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Effect of protein reduction with indispensable amino acid supplementation at different levels in practical diets of Nile tilapia Oreochromis niloticus fish.

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ABSTRACT

Objective: To estimate the effect of reducing dietary protein levels in Nile tilapia *(Oreochromis niloticus)* with dietary supplementation of commercially available synthetic amino acids at different levels on growth performance, whole-body composition, morphometric indices, and serum metabolites.
Design: Randomized controlled experimental study.
Animals: One hundred eighty male Nile tilapia fingerlings.
Procedures: One hundred eighty male Nile tilapia fingerlings were equally distributed in twelve glass aquaria (15 fish per aquarium) and fed the formulated diets at 4-5% of the body weight. Every two weeks, tilapia was weighed, and the amount of feed intake was adjusted according to the increase in the tilapia weight.
Results: By reducing dietary protein levels to 26%, final body weight (FBW), weight gain (WG), weight gain percent (WG %), specific growth rate (SGR), protein efficiency ratio (PER), and feed intake (FI) were significantly reduced, while feed conversion ratio (FCR) was significantly increased. Indispensable amino acid (IAA) supplementation at 120% of the NRC requirements to low protein diets (26 and 28%) resulted in a significant improvement in all growth performance parameters. The reduction of dietary protein to 26% resulted in a significant increase in triglycerides (TG), cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine concentrations. Serum IgG, IgM, and reduced glutathione (GSH) showed a significant improvement with IAA supplementation at 120% of the NRC requirements.
Conclusion and clinical relevance: Dietary protein level could be lowered to 26% with supplementation of IAA at 120 % of the NRC recommendation to maintain comparable growth performance of Nile tilapia.

Keywords: Nile tilapia, indispensable amino acid, dietary protein, commercial amino acid, growth performance, serum metabolites.

1. INTRODUCTION

Globally, tilapia is considered the second most farmed fish species after carps. It has gained popularity as an ideal species for fish farming. This is attributed to its fast growth rate and its high tolerance to a variety of environmental conditions. Moreover, tilapia can feed on a wide variety of feed items and can withstand stress and diseases [1-3]. The sustainability of aquaculture relies on the production of a well-balanced, cost-effective feed. Generally, feed cost constitutes approximately 60 % of the total operational cost in intensive fish farms, with protein sources being the most expensive component in aquafeed. Like other animals, fish have a dietary requirement for a well-balanced mixture of indispensable amino acids (IAAs) and dispensable amino acids (DAAs) instead of having a dietary requirement for protein [4, 5].

Fish meal (FM) is considered the principal protein ingredient for most commercially farmed fish species because of its high content of protein with an excellent amino acid profile. However, its price has increased dramatically in the last 20 years, and it is expected to increase even more, especially because of the limitation in the world production of FM along with increasing demand and competition with domestic animal requirements. Therefore, it has become essential to minimize the reliance on FM by using less expensive protein ingredients and/or reducing dietary protein levels in fish diets, but in both cases, the deficiency in some essential amino acids, which are known as "limiting amino acids" is the most prominent concern. Therefore, the strategy of crystalline amino acid (CAA) supplementation has been widely used in tilapia diet formulation based on the ideal concept to ensure a well-balanced amino acid profile for optimum growth performance [6-8].

Several studies have confirmed that reducing dietary protein levels in fish diets with CAA supplementation is considered one of the solutions in order to overcome the high cost of FM and deal with its limited supply as well as produce a cost-effective fish diet without affecting fish growth performance and health [7, 9-11].

Puppo, Haese [10] concluded that based on the ideal protein concept, it is possible to lower the protein content in Nile tilapia diets without having any negative effects on growth performance or hematological parameters since amino acids are supplemented. Additionally, Nguyen, Dinh [11] stated that applying the ideal protein concept with IAAs supplementation was an effective way for reducing the protein content in fish diets. According to their study, intact protein levels could be reduced from 32% to 27.2% in tilapia diets with IAAs supplementation (methionine, lysine, tryptophan, histidine, and valine) up to the requirement of the NRC [12] without affecting the growth performance of fish.

Therefore, the aim of this study focused on evaluating the effect of reducing dietary protein levels in Nile tilapia *(Oreochromis niloticus)* with dietary supplementation of commercially available synthetic amino acids at different levels on growth performance, whole-body composition, morphometric indices, and serum metabolites.

2. MATERIALS AND METHODS

2.1. Experimental design and diet preparation

The experiment consisted of six isoenergetic (3000 Kcal DE / Kg) dietary treatments with two replications per each. The formulation of the experimental diets is illustrated in table 1. The first four groups of diets were formulated to contain different levels of dietary protein (32, 30, 28, and 26%) in which the IAA profile covered the IAA requirements for Nile tilapia as recommended by the NRC [13], except for the low protein diet (26%), in which methionine was supplemented to raise its level up to the requirements as reported by Nguyen, Dinh [11]. The last two groups of diets were formulated to contain 26 and 28% crude protein (CP) in which IAAs were supplemented at 120% of the NRC requirements.

All the dietary ingredients were finely ground, sieved, weighed, and thoroughly mixed together with oil and water, and then put in the laboratory pelletizer machine with a 4-mm diameter die for the preparation of the pellet. The formulated pelletized experimental diets were packed in airtight plastic bags and preserved in the freezer at -20°C until used. 30 gm of each sample of the experimental diets was taken for the analysis of moisture, crude protein, ether extract, and ash following the standard methods of AOAC [14].*Culture environment*

A fifteen-week growth trial was carried out at the laboratory of Fish Nutrition at Nutrition and Nutritional Deficiency Diseases Department, Faculty of Veterinary Medicine, Mansoura University. In this study, a total of 180 Nile tilapia male fingerlings with an average initial weight of 6.64 ± 0.04 g were used. Upon arrival, tilapia fingerlings were adapted to the laboratory conditions for about 2 weeks in which they were fed the basal diet. After the adaptation period, tilapia fingerlings were equally distributed in twelve glass aquaria (15 fish per aquarium). Throughout the experiment, the water quality parameters; temperature, pH, and dissolved oxygen (DO) were monitored daily to be maintained within the optimal levels for Nile tilapia at 24-27 ºC, 7.25 to 8.25, and 3-5mg/L, respectively. The photoperiod was kept at 12 hours of light and 12 hours of darkness.

During the growth period, experimental diets were offered to tilapia at a rate of 4-5% of the body weight and divided into meals per day (at 8:00 am & 2:00 pm). Every two weeks, tilapia was counted and weighed, and the amount of feed intake was adjusted in response to the increase in the tilapia weight.

At the end of the experiment, tilapia was counted and weighed, and growth performance parameters were calculated by using the following equations:

2.2. Growth performance measures

- **Mean weight of tilapia in the aquarium** = Total tilapia weight in each aquarium/ number of tilapias in the same aquarium.
- **Weight gain (WG)** = W2 – W1

Where W1 and W2 are initial tilapia weight and final tilapia weight, respectively.

- **Specific growth rate (SGR)** = $\frac{\text{Ln (W2)} - \text{Ln (W1)}}{t} \text{X } 100$ **[9]**.

Ln (W2) = the natural logarithm of the final weight

Ln (W1) = the natural logarithm of the initial weight

t= the duration of the experiment in days.

- **Survival rate (SR) %** = final number of tilapia / initial tilapia number × 100.
- **Feed intake g /fish =** feed (DM) offered in a known period/number of fish.
- **Feed conversion ratio (FCR)** = Feed intake (g)/weight gain (g).
- **Protein efficiency ratio (PER)** = Weight gain (g) / protein intake (g).

2.3. Serum metabolites, immune, and antioxidative enzymes parameters:

Six tilapia were randomly selected from each group, anesthetized with lidocaine (10%), and then blood samples were collected from the caudal vein of each fish using 1 ml disposable syringes and centrifuged at 3000 rpm for 15 minutes to obtain the serum. Blood serum analysis was performed in the central lab at the Faculty of Veterinary Medicine, Mansoura University.

Serum total protein (TP) g/dl, Albumin (ALB) g/dl, Triglycerides (TG) mg/dl, Cholesterol (mg/dl), and Glucose (mg/dl) were estimated following the instruction use of Spinreact procedures, while Alanine aminotransferase (ALT) U/L, Aspartate aminotransferase (AST) U/L, Creatinine (mg/dl), and Urea (mg/dl) were measured by using diagnostic kits obtained from (Diamond).

Serum immunoglobulins, including IgG (mg/dl) and IgM (mg/dl) were determined according to Dati, Lammers [15]. Also, reduced glutathione (GSH) mg/dl and serum superoxide dismutase (SOD) mg/dl were analyzed spectrophotometrically by using Bio-diagnostic kits (Egypt) following the manufacturer's instruction.

2.4. Morphometric indices analysis:

Two tilapia from each aquarium were randomly collected, anesthetized with lidocaine (10%), and individually weighted, then the total tilapia length was measured for the calculation of the condition factor (K). Thereafter, they were dissected and viscera and liver were removed and weighted for the determination of viscero-somatic index (VSI) and hepato-somatic index (HSI), respectively. Additionally, carcass weight and intestine weight were measured for the calculation of the carcass yield, and intestinal index, respectively.

- **Condition factor (K)** = (W/ L^3) × 100

W = fish body weight in grams & **L** = fish body length in cm **[9]**.

- **Viscero-somatic index (VSI) =** (viscera weight [g]/whole fish weight [g]) x 100.
- **Hepato-somatic index (HSI) =** (liver weight [g] /whole fish weight [g]) x 100.
- **Carcass yield =** (carcass weight [fish weight without viscera]/ total fish weight) x 100.
- **Intestinal index** = (intestine weight [g] /total fish weight [g] x 100.

2.5. Proximate chemical analysis of tilapia body composition:

At the end of the experimental period, six tilapia from each group were collected, cut into pieces, and dried in the hot air oven at 70 ºC for 72 hrs. The dried samples were minced in a meat grinder into powder, then analyzed for proximate chemical analysis of the whole tilapia body according to the standard methods described by AOAC [14].

2.6. Statistical analysis:

The statistical analysis of the collected data was performed using the software GraphPad Prism version 8.0.2(263). All data were validated for normality and equality of variances by using Shapiro-Wilk tests. Then, the normally distributed data were subjected to one-way analysis of variance (ANOVA) to determine significant differences ($P < 0.05$) among the experimental dietary treatments, which was followed by Tukey's multiple comparison test to distinguish significant differences among treatment means.

3. RESULTS

3.1. Growth performance and feed utilization

The results of allover growth performance parameters of juvenile Nile tilapia; FBW, WG, WG %, SGR, PER, FI, and FCR are presented in table 2. It was noticed that FBW, WG, WG%, SGR, PER, and FI were significantly reduced with reducing dietary protein levels to 26%, while FCR was significantly increased. However, with IAAs supplementation to both dietary protein levels (26 and 28%) at 120% of the NRC requirements, all growth performance parameters were significantly improved compared to 26% CP at 100% NRC.

3.2. Whole fish body proximate composition

The whole-body composition of juvenile Nile tilapia is shown in figures 1 and 2. No significant differences were found in the whole tilapia body moisture and crude protein by reducing the dietary protein levels from 32 to 26% and with IAA supplementation at 120% of the NRC requirements to low protein diets (26 and 28%), while fat and ash contents were significantly increased as dietary protein was reduced to 26% and with the supplementation of IAA.*Morphometric indices*

The results of the morphometric indices (HSI, VSI, intestinal index, and carcass yield) are shown in table 3. Reducing dietary protein levels to 26% caused an inverse increase in HSI, VSI, and intestinal index. Meanwhile, carcass yield showed a significant decreasing trend. However, with IAAs supplementation to both dietary protein levels (26 and 28%) at 120% of the NRC requirements, HSI, VSI, and intestinal index showed a slight reduction, while carcass yield was significantly improved.

3.3. Serum biochemical, immune, and antioxidative enzymes parameters

Serum biochemical, immune, and antioxidative enzymes parameters are presented in tables 4 and 5. Serum TP, ALB, glucose, and SOD activity did not show any significant differences ($p > 0.05$) among tilapia fed different dietary treatments, while TG, cholesterol, AST, ALT, urea, and creatinine concentrations were significantly increased with the reduction of dietary protein to 26%. Also, by reducing dietary protein content to 26%, serum immunoglobulins (IgG and IgM) and GSH showed a significant reduction. Meanwhile, with 1AA supplementation at 120% of the NRC requirements, they showed significant improvements.

4. DISCUSSION

Currently, with the fast growth of the aquaculture industry and increasing the cost of FM worldwide, it has become inevitable to partially or totally replace FM with less expensive protein ingredients or reduce dietary protein levels in fish feed, but the problem of limiting amino acids will be a big challenge in both cases. Now, crystalline amino acids (CAAs) have been widely used as supplements to restore the amino acid balance and overcome the problems associated with limiting amino acids, which is considered an effective way to develop cost-effective aquafeeds [7, 16].

In the first four groups of this study, dietary protein levels were reduced from 32 up to 26%, in which the IAA profile covered the IAA requirements for Nile tilapia as recommended by the NRC [13], except for the low protein diet (26%), in which methionine was supplemented to raise its level up to the requirements as reported by Nguyen and Allen Davis [17]. Our results showed that with the gradual reduction in dietary protein levels (32, 30, 28, and 26%), FBW, WG, WG% SGR, PER, and FI showed a significant reduction, while FCR exhibited a significant increase Table (2).

Table 1. Ingredient compositions (100 g/kg^{-1}, as fed) of six experimental diets offered to juvenile Nile tilapia (6.64 ± 0.04 g) over a 15-week growth period.

	Dietary treatments					
	32	30	28	26*	26**	28**
Ingredients (%)						
Corn, yellow	10.00	15.5	26.3	37.02	35.7	25.00
Wheat bran	37.74	35.48	28.18	20.84	20.95	28.66
Soybean meal	30.00	28.00	26.00	24.00	24.00	26.00
Fish meal	13.00	12.00	11.00	10.00	10.00	11.00
Corn gluten meal	5.5	5.00	4.5	4.00	4.00	4.5
Soybean oil	0.5	0.6	0.5	0.4	0.8	0.8
Gelatin	1.00	1.00	1.00	1.00	1.00	1.00
Min &vit, premix+	1.00	1.00	1.00	1.00	1.00	1.00
Common salt	0.3	0.3	0.3	0.3	0.3	0.3
Antioxidant	0.02	0.02	0.02	0.02	0.02	0.02
Stay.C	0.1	0.1	0.1	0.1	0.1	0.1
Di Ca phosphate	0.84	1	1.1	1.3	1.3	1.1
Methionine	-	-	-	0.02	0.13	0.09
Lysine	-	-	-	-	0.39	0.22
Threonine	-	-	-	-	0.28	0.21
Tryptophan	-	-	-	-	0.03	-
Chemical composition++						
Crude protein %	32.04	30.31	28.3	26.3	26.2	28.3
DE(Kcal/kg)	3000	3000	3005	3007	3001	3003
EE%	6.48	6.47	6.44	6.41	6.37	6.41
Crude fiber %	5.15	4.98	4.42	3.86	3.84	4.44
Ash%	6.45	6.09	5.52	4.96	4.95	5.53
Calcium %	1.01	0.99	0.95	0.93	0.93	0.95
Phosphorus%	0.54	0.53	0.51	0.50	0.50	0.51
Chemical analysis+++						
Crude protein %	31.90	29.80	28.00	26.00	26.00	28.00
EE %	6.20	7.31	7.03	7.22	7.31	7.71
Ash %	6.38	7.08	6.19	5.23	5.86	5.86

*IAA was supplemented to meet the requirement according to the NRC (1993).

**IAAs were supplemented to meet 120% of the NRC (1993) requirements.

+Trace minerals & vitamins premixes were supplemented to cover the levels of the microminerals &vitamins for tilapia fish as recommended by NRC [13]. Vitamins premix; vit. A 5000 IU, Vit. D3 1000 IU, vit. E 20 IU, vit. k3 2 IU, vit. B1 2 mg/kg diet, vit. B2 5 mg/kg diet, vit. B6 1.5 mg/kg diet, vit. B12 0.02 mg/kg diet, Pantothenic acid 10 mg/kg diet, Folic acid 1 mg/kg diet, Biotin 0.15 mg/kg diet, Stay-C® (L-ascorbyl-2-polyphosphate 25% Active C), Mineral mixture (mg/kg diet); Fe 40, Mn 80, Cu 4, Zn 50, I 0.5, Co 0.2 & Se 0.2.

++ Calculated values from the feed composition tables, nutrient requirement of fish [13].

+++Analysed values according to AOAC [14] procedures.

The reduction in growth could be due to the limitation or the imbalance of the dietary IAA or the level of IAA in the low protein diet (26%) was not enough to meet the physiological or metabolic needs or to synthesize DAA. As stated by Wilson [18], an imbalance or deficiency of amino acids results in a reduction in the utilization of the remaining amino acids, which in turn affects protein synthesis and other physiological activities. The imbalance or deficiency of some amino acids may also be due to the effect of leaching. Some reports have demonstrated that the efficiency of using of CAAs by tilapia might be poor in comparison to protein-bound amino acids. This could be attributed to the rapid absorption of free AA and losses of supplemental amino acids because of leaching prior to consumption. Supplemental amino acids might be especially at high risk of leaching because of their comparably high solubility. On average, methionine and lysine are leached at higher rates than other essential amino acids [19, 20].

From our results, we found that amino acid requirements as recommended by the NRC [13] for Nile tilapia still need revaluation and further studies for efficient utilization in case of reducing the level of protein ingredients in fish feeds. Therefore, the last two groups of the experiment aimed to investigate the effectiveness of IAAs supplementation to be 20 % above the NRC [13] requirements in the low dietary protein levels (26 and 28%). It was shown that growth performance parameters were significantly improved with the supplementation of IAAs at both protein levels (26 and 28%) Table (2), but numerically the best values were obtained at the protein level of 28%. From an economical point of view, it is better to use the low dietary protein level (26%) supplemented with IAAs at 120% of the NRC requirements to reduce the inclusion of the expensive protein ingredients (FM and SBM) since the two groups (26 and 28%) showed no significant difference in growth performance parameters with supplementation of IAA at 120% of the NRC requirements. Comparable results are obtained by De, Lobão [21], who confirmed the possibility of reducing dietary protein levels for Nile tilapia fingerlings from 32 to 28% without any adverse effects on growth performance as long as indispensable limiting amino acids are supplemented to restore the proper balance.

Table 2. Allover growth and feed utilization parameters of juvenile Nile tilapia *(Oreochromis niloticus)* fed on experimental diets with reduced dietary protein levels supplemented with IAAs at different rates over a 15-week growth period.

	Experimental diets							
	32	30	28	26*	26**	28**	*P-value*	PSE⁺
Initial weight	6.70	6.58	6.63	6.67	6.61	6.63	0.157	0.394
Final weight	46.31^{a}	44.22^{a}	40.00^{ab}	34.66^{b}	42.82^{a}	45.81^{a}	0.002	1.114
Weight gain	39.64^{a}	37.59^{a}	33.42^{ab}	27.96^{b}	36.21^{a}	39.17^{a}	0.002	1.117
Weight gain %	595.03^{a}	566.39^{a}	507.64^{ab}	416.11^{b}	548.03^{a}	590.43^{a}	0.002	17.20
Feed intake	72.67	69.35	63.89	60.72	63.78	69.83	0.045	1.33
FCR	1.839^{bc}	1.846^{bc}	1.911^{b}	2.186^{a}	1.760^{c}	1.781^{c}	0.000	0.035
SGR	0.79^{a}	0.78^{a}	0.74^{ab}	0.68^{b}	0.77^{a}	0.79^{a}	0.002	1.06
PER	1.24^{ab}	1.25^{ab}	1.19^{ab}	1.07^{b}	1.39^{a}	1.40^{a}	0.016	0.033
Survival	63.33	60.00	86.67	73.33	86.66	63.33	0.04	3.49

Means (n = 4) in the same row with different superscripts are significantly different at *P < 0.05* based upon analysis of variance followed by Tukey's multiple range test.
*IAA was supplemented to meet the requirement according to the NRC [13].
**IAAs were supplemented to meet 120% of the NRC (1993) requirements.
⁺PSE: Pooled standard error.

Table 3. Morphometric indices of juvenile Nile tilapia *(Oreochromis niloticus)* fed on experimental diets with reduced dietary protein levels supplemented with IAAs at different rates over a 15-week growth period.

	Experimental diets							
Items (%)	32	30	28	26*	26**	28**	*P*-value	PSE⁺
HIS	2.096	1.580	1.978	2.440	2.124	2.343	0.517	0.130
VSI	7.759	6.827	7.696	8.612	6.632	6.991	0.158	0.244
Intestinal index	3.813^{abc}	3.233^{bc}	4.025^{ab}	4.658^{a}	2.680^{c}	3.685^{abc}	0.002	0.163
Carcass yield	86.75^{a}	85.76^{a}	85.35^{ab}	79.37^{b}	85.53^{a}	87.31^{a}	0.008	0.735

Means (n = 4) in the same row with different superscripts are significantly different at *P < 0.05* based upon analysis of variance followed by Tukey's multiple range test.
*IAA was supplemented to meet the requirement according to the NRC [13].
**IAAs were supplemented to meet 120% of the NRC [13] requirements.
⁺PSE: Pooled standard error.

In our study, the highest values of PER (1.39 and 1.40) were obtained in group 5 and group 6, respectively with IAAs supplementation, indicating that Nile tilapia fed on diets supplemented with IAA at 120% of the NRC requirements had better efficiency of protein utilization, which could be due to the fact that amino acids in the diet were used efficiently for protein synthesis rather than as an energy source. These results suggested that adding IAAs to 120% of the NRC nutritional recommendation restored the proper balance or the exact balance required for efficient amino acid utilization. This is further confirmed by the poor growth performance of Nile tilapia, which fed the 26% protein diet without supplementing IAA 20% above the NRC requirements. Comparable results were reported by Zhou, Dan [9], who found that the PER of Nile tilapia was significantly improved (p<0.05) by lowering dietary protein levels from 33.5 to 30.3% with supplementation of IAAs.

Table 4. Analyzed serum biochemical parameters of juvenile Nile tilapia *(Oreochromis niloticus)* fed on experimental diets with reduced dietary protein levels supplemented with IAAs at different rates over a 15-week growth period.

	Experimental diets							
Items	**32**	**30**	**28**	**26***	**26****	**28****	***P*-value**	**PSE⁺**
Total Protein (g/dl)	4.45	4.82	4.30	3.32	3.66	4.55	0.071	0.181
Albumin (g/dl)	1.98	1.97	1.89	1.41	1.69	1.77	0.379	0.082
Triglycerides(mg/dl)	183.75^{ab}	145.85^{ab}	225.13^{ab}	292.65^{a}	159.72^{ab}	124.00^{b}	0.043	19.01
Cholesterol (mg/dl)	173.10^{bc}	163.10^{bc}	222.85^{ab}	265.75^{a}	116.80^{c}	153.50^{bc}	0.006	15.46
AST (U/L)	23.00^{ab}	24.00^{ab}	20.50^{b}	53.25^{a}	24.00^{ab}	30.00^{ab}	0.045	3.77
ALT (U/L)	35.00^{ab}	11.50^{b}	12.00^{b}	51.00^{a}	18.50^{ab}	25.50^{ab}	0.024	4.60
Urea (mg/dl)	13.50^{b}	12.50^{b}	14.50^{b}	21.00^{a}	16.50^{ab}	15.50^{b}	0.004	0.865
Creatinine (mg/dl)	0.55^{b}	0.40^{b}	0.65^{ab}	0.90^{a}	0.55^{b}	0.35^{b}	0.005	0.056
Glucose (mg/dl)	52.70	49.10	54.17	63.93	44.80	52.70	0.189	2.19

Means (n = 4) in the same row with different superscripts are significantly different at *P < 0.05* based upon analysis of variance followed by Tukey's multiple range test.
*IAA was supplemented to meet the requirement according to the NRC [13].
**IAAs were supplemented to meet 120% of the NRC [13] requirements.
⁺PSE: Pooled standard error.

Table 5. Serum immune biomarker and antioxidant enzymes of juvenile Nile tilapia *(Oreochromis niloticus)* fed on experimental diets with reduced dietary protein levels supplemented with IAAs at different rates over a 15-week growth period.

	Experimental diets							
Items (mg/dl)	**32**	**30**	**28**	**26***	**26****	**28****	***P*-value**	**PSE⁺**
IgG	12.25^{ab}	9.00^{ab}	8.00^{b}	7.50^{b}	13.35^{ab}	17.00^{a}	0.031	1.122
IgM	15.63^{ab}	13.50^{ab}	12.09^{ab}	11.50^{b}	13.39^{ab}	17.90^{a}	0.039	0.732
SOD	261.50	274.50	265.00	243.50	258.50	299.50	0.165	6.370
GSH	6.70^{ab}	7.15^{ab}	4.25^{ab}	3.50^{b}	5.90^{ab}	7.70^{a}	0.036	0.510

Means (n = 4) in the same row with different superscripts are significantly different at *P < 0.05* based upon analysis of variance followed by Tukey's multiple range test.
*IAA was supplemented to meet the requirement according to the NRC [13]
**IAAs were supplemented to meet 120% of the NRC [13] requirements.
⁺PSE: Pooled standard error.

Our results indicated that using higher IAAs at 120% NRC in the low protein diets promoted better growth performance in tilapia than those fed low protein diets at 100% NRC. Comparable to the findings of our study Nguyen, Salem [22] noticed that the supplementation of IAA at 110% of the NRC [12] requirements in Nile tilapia diets resulted in better growth performance compared to fish fed 100% NRC. The discrepancies between their results and ours are attributed to the difference in diet formulation, which in their study was carried out according to the requirements stated by NRC [12], while in our study, the diets were formulated based on the NRC [12] requirements as well as there were variations in the experimental conditions in the two studies.

The whole-body moisture and protein contents of tilapia were not significantly affected as the dietary protein was reduced from 32 to 26% and with IAA supplementation at 120% of the NRC requirements in the low protein diets (26 and 28%), while fish body fat and ash contents were significantly increased (Fig. 1, Fig. 2). The increase in the body fat content as a result of reducing dietary protein levels could happen due to the imbalance or the deficiency of amino acids. Amino acids catabolized and got deposited as body fat. Also, it may be because increasing the DE/DP ratio above the required level as dietary protein levels decreased led to an increase in the inclusion level of carbohydrates (yellow corn) Table (1), which consequently led to extra fat deposition. In agreement with our study, Nguyen, Dinh [11] found that the whole-body moisture, protein, and ash were not significantly affected by dietary protein levels except for the lipid content of tilapia. This was due to the fact that reducing protein content from 32 to 24% in their study with IAA supplementation resulted in increasing the inclusion levels of corn starch from 10.75 to 26.87%, which consequently increased body fat deposition.

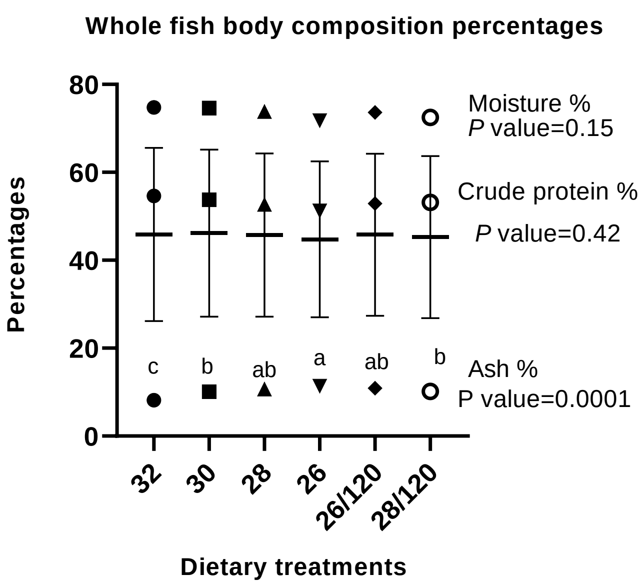

Fig.1 Whole body composition of juvenile Nile tilapia *(Oreochromis niloticus)* fed on experimental diets with reduced dietary protein levels supplemented with IAAs at different rates over a 15-week growth period.

In our study, reducing dietary protein levels to 26% caused an inverse increase in HSI, VSI, and intestinal index, while carcass yield showed a significant decrease. However, with IAAs supplementation at 120% of the NRC requirements, HSI, VSI, and intestinal index showed a slight reduction, while carcass yield was significantly improved Table (3). The greatest carcass yield was observed in tilapia fed 28% CP at 120% of the NRC requirements. The increase in the HSI, VSI, and intestinal index could be because reducing dietary protein levels from 32 to 26% resulted in increasing the use of yellow corn from 10 to 37.02%, and the high levels of yellow corn or carbohydrates consequently got deposited in the liver and viscera as fat. Similar to our results, Zhou, Dan [9] stated that HSI, IPF, and VSI of tilapia were inversely increased by reducing dietary protein content, indicating that more fat got deposited in the liver and in the abdominal cavity.

Serum TP, ALB, glucose, and SOD activity were not significantly affected as dietary protein was reduced from 32 up to 26% and with IAAs supplementation at 120% of the NRC requirements. Meanwhile, TG, cholesterol, AST, ALT, urea, and creatinine concentrations showed a significant increase with reducing dietary protein levels to 26%. The highest AST and ALT activities were observed in fish fed on the lowest protein diet (26%) at 100% NRC (Table 4, 5). These findings were likely attributed to the imbalance of amino acids, which could happen with reducing dietary protein levels. Excess amino acids cannot be directly stored in fish and will be then catabolized, deaminated, and converted into energetic compounds [23]. Hence, high levels of AST and ALT may reflect the use of hydrocarbons from these catabolized amino acids to supply energy requirements. Thus, the above results indicated that excess amino acids in the diet were not used for growth or protein deposition of Nile tilapia, but for energy expenditure towards deamination and excretion. Similar to our findings, Salem, Nguyen [7] demonstrated that by reducing dietary protein levels from 32 to 24% in channel catfish diets, serum TP, ALB, total immnuoglobulins, and glucose were not significantly affected. However, serum TG showed a significant increase with lowering intact protein to 24%.

It was noticed that immunoglobulins (IgG and IgM) showed a significant reduction with reducing dietary protein content to 26%, which could be attributed to the imbalance or deficiency of amino acids. As stated by Kiron, Fukuda [24], adequate dietary protein content is essential to maintain non-specific defense mechanisms. The highest values of both IgG and IgM were observed in tilapia fed on 28% CP diets supplemented with IAA at 120% of the NRC requirements. These results suggested that IAAs supplementation may enhance immune response and overall fish health status. As reported by Li, Mai [25], dietary lysine supplementation improves the immune response of agastric fish (Jian carp).

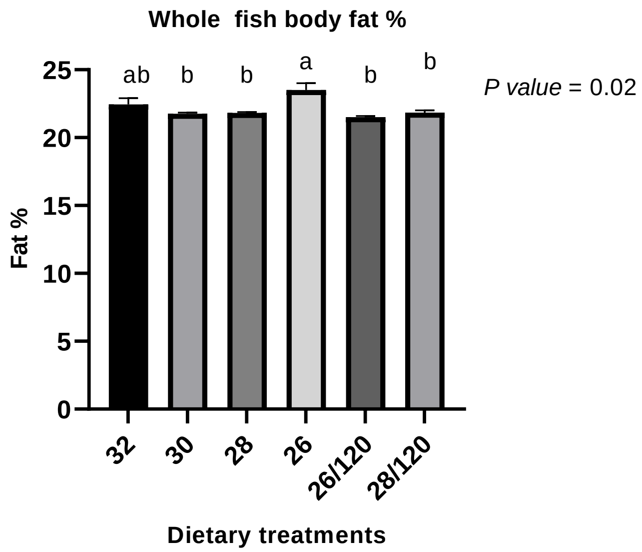

Fig.2 Whole body fat percent of juvenile Nile tilapia *(Oreochromis niloticus)* fed on experimental diets with reduced dietary protein levels supplemented with IAAs at different rates over a 15-week growth period.

Superoxide dismutase (SOD) and reduced glutathione (GSH) enzymes, both are important components of the tilapia antioxidative defense system. There was no significant difference in SOD activities in tilapia fed different dietary treatments, while GSH activity exhibited a significant reduction in fish fed on the lowest protein diet (26%), which may be because of the imbalance of amino acids. However, with IAAs supplementation at 120% of the NRC requirements, GSH activities showed a significant increase, which may be due to the balance of the amino acid profile. Besides, methionine plays a vital role in the defense against oxidative stress. This is attributed to the fact that methionine is a precursor of cysteine, which is involved in the synthesis of glutathione (GSH), which is considered an important antioxidant component in fish as reported by Séité, Mourier [26].

Conclusion

In conclusion, the results of our study confirmed that in Nile tilapia practical diets, dietary protein content could be reduced from 32 to 26% with IAA supplementation at 120% of the NRC nutritional recommendation while maintaining comparable tilapia growth, body composition, immune response, antioxidative activity, and healthiness of the internal organs.

Conflict of interest declaration

The authors declare that there is no conflict of interest to disclose.

Research Ethics Committee Permission

The study was ethically approved by the Research Animal Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University.

Authors' contribution

Sara Youssef designed the experiment, carried out the practical work, wrote and edited the manuscript. Shimaa M.R. Salem participated in the study design, made the data analysis, and reviewed the manuscript critically for its scientific content. Rania E. Mahmoud revised the manuscript. Tarek I. Mohamed supervised all the work. All authors read and approved the final manuscript.

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