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Original Article

Exogenous β-mannanase and Dl-methionine as feed additives to improve growth, feed efficiency and hematological indices of Nile tilapia *Oreochromis nilotucs* fed dietary plant protein

Ashraf Y El-Dakar[1] , Shymaa M Shalaby[1,2], Samar A. Abdel-Salam[1], Shaimaa S. Gomaa[1] and Mohamed F Abdel-Aziz[1]

[1], Aquaculture and biotechnology dep., Faculty of Aquaculture and Marine Fisheries, Arish University, Egypt

[2], Aquaculture dep., Faculty of Fish Resources, Suez University, Egypt

ABSTRACT: The present study was done to determine the effects of exogenous β-mannanase (Hemicel® enzyme) and methionine on growth performance and blood parameters of Nile tilapia *Oreochromis niloticus* fed plant protein diet. Two basal diets were formulated to contain 27% CP one of them contained fishmeal (A) and other was plan protein (B). This study included six treatments were as the following: T1: (negative control) fish fed with diet A, T2: (positive control) fish fed on diet B, T3 and T4: fish fed on diet B with adding 0.025 and 0.05 % of Hemicel®, respectively, and T5: fish fed the diet B with 0.025% of Hemicel® and 5% methionine and T6: Diet B with adding 0.05 % Hemicel and 5% methionine. 120 juveniles were randomly divided into 12 plastic tanks (water size: 35 liters) with density of 10 fish/tank. Fish reared in saline of 3ppt, and fed two times daily for six days weekly with rate of 4% of body weight. Results confirmed that feed additives of Hemicel and Dl-methionine significantly improved nutritional utilization of plant protein diets. One way ANOVA analysis did not show significant differences in growth performance between fish fed with a soy-based diet containing 0.025% Hemicel® and Dl-methionine 0.5% (T5) and those fed with the diet containing fish meal (T1). Fish in T5 and T4 had highest body content of protein and the best blood indicator. Generally it can be totally replaced fish meal by soy bean with adding Hemicel® and Dl-methionine at levels of 0.025% and 0.5% respectively.

Key word: fishmeal, soybean meal, methionine, Hemicel, Nile tilapia *Oreochromis nilotucs*

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1. INTRODUCTION

Aquaculture is a way to achieve sustainability in the production of aquatic products with the continued unsustainable harvests from capture fisheries; the sector is seen as the only solution to meeting the rising demand for aquatic products global

Correspondence :

Mohamed F Abdel-Aziz

Faculty of Aquaculture and Marine fisheries, Arish, Egypt

Mail: M_fathy8789@yahoo.com

Aquaculture's contribution to global fish production to 82.1 million tons (46%) out of the estimated 179 million tons of global production (FAO, 2020).In the last decade, has become Egyptian aquaculture has rapidly developed and has become about 80% of total seafood production and tilapia production represents over 88% of aquaculture production (GAFRD, 2020). However, increasing feed prices is considered the main challenge and the factor limiting profitability in tilapia farms. This because of the production of fishmeal which considers a conventional protein source in aqua-feeds is less stable because of restricting its production and impact of climate change on capture fisheries through alterations in the abundance and distribution of fish species which causing a significant effect on fishmeal and fish oil supplies. (Hardy, 2010; Mazurkiewicz, 2009). To overcome this change has to use more innovative and sustainable ingredients in aqua feeds (Tacon & Metian, 2015). The use of plant protein ingredients is currently increasing with replacing fishmeal is necessary to reduce the feed cost. Whereas the commercial fish feed usually contain high fish meal content ranging from 30-50% leading to enhance the feed cost (Davies and Gouveia, 2008). But plant ingredient sources contain with high levels of antinutrition factors which prevent and reduce the feed utilization such as hemicellulases, xylan, galactan and mannans (Dhawan and Kaur, 2007). Furthermore, plant protein sources are poor indispensable amino acids, impaired phosphorus availability (Bonalod et al., 2015). Soy bean meal is a main source of plant protein in fish feed it contain β-mannans, allergenic factors which act 30% of soy bean and has negative effect on immune system (Utsumi et al., 1997). In addition Soybean meal is deficient in sulfur-containing amino acids, and contains endogenous ant nutrients, including protease (trypsin) inhibitor, phytohaemagglutinin and anti-vitamins. Some of the factors can be destroyed or inactivated during thermal processing (Tacon, 1993). Hence, improving the efficiency of partially or totally replacement fish meal without negative effect on fish growth is considered other challenge. Using exogenous enzyme can increase improve nutrients utilization in plant protein sources and thus growth parameters leading to reduce feed cost (Karimi et al. 2009). B-mannanase hydrolyzed β-mannan in soybean meal to mannan-oligosaccharides that improve absorption of many nutrients in fish intestinal (Shao et al., 2000). B-mannanase increases the utilization of total carbohydrate and then saving the dietary protein and directs it to build muscle instead of carbohydrates which are used as a source of energy.

On the other hand, methionine was identified as the limiting amino acid, when the level of fish meal decreases below 60% in diet (NRC, 1993). Many works showed that methionine supplementation improved intestinal c-glutamyltransferase and creatine kinase activity, lysozyme activity, lectin potency, and declined Aeromonas counts (Liao et al. 2014). The effect of methionine on growth and feed efficiency of fish has been demonstrated in several species such as *Oreochromis niloticus* (He et al., 2017) *Pelteobagrus fulvidraco* **(Elmada et al., 2016**) *Cyprinus carpio* (Tang et al., 2009). But no study has assessed the combine exogenous βmannanases enzyme with Dl-methionine as supplementary diet to improve the utilization of plant protein-based diets and dispense with fish meal. Accordingly, the present work aimed to assess the possibility of dispensing with the fish meal from tilapia diets on growth, utilization and blood indexes, through using β-mannanase (Hemicel®) with or without adding methionine in plant protein diets that were offered to Nile tilapia Juveniles.

2. MATERIAL AND METHOD

This experiment was carried out in the Fish nutrition Laboratory, Faculty of Aquaculture and Marine Fisheries, Al-Arish University, Egypt, 120 Tilapia Juveniles, Oreochromis niloticus, were obtained from the Fish Research Center, that located in Arish and transported to the site of works in carful. Fish were acclimatized for 1 week.

2.1. Design of the experiment

This trial was designed of 6 treatments, to estimate dietary plant protein with exogenous enzymes and methionine on biological, nutritional, and hematological indices. The description of treatments was as the following: T1: (negative control with fish meal) fish fed with diet A, T2: (positive control without fish meal) fish fed on diet B, T3 and T4: fish fed on diet B with adding 0.025 and 0.05 % of Hemicel®, respectively, and T5: fish fed the diet B with 0.02% of Hemicel® and 5% methionine and T6: Diet B with adding 0.05 % Hemicel and 5% methionine. Each treatment had two replicates. 12 plastic storage tanks were used to accomplish this work with dimension 54cm x 38 cmx 28 cm (L x W x H) and with a volume of 35 liters, also it were equipped with 3 air pumps (220 v, 50 Hz, 5 w) supplying approximately 3.4 liters of oxygen per minute for each one. Fish were randomly distributed at stocking of 10 fish per tank with initial weight 15±20 fish were fed twice daily at 9 a.m and 5 p.m for 6 days weekly. The feeding was done manually with feeding rate 4%of fish biomass. The water exchange rate was 40% of water volume daily. Fishes were weighted every two weeks to control the optimum feeding rate. Experimental period was a 42 day.

2.2. Preparation of diets

Two basal diets were prepared to contain 27 % crude protein and named A and B, Diet A contained fish meal with rate of 33% of their proteins (Table 1). Diet B was plant protein and soy bean was their main source. The ingredients (Fishmeal 64% as an animal protein source. Soybean meal 44% was used as a plant protein source. Yellow corn, wheat bran, and sunflower oil were served as energy sources) were collected from the local market of medicinal and herbal products. The feed was formed in the nutrition lab at the Faculty of Aquaculture and Marine Fisheries, Al-Arish University, and the ingredients were milled in which feed additives of Hemicel® and methionine were added into the milled ingredients then well mixed with water and then pelleted using a meat mincer with a 1-mm diameter. The pellet was dried in oven at temperature of 70° C for 6 hours and stored at 1°C until use.

Table (1): Ingredients and a proximate composition of the basal diets

Ingredients	***Diets***					
	Diet A	**Diet B**				
	T1: (control negative)	T2: control positive	T3	T4	T5	T6
Fish meal	14.3	-	-	-	-	-
Soy bean meal	25	46	46	46	46	46
corn	26	9.3	9.3	9.3	9.3	9.3
Wheat bran	30	40	40	40	40	40
Plant oil (sun flower oil)	1.4	1.4	1.4	1.4	1.4	1.4
[1]**Vitamin premix**	3	3	3	3	3	3
starch	0.3	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100	100
Feed additives						
[2]**Hemicel %**	**-**	**-**	**0.025**	**0.050**	**0.025**	**0.050**
[3]**Methionine %**	**-**	**-**	**-**	**-**	**5**	**5**
Chemical composition of a basal diets without additives on basis dry matter, %						
Dry matter	11.40	11.90				
ash	5.60	5.11				
Fat	5.06	3.90				
Fibers	5.49	7.35				
Protein	26.8	27.07				
Methionine	0.51	0.37				
Lysine	1.61	1.50				
Methionine and cysteine	0.89	0.82				
[4]**Nitrogen free extract**	57.05	56.57				
[5]**Gross energy Kcal/kg**	4560.0	4521.60				

[1]Vitamins and minerals mixture each 1Kg of mixture content: 62.5m I.U. vit A, 25m I.U. vit D3, 25 g vit E, 1.75g vit K, 0.5g vit. B1, 2.75g vit. B2, 1.25g vit. B6,10 mg.vit. B12, 20g.niacin, 500mg.Folic acid, 50mg.Biotin, 37g Zinc, 22g. Iron, 31g.Manganese, 2.5g Copper, 50mg.Coblat, 113 mg Selenium, 650 mg iodine.

[2] MetAMINO, Dl-Methionine, Feed grade 99%, Manufactured by Evonilk methionine sea PTCLTD, and item code 165595288.

[3]β-mannanase is as an exogenous enzyme (A commercial product, named Hemicel® which is a registered trademark of ChemGen Corp., Gaithersburg, MD. Recommended usage rate is 0.05% of feed). Hemicell is well known as a fermentation product of *Bacillus lentus* and contains high content of β-mananase.

[4] NFE was calculated by difference

[5] Gross energy was calculated according (NRC, 1993).

2.3. Examinations of water physiochemical

Water temperature, pH, and dissolved oxygen (mg/l) were recorded daily at 1p.m using a multi-parameter water quality analyzer (MULP-8C) while standard methods of APHA (1995) were used to estimate total ammonia nitrogen (mg/l) every two weeks.

2.4. Growth and feed utilization

- Total gain (TG, g) = W2-W1; Where, W2 is final body weight (g) W1 is initial body weight (g).
- Average daily gain (ADG, g) = $\frac{TG,g}{days}$
- Specific growth rate (SGR, %) = $\frac{\ln W2-\ln W1}{days}$ ×100 (ln is natural logarithm)
- Survival rate (SR, %) = $\frac{NO.of\ fish\ at\ the\ end\ x\ 100}{NO.of\ fish\ at\ the\ beginning\ of\ trial\ period}$
- Feed conversion ratio (FCR) = $\frac{Feed\ consumed\ ,g/fish}{TG,g}$
- Protein efficiency ratio (PER) = $\frac{TG,g}{Protein\ consumed\ ,g/fish}$

2.5. Chemical composition

The methods of (AOAC, 2000) were used to determine the approximate chemical composition of fish and diets. The samples were dried in an oven (105° C) till weight stability to calculate the moisture. Ash was estimated by incinerating of dray matter samples at 550° C for 3 hours. Crude fat was evaluated by Soxhlet extraction with petroleum ether (boiling 40-60°C) and crude protein measured by the micro-Kjeldahl technique, N%× 6.25. Total carbohydrate was calculated by differences and Gross energy was estimated using the factors 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and carbohydrates respectively were used (NRC, 1993). Amino acids concentrations in diets were determined by using an amino acid analyzer (Biochrom 30 Amino Acid Analyzer, Biochrom, Cambridge, UK)

2.6. Blood sampling

At the end of the experiment, three fish were randomly selected from each replicate and anesthetized with clove oil 0.05 ml/l (El-Dakar et al., 2021) blood samples were collected from the caudal vein of fish using 3ml syringes. The obtained blood was placed into two tubes, on with heparin (EDTA 10%) to evaluation blood hematology and other without EDTA to evaluate serum biochemical. All samples were immediately transferred to the analysis laboratory. Hematological assay such as red blood cells count (RBCs) Hemoglobin (Hb) Hematocrit (Hct) white blood cells (WBCs) were determined according to (Blaxhall and Daisley, 1973). Commercial test kits (Bio-diagnostic, Egypt) were used to determine serume assay such as aspartate aminotransferase (AST), Alanine aminotransferase (ALT), urea, ceratinine and serum glucose were estimated using colorimetric method (Reitman Frankel, 1975 and Tietz et al., 1994) and total protein (Wootton, 1964).

2.7.Statistical analysis

All the obtained data were statistically analyzed by using SPSS software version 21. One way ANOVA were used to analyze results then following by Duncan's multiple range tests to compare the differences among the treatments means at significant level (*P≤0.05*).

3. Results

3.1. Water quality

As presented in table (1) No significant differences were found among groups in temperature degree and total ammonia nitrogen mg/l. even though the ranges of temperature and TAN between (24.4-26.5 C) and (0.18-0.31 mg/l) respectively.

Table (2): Averages of some indicators of water physiochemical

Items	Treatments						*P-value*	PSE*
	T1	T2	T3	T4	T5	T6		
Temperature° C	24.4	25.5	24.95	26.3	27.5	25.3	0.48	0.44
pH	8.39[a]	8.31[a]	8.37[a]	8.09[b]	8.08[b]	8.30[a]	0.01	0.04
Dissolved oxygen, mg/l	7.32[a]	6.77[b]	7.07[ab]	6.76[b]	6.22[b]	6.83[b]	0.004	0.106
Total ammonia nitrogen (TAN), mg/l	0.33	0.31	0.18	0.19	0.29	0.3	0.06	0.021

a, b are as different letters in the same row implied the significant differences ($P \leq 0.05$)
*, Pooled standard error

Conversely, values of pH and DO, mg/l significantly changed at ($P \leq 0.05$), T5 had lowest pH than other groups and T1 and T3 had the highest DO, mg/l compared to the other treatments.

3.2. Growth performance

Significant differences were detected in the growth performance of *O. niloticus* fed with different diets except W1 and survival rate (Table 3). Results showed T5 had the highest values in W2 (34.97 g), TG (19.37, g), ADG (0.460 g) compared with the treated groups with additives, followed by T4, T3 and T6 while T2 (Positive control) had the lowest. Furthermore, SGR did not significantly vary among T5, T4, and T1 and they significantly higher in the indicator than T3, T6, and T2 respectively.

Table (3): Means of growth and nutritional indices of Nile tilapia fed on Soybean-based diets with adding Hemicel® and Dl-methionine in comparison with control diets for 6 weeks.

Items	Treatments						*P-value*	PSE*
	T1	T2	T3	T4	T5	T6		
W1, g	16.80	15.5	16.60	15.00	15.60	16.30	0.403	0.270
W2, g	36.60[a]	25.90[e]	29.70[c]	33.30[b]	34.97[a]	27.75[d]	0.001	1.050
TG, g	19.80[a]	10.40[d]	13.10[c]	18.30[ab]	19.37[a]	11.45[d]	0.004	1.070
ADG, g	0.471[a]	0.245[c]	0.315[b]	0.435[a]	0.460[a]	0.270[c]	0.001	0.025
SGR, %/day	1.85[a]	1.22[d]	1.38[b]	1.90[a]	1.92[a]	1.26[d]	0.090	0.008
FCR	1.05[b]	1.70[a]	1.35[ab]	1.20[ab]	1.10[b]	1.58[a]	0.060	0.090
PER	3.55	2.18	2.74	3.09	3.36	2.34	0.001	0.190

a, b, c, e are as different letters in the same row implied the significant differences ($P \leq 0.05$)
*, Pooled standard error

Regarding feed utilization, FCR was not significantly differed among T5 (1.10), T1 (1.05), and T4 (1.20) and these treatments were the best in this parameter while T2 had the highest value in FCR (1.70). Insignificant differences were found in PER but it was in the same trend of FCR.

3.2. Composition of whole body fish

The proximate composition of whole body of *O. niloticus* among all treatments is shown in table (4). Significant differences were found in protein, ash and moisture, fat contents. Moisture content was not statistically different among T1, T6, T5 and

T3 and these groups were significantly higher than T4 and T2. Fat content did not statistically vary among T4, T1, and T5 and these treatments were significantly lower than T3 and significantly higher than T2 and T6. The highest value of ash content was recorded by T6 and T3 followed by T1, T2, T4, and T5 respectively. T5, T4, and T5 had higher protein content than T1 and T6 while T3 had the lowest protein content.

Table (4): Means of body contents of Nile tilapia fed on Soybean-based diets with adding Hemicel® and Dl-methionine in comparison with control diets for 6 weeks.

Items	*Treatments*						*P-value*	PSE*
	T1	**T2**	**T3**	**T4**	**T5**	**T6**		
Moisture, %	85.30^{a}	65.06^{b}	79.09^{a}	75.15^{ab}	78.59^{a}	82.90^{a}	0.050	2.22
Fat, %	12.12^{ab}	9.77^{bc}	13.43^{a}	12.14^{ab}	11.80^{ab}	8.73^{c}	0.096	0.55
Ash, %	19.73^{ab}	17.71^{bc}	22.64^{a}	15.45^{bc}	13.28^{c}	23.65^{a}	0.008	1.18
Protein, %	67.65^{ab}	72.32^{a}	63.84^{c}	72.40^{a}	74.89^{a}	67.22^{ab}	0.082	1.32

a, b, c, e are as different letters in the same row implied the significant differences ($P\leq 0.05$)
*, Pooled standard error

3.4. Hematological assay

Hematological indicators are set out in table (5) which showed that, there were significant differences in Hct and no significant variations in Hb and RBC, T4 had the highest means of Hct, Hb and RBC followed by T5, T1, T2 and then T3 while T6 had the lowest. The highest count of WBC was recorded by T6and T4 then T1 and T3 while the lowest values were within T5 and T2**.**

Table (5): Means of Hematology assay of Nile tilapia fed on Soybean-based diets with adding Hemicel® and Dl-methionine in comparison with control diets for 6 weeks.

Items	*Treatments*						***P-value***	**PSE***
	T1	**T2**	**T3**	**T4**	**T5**	**T6**		
Hb, g/dl	4.51	4.1	3.8	4.8	4.3	3.3	4.51	0.2
HCT, vol %	12.9^{bc}	12.3^{bc}	11.4^{cd}	14.4^{a}	13.45^{ab}	10.1^{d}	0.01	0.44
RBC, 10^{6}/cmm	1.43	1.36	1.25	1.6	1.55	1.1	0.66	0.08
WBC, 10^{3}/cmm	31.4^{ab}	27.9^{c}	$29.50b^{c}$	31.95^{a}	28.60^{c}	32.8^{a}	0.006	0.57

a, b, c, are as different letters in the same row implied the significant differences ($P\leq 0.05$)
*, Pooled standard error

3.5. Serum assay

Table (6) Means of blood glucose, AST, and ALT significantly differed among groups. T5 had the lowest plasma glucose while T2 recorded the highest concentrations, and no statistical significant among the other groups. The highest AST was obtained by T1 followed T2 and T3, but The lowest AST values were within T5 and T4. GPT was insignificant variations among T2, T2. T6, T4, and T3 however these treatments were significantly lower than T1. There were not statistical variations in total protein, urea, and createnine.

Table (6): Means of Serum assay of Nile tilapia fed on Soybean-based diets with adding Hemicel® and Dl-methionine in comparison with control diets for 6 weeks.

Items	*Treatments*						*P-value*	PSE*
	T1	T2	T3	T4	T5	T6		
Glucose, mg/dl	58^{ab}	67^{a}	62^{ab}	57^{ab}	55^{b}	61^{ab}	2.57	1.53
GOT, U/l	51^{a}	43^{b}	40^{b}	29^{c}	31^{c}	31^{c}	0.002	2.46
GPT, U/l	45^{a}	29^{b}	27^{b}	25^{b}	28^{b}	23^{b}	0.006	2.3
Total protein g/dl	7.80	7.70	7.10	6.80	6.65	7.30	0.01	0.33
Urea, mg/dl	13	14	15	11	10	13	0.82	1.01
Create mg/dl	0.19	0.21	0.17	0.19	0.2	0.18	0.97	0.011

a, b, c, are as different letters in the same row implied the significant differences ($P\leq0.05$)
*, Pooled standard error

4. DISCUSSION

From table 2 it can be said that means of water temperature and total ammonia were within the acceptable limits of tilapia cultured as reviewed by (Kausar & Salim, 2006 and Lawson, 1995). Despite the significant differences which were appeared among the means of DO, mg/l or pH values, the ranges of them fell in the recommended side for growth and health statues of tilapia (Makori et al., 2017).

Obviously, table (3) illustrated control positive (T1) was significantly better control negative (T2) in all growth measurements, naturally, fish fed a soy bean-based diets as a main sources of protein had the lowest growth, Although Soybean meal (SBM), has a high protein content with good amino acid profile and low cost, However, it is considered limiting in methionine, contains some anti-nutrients such as trypsin inhibitor, phytic acid, saponin, hemagglutinin, anti-vitamins and low palatability (Bonaldo et al.,2011; Hardy 2010). In view of table (1) it can be noted that addition of fish meal to contribute 1/3 of dietary protein in diet A leading to provide Methionine (0.51%) as required of tilapia according to previous reports of (Nguyen, 2007; Polat, 1999) compared with the diet B which contained 0.37% of Methionine. It is known Methionine cannot be produced by fish and has a vital role for the synthesis of choline hence phosphatidylcholine and acetylcholine or other phospholipid accordingly it is essential for nerve functions and leucocyte metabolism. Moreover methionine deficiency in aquatic feed decreased antioxidant causing oxidative stress, consequently reducing the immune system and poor growth and FCR (NRC, 2011; Ahmed et al., 2003).

Diets plant protein in special soya-based diets contain high levels of Non starch-polysaccharides that may reduce feed intake, digestibility, and thus growth (Korgdahl et al, 2010) also soybean in diets changes the intestinal content of micro flora and reducing these microbial population of adherent bacteria throughout the intestine (Refstie et al., 2006). All these reasons resulted in decreasing the performance of fish fed a soy-based diet (T2) compared with T1 (negative control). Our results are correspond with several works which affirmed, total replacement of animal protein with plant protein in fish diets had negative effects on feed utilization and growth rates (Mahmoud et al., 2014; Lin and Luo, 2002; Gomes et al., 1995).

Adding β-mannanase as exogenous enzyme (Hemicel) at doses 0.025 and 0.05% with or without Dl-methionine at concentration of 5% led to improve biological performance of all fish fed the diet B in comparison to fish in positive control (T2). Especially T5 which fed soy based dies containing 0.025 % (Hemicel) with 5% methionine and T4 which fed a high dose of Hemicel (0.05%). In the line these results Gatlin et al. (2007) and Flix et al. (2004) confirmed that using the exogenous enzymes with essential amino acid as supplementary diet can improve nutrient utilization in plant protein source and are more effective in replacing fish meal.

It is known, tilapia are omnivores fish and most of their diet ingredients are plant protein sources corn, wheat bran, rice bran and plant protein sources therefor their feeds contains a high amount of Non starch-polysaccharides which like substances of hemicellulose, cellulose, pectin, these components are able to reduce nutrients digestibility in fish gut. B-mannan is one of hemicellulose compounds have harmful effects on fish performance and which are present in two forms galactomannanas and glucmannans in the cell walls of many plant ingredients like soybean (Dhawan and Kaur, 2007; Choct, 1999). Using exogenous β-mannanase in fish diet to hydrolyze β-mannan to mannan oligosaccharide that improved the utilization of total carbohydrates in plant ingredient and insulin secretion through improving fibers digestion as a result of reducing the viscosity of intestinal ingest, whereas increasing viscosity can reduce water and glucose absorption as reported by (Zou et al., 2006; Alimirall et al., 1995). Not only B-mannanase stimulates the excretion of digestive enzymes from intestinal tracts but also mitigates the inhibitory impacts on these enzymes activities and improves morphological traits of gastrointestinal tract as illustrated by (Gutierrez et al., 2008). Mannan oligosaccharide boosts bifocal micro flora in intestinal, improves the intestinal absorption of Zinc, copper, and selenium (Saeed et al., 2019; Shao et al. 2005). Additionally, β-manganese is capable of increasing monocytes, macrophage, and cytokine accordingly improving the innate immunity (Lee et al., 2003).

As a result of enhanced utilization of dietary carbohydrate by β-mannanase, dietary protein is more used to muscles building and not as an energy source leading to improve the growth performance.

Our results are in accordance with finding of Jackson et al. (2004) β-mannanase had positive impact on birds fed corn, soy meal as a result of improving the utilization dietary energy. **Ghobadi et al. (2009**) using multi-enzymes in trout diet lead to increase growth indices by decreeing negative effects of dietary soy bean. **Rahmen et al. (2013)** β-mannanase supplementation in diets containing a low level of energy had positively impacts on FCR. Additionally, our findings were together in accordance with Cladas et al., (2018) β-mannanase at level 0.02-0.04 % in poultry diet improves digestible energy and absorption of amino acids in intestinal tract. On contrary, Yigite et al. (2016) and Yigite and Olmez (2011) said that fish performance did not significantly differ with including exogenous enzymes in their feeds.

As it was above showed in table 3, using a mixture of 0.025% Hemicel® and 5% methionine as supplementary diet on a soybean meal –based diet achieved statistically quite similar results in growth and feed efficiency comparable to fish fed diet containing fish meal (T1). This cleared that the used doses of enzyme and Dl-methionine were the most suitable to achieve the maximum utilization of soybean-based diet and feed additives did not negatively conflict in some of them. Whereas, there are many factor may be effect on effectiveness of the dietary methionine such as dose of methionine, doses of Hemcile, diet ingredients and their content of protein, and which resulted in decreasing the growth of T6.

As it is known that methionine are the first limiting amino acids in the soybean-based diets, also higher replacement levels usually require supplementary diet with limiting amino acids, mainly methionine and lysine lead to negative effect on growth, FCR (Li et al., 2009). Methionine is required for protein synthesis and several methylation reactions like DNA and a precursor of polyamines, L-caranitine and cysteine (Espe et al., 2011). A substantial of the established methionine requirement may be driven for cysteine synthesis accordingly diets that contain adequate amount of cysteine have a methionine-saving effect (Baker, 2006). Methionine is a source of sulphure and other compounds require by fish for normal metabolism (NRC, 2011). Moreover, methionine can effect on the balance of gut micro flora and improve the morphological of intestinal (Yan and Zhou, 2006 and Zhou and Li, 2004). Several works supported our observations like He et al. (2017) growth and FCR of tilapia juvenile improved as increasing dietary methionine level up to 0.75%. In the same manner, Tang et al. (2009) found that growth and feed efficiency of juvenile Jian carp gradually rise with methionine supplementation. Presumably, methionine supplementation was lesser effects on growth than Hemicel enzyme whereas performance of groups T3 or T4 which fed 0.025 or 0.05% Hemicel supplementation only was better than T6 which fed 0.05% Hemicel with methionine. This result manifested that fish may be able to overcome the apparent marginal methionine (Diet B containing 0.37% methionine). In addition efficiency of methionine utilization fish is increased by fish fed a low dietary methionine as observed by (Peres and Oliva-Teles, 2008). In the same context, Viola and Arieli (1983) affirmed that methionine supplemented feed did not impact on growth when replacing the fish meal in the diet totally. Also, as was aforementioned, improving the degradation of dietary carbohydrate and non-starch polysaccharides led to improving the utilization of dietary energy, hence saving dietary protein and directing it to the growth process.

Concerning body composition, there was a line relationship between growth performance and body protein content. T5 and T4 had the highest protein content with lowest ash content. These results were in contrast to Goda et al. (2012) found dietary exogenous enzyme did not change the moisture content and control group was higher in protein content. Similarly, Ng and chong (2002) no variation in body composition of fish fed diets containing exogenous multi-enzymes. It should be noted that fish-fed Hemicel only (T3 and T4) diets were relatively higher in fat content, which may be due to the increased digestible energy released by the Hemicel effect (Sinha et al., 2011). Partially in agreement with these data Elmada et al. (2016) and Figueiredo-Silva et al. (2015) protein content increased with fish fed a soybean diet with methionine supplementary and lipid content is the vice versa as a result of enhancing the utilization of protein, this trend may be found with T5. Besides, decreasing fat content in T2 or T6 may result in reducing the growth as reported by (Mahmoud et al., 2014).

Regarding hematology and serum assay, as it was known that blood is a pathophysiological reflector of the entire body, blood parameters are critical in determining the health status of fish. Fish in T4 had the best hematology parameters followed by T5. This observation could be related to enhance growth rate and it may provide an efficient level of blood oxygen carrier system also it was found the highest WBC was obtained with T4 and T6. Using Hemicel at 0.05% as feed additive led to crossing the β-mannanase the intestinal mucosa which is a powerful stimulator of the innate immune system, resulting in increased macrophage and monocyte proliferation and cytokine production as reviewed by (Elmada et al., 2016; Zamini et al., 2014).

It is noteworthy that reducing plasma glucose is indicator for decreasing stressful condition and fish are in welfare status (Mohapatra et al., 2014). Additionally elevating GOT and GPT concentration is an indicator of hepatocellular damage leading to a leakage of the enzymes into circulation (Samanta et al., 2014). The showed results in table (6) stated that T5, T4and T6 had the lowest plasma glucose compared other treatments as well as T4, T5, and T6 had the lowest Got and GPT this confirmed that Hemicel at 0.05% and methionine supplementation improved serum traits. A similar trend was observed also with Caldas et al. (2018) Hemicel® supplemented feed in diet poultry at 0.02-0.04% improved blood glucose and anabolic hormone. Moreover, Elmada et al. (2016) and Xie (1992) they reported that GOT of decreased with increasing dietary methionine and the optimum methionine level improve liver function.

5. CONCLUSION

From the obtained results and their discussion, it can be concluded that, 1- Adding exogenous β-manganese at 0.025 or 0.05% with or without Dl-methionine at dose of 5% soybean-based diets can improve of biological performance and blood indices of tilapia juveniles which fed soybean –based diets compared with those fed a soybean –based diet without additives (positive control T2). 2- the best growth, feed conversion ratio and body protein was obtained by juveniles fed a diet containing 0.025% of Hemicel with 5% supplementation this treatment (T5) followed by fish fed a diet containing 0.05 % Hemicel only (T4). T5: did not significantly vary in growth parameters when comparing with those fed a diet containing fish meal (negative control T1). Besides T4 and T5 had the best hematological and serum biochemical parameters. Finally, it is possible to dispense with fishmeal from Tilapia feed (27% crude protein) and rely on soybean meal as a main source of protein with the addition of β-mannanase and Dl-methionine in proportions of 0.025% and 0.5%, respectively. This study also suggested that the interaction between exogenous enzymes and amino acids is poorly understood and needs further study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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