blood sample profiles showed an increase in white and red blood cells in fish fed 15 g/kg yeast extract in comparison with the other treatment groups. In contrast, Hoseinifar et al., (2011) reported that hematological parameters and serum biochemical parameters were not significantly affected by dietary yeast.

The concentration of total plasma protein is used as a basic index for the health status of brood fish (Hille et al., 1982; Rehulka et al., 1996). Most serum protein synthesis occurs in the liver and therefore, serum protein can be used as an indicator of liver dysfunction. A reduction in total protein concentration is an obvious feature of many diseases and may occur due to liver disease, absorption reduction, or the loss of protein (Bernet et al., 2001). Our results revealed that compared to those fed PL-Ulva alone, Nile tilapia fed the FM diet and the PL-Ulva diets supplemented with enzymes or yeast had higher values of serum total protein and albumin, with no significant difference between them.

These results are in agreement with these of Xing et al. (2007) who demonstrated that fish fed diets containing 0.05%, and 0.10% xylanase had significantly higher total serum protein and albumin (ALB) levels than the control

fish. A similar trend was observed by Liu et al. (2013) who reported that ALB content increased in grass carp and gibel carp fed with phytase-supplemented diets. Hassaan et al. (2018) also found that fish that were fed diets supplemented with 10 and 15 g/kg yeast extract had significantly higher albumin and globulin levels than the control group.

The determination of glucose concentration in blood serum is a well-known indicator of stress in fish (Martin and Black, 1998) but can also occur due to malnutrition or an injured kidney (Jacobson and Keller, 2001). There were no significant differences in serum glucose and cholesterol between the fish fed experimental diets in the present study. Similarly, Hassaan et al. (2018) reported that the addition of 15 g/kg of yeast extract to the diet caused decreased levels of cholesterol and triglycerides in fish. This finding is supported by Mahmoud et al. (2014) who reported that fish fed soybean meal plant-based diets containing exogenous multi-enzyme showed no significant difference in cholesterol, uric acid, and creatine while glucose level decreased.

In the present study, lower levels of AST and ALT were noted in fish fed the FM diet and PL-Ulva diets supplemented with different enzymes or yeast, which might indicate improved liver function. Similarly, Hassaan et al. (2018) revealed that decreased levels of aspartate aminotransferase and alanine aminotransferase were noted in fish fed the diet with 15 g/kg yeast extract. Mahmoud et al. (2014) reported that there were no significant differences in ALT and AST between groups fed soybean meal plant-based diets containing exogenous multi-enzymes.

V -CONCLUSION

Supplementation of exogenous enzymes (mono- or multi-enzyme complex) and yeast to the Ulva lactuca plant-based diets of Nile tilapia can enhance nutrient utilization and improve fish growth performance. Indeed, exogenous enzymes provide functional feed additives and supplements to enhance digestion and growth in fish in the commercial sector.

Conflict of interest

'Declarations of interest: none'.

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