Brazilian Journal of Biology
ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

https://doi.org/10.1590/1519-6984.189413
Original Article

The effect of replacement of fish meal with Amino Acids and Optimized Protein Levels in the diet of the Nile Tilapia *Oreochromis niloticus*

*A-W. Abdel-Warith[1,2], N. Al-Asgah[1], Y. El-Sayed[1], A. El-Otaby[1] and S. Mahboob[1,3]**

[1]Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia
[2]Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt
[3]Department of Zoology, GC University, Faisalabad, Pakistan
*e-mail: shahidmahboob60@hotmail.com

Received: December 21, 2017 – Accepted: February 19, 2018 – Distributed: November 30, 2019

Abstract

This study was conducted to determine an appropriate replacement of fishmeal with amino acids (AAs) and optimized protein levels in practical diets for *Oreochromis niloticus* with mean initial body weight 12.52±0.63g. Six experimental and a control diet (total 7 diets) divided into two groups, and a control diet (D1) containing 32% protein. The first group contained three diets that included different dietary protein levels, *viz*. 20 (D2), 25 (D3), and 30% (D4) with AAs when replacing fishmeal by plant protein sources. In the second group, the diets were contained 20 (D5), 25 (D6), and 30% (D7) without AAs. The best growth performance was achieved in fish fed with diet D1. Total feed intake was increased with an increase in dietary protein level with AAs. The specific growth rate showed a similar pattern with a significant difference between control, D4 and D7 compared to other groups. The feed conversion ratio decreased when protein levels in the diets increased. The protein efficiency ratio showed a similar performance, with a slight increase between the control diet and diets with AAs. However, insignificant differences (P>0.05) were observed between diets with and without AAs. An economic evaluation indicated that inclusion of low fishmeal in tilapia diets reduced the price/kg of diets compared to control.

Keywords: amino acids supplementation, economic evaluation, growth performance, plant ingredients.

O efeito da substituição da farinha de peixe por Aminoácidos e Níveis Otimizados de Proteína na dieta da tilápia do Nilo Oreochromis niloticus

Resumo

Este estudo foi realizado para determinar uma substituição adequada de farinha de peixe com aminoácidos (AA) e níveis otimizados de proteína em dietas práticas para Oreochromis niloticus com peso corporal inicial médio de 12,52 ± 0,63 g. Seis dietas experimentais e controle (total de 7 dietas) divididas em dois grupos, e uma dieta controle (D1) contendo 32% de proteína. O primeiro grupo continha três dietas que incluíam diferentes níveis de proteína na dieta, viz. 20 (D2), 25 (D3) e 30% (D4) com AAs ao substituir a farinha de peixe por fontes de proteína vegetal. No segundo grupo, as dietas continham 20 (D5), 25 (D6) e 30% (D7) sem AAs. O melhor desempenho de crescimento foi alcançado em peixes alimentados com dieta D1. O consumo total de ração foi aumentado com o aumento do nível de proteína na dieta com AAs. A taxa de crescimento específico mostrou um padrão semelhante com uma diferença significativa entre o controle, D4 e D7 em comparação com outros grupos. A taxa de conversão alimentar diminuiu quando os níveis de proteína nas dietas aumentaram. A taxa de eficiência protéica apresentou desempenho semelhante, com discreto aumento entre a dieta controle e as dietas com AAs. Entretanto, diferenças insignificantes (P> 0,05) foram observadas entre dietas com e sem AAs. Uma avaliação econômica indicou que a inclusão de farinha de peixe baixa em dietas de tilápia reduziu o preço / kg de dietas em relação ao controle.

Palavras-chave: suplementação de aminoácidos, avaliação econômica, desempenho de crescimento, ingredientes vegetais.

1. Introduction

The increasing cost of the strategic use of fish meal for higher value fish species means that it is necessary to consider economic diets for aquaculture and this is especially important for the non-carnivorous fish, other than salmonids and sea basses that require high specification diets and where fishmeal is widely used. Considerable scholarly attention has, therefore, been concentrated on evaluating the use of plant protein sources, such as oil seeds, pulses, and cereals, in the context of commercial diets for fish.

Oreochromis niloticus is one of the most promising fish for freshwater culture, especially in South Asian countries (FAO, 2004) also, since it is an omnivorous fish with low mortality and rapid growth, it accepts artificial diets for all stages of production (El-Sayed, 2006). Fish meal has a sufficient and balanced content of amino acids when compared with plant protein sources that are often limited in some of the essential amino acids with consequent negative effects on growth and feed utilization when substituted for fish meal in diet formulations (Wilson, 1989). Economic feeds of tilapia contain about 5–10 percent of fish meal in the diet, but continuous price hike of fish meal forced the fish culturists to use certain supplementations (Naylor et al., 2000). The consideration has been given to tilapia nutrition with an attention on the partial or total substitution of fish meal by cheap vegetable protein sources in tilapia feeds (El-Sayed, 1999) such high levels of replacement plant vegetable sources, however, require supplementation of essential AAs in fish diets.

Fish body weight and age are important parameters for determining the content of protein diets to support efficient fish production. Some studies, however, have suggested that the total replacement of fish meal by vegetable protein affected growth performance of rainbow trout (Gomes et al., 1995) and Atlantic salmon (Espe et al., 2006). However, 100% replacement of fish meal in diets was possible for salmon with no negative impact on growth. The feed consumption of diet supplemented with amino acids in salmon was better compared to high concentrate fish meal feed (Espe et al., 2007; Ayisi et al., 2017).

Knowledge of the protein demands of fish during the rapid growth period is necessary for aquaculture practice to achieve maximum feed conversion and utilization, saving some of the costs, and reducing protein load in the aquatic environment (Abdel-Tawwab et al., 2010). Many researchers attempted to determine the substitution of fishmeal in feed for tilapia with cheap, indigenous plant, and animal sources of proteins (El-Saidy and Gaber, 2003; Gonzales Júnior et al., 2007; Lim et al., 2007; Zuo et al., 2017). However, more investigations are required to evaluate the performance of different combinations of alternative constituents to prepare a balanced tilapia diets. Koch et al. (2016) demonstrated that the available economical feeds for *O. niloticus* entail nutrient reduction or are unbalanced, such that the decreased feed utilization is likely to release unutilized protein, carbohydrates, and other constituents into, and cause eutrophication of, adjoining water reserviors (Montanhini Neto and Ostrensky, 2015). Meanwhile, Webster et al. (1999) reported that compounding animal and plant protein sources with additional amino acid profiles may enhance the possibility of nutrient inadequacy that could adversely affect fish growth. Fishmeal is successfully substituted with other vegetable protein sources in fish feeds, there is some concern that the current evaluations of essential amino acid requirements are unsatisfactory goal (Furuya et al., 2004), Some AAs may also turn into a limiting factor and thus need to be supplemented in feed for important species of fish (El-Sayed, 2014; Liu et al., 2017).

The main aim of this study were to define the effect of dietary protein level, Essential Amino Acid (EAA) supplementation as a replacement of fishmeal, and their interaction on growth, feed utilization, carcass composition, and the potential cost benefit to the aquaculture of Nile tilapia.

2. Material and Methods

2.1. Collection of fish and growth trial

Fingerlings of Nile tilapia, *O. niloticus*, were procured from the fish seed hatchery of King Abdulaziz City for Sciences and Technology, Riyadh, Saudi Arabia. Fish were acclimatised for two weeks before the experiment. A total of 315 fingerlings of *O. niloticus*, with an average body weight of 12.52±0.63g and body length of 9.13±0.27cm, were assigned to this study. The fish were stocked into 21 glass aquaria containing 75 litres of dechlorinated tap water. Each feed was given with three replicates in factorial design. The water temperature was maintained at 28 ±1°C by thermostatically controlled immersion heaters. Water quality parameters were held within the desirable range for tilapia: pH 6.9–8.0, ammonia (NH_3) 0.08–0.21 mg/L, nitrite (NO_2) 0.17–0.36 mg/L, nitrate (NO_3) 4.28–5.71 mg/L, and dissolved oxygen 5.9–7.4 mg/L (El-Sayed, 2014).

2.2. Diet formulation

Seven diets were formulated to include a control diet (D1), which contained 32% protein, while the other six diets were divided into two groups: a group containing three diets that included different dietary protein levels, that is, 20 (D2), 25 (D3) and 30% (D4) with AAs supplementation in the form of methionine (Met 2%) and lysine (Lys 1%) achieved by replacing fishmeal with plant protein sources. In the second group, another three diets contained the same protein levels i.e. 20 (D5), 25 (D6) and 30% (D7), replacing fishmeal by plant protein sources but without EAA supplementation. Table 1 displays the proximate analysis of the major dietary ingredients performed prior to the formulation of the experimental diets. The formulation and proximate composition of the diets are presented in Table 2.

2.3. Growth studies

Fish were weighed fortnightly and fed at 3% of body weight twice daily, in the morning at 8.00 am and evening at 6.00 pm. The feeding trial was conducted for 12-week. At the end of the feeding experiment, five fish from each aquarium were killed for the determination of the proximate composition of fish meat. Growth and nutrient efficiency were calculated by following the equations 1-4 as described by Guo et al. (2012):

$$Weight\ gain\ (WG) = (BWf - BWi) \tag{1}$$

Where BWi = Initial body weight and BWf = Final body weight.

$$Feed\ conversion\ ratio\ (FCR,\ g) = \frac{Feed\ intake\ (g)}{Weight\ gain\ (g)} \quad (2)$$

$$Protein\ efficiency\ ratio(PER) = \frac{Biomass\ (g)}{Protein\ intake\ (g)} \quad (3)$$

Specific growth rate (SGR)

Specific growth rate (SGR) was estimated according to Dhwan and Kaur (2002); that is:

$$SGR = \frac{In(Final\ wet\ body\ weight) - In(Initial\ wet\ body\ weight) \times 100}{Time\ duration\ (days)} \quad (4)$$

Protein productive values (PPV (%): retained protein (g)/total protein intake (g) × 100.

2.4. Proximate Composition

Proximate chemical compositions of diets and fish tissue for moisture, protein, lipid, and ash were determined according to AOAC (1995).

2.5. Determination of amino acids

The amino acid analysis was performed with High performance liquid chromatography (HPLC, Shimadzu Co. Ltd, Kyoto, Japan) so as to analyze and quantify the amount of 15 amino acids in feeds +fertilizers +Pure Amino Acids (Feed Grade) and Premixes. Approximately 0.2-0.5 g of dry sample was weighed and hydrolysed with 50 ml of 50% HCl for 18 hours at 110°C. A Sodium citrate buffer, PH = 2.2, was used to make the solution up to 100ml. The sample was then dervetized in the column oven before the separation into the HPLC column using a Borate buffer and O-phthaldialdeyde (OPA). Elution entailed using three buffers as mobile phases and was detected with a fluorescence detector, at EX = 348 nm and EM = 480 nm wavelengths. The amino acid compositions in the diets (g/100g diet) are presented in Table 3.

Table 1. Chemical composition of ingredients used in diets formulation fed to Nile tilapia *Oreochromis niloticus.*

	Moisture	Protein	Lipid	Ash	NFE[1]	Gross energy (Kcal/kg)[2]
Fish meal	8.89	67.74	10.33	20.80	1.13	485.10
Soybean	6.84	50.12	2.91	6.77	40.20	479.52
Yellow corn	10.60	8.80	3.10	4.40	83.70	430.56
Wheat bran	7.00	15.81	5.25	4.55	74.39	451.35
wheat	11.20	17.58	1.40	1.59	79.43	356.18

[1]Nitrogen free extract (NFE) = 100-(CP+EE+Ash). [2]Gross energy (GE) was calculated as 5.65, 9.45 and 4.2 Kcal/g for CP, EE, and NFE, respectively according to Hepher et al., 1983.

Table 2. Proximate composition for experimental diets (g100 g^{-1} dry weight) fed to Nile tilapia *Oreochromis niloticus.*

Ingredients	Diets						
	D1 (control)	D2 (20%CP+AA)	D3 (25%CP+AA)	D4 (30%CP+AA)	D5 (20%CP)	D6 (25%CP)	D7 (30% CP)
Fish meal	40	3	10	18	3	10	18
Soybean meal	----	25.00	25.00	25.00	25.00	25.00	25.00
Wheat meal	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Wheat bran	20.00	22.00	20.00	20.00	20.00	20.00	20.00
Yellow corn	20.00	25.00	20.00	12.00	30.00	23.00	15.00
corn oil	2.00	4.00	4.00	4.00	4.00	4.00	4.00
Methionine	----	2.00	2.00	2.00	----	----	----
Lysine	----	1.00	1.00	1.00	----	----	----
Vitamin mix[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Proximate composition (% as fed)							
Moisture	7.61	8.21	7.98	7.71	7.92	7.88	7.21
Protein	31.85	21.85	25.40	29.72	21.89	25.67	29.99
Lipid	6.05	4.64	5.09	5.75	5.64	5.28	6.73
Ash	13.24	7.96	8.91	9.42	5.66	6.79	8.54
NFE[3]	48.40	65.55	60.60	55.11	66.87	62.26	54.74
GE (Kcal/kg) [4]	443.00	442.61	446.13	453.72	457.83	456.43	462.65

[1]Vitamins mixture contained (as g/kg premix): Thiamine 2.5; Riboflavin2.5; pyridoxine 2.0; Inositol 100.0; Biotin 0.3; Pantothenic acid 100.0; folic acid 0.75; Para-aminobenzioic 2.5; Choline 200.0; Nicotinic acid 10. Cyanocobalmine 0.005; Tocopherol acetate 20.1; Ascorbic acid 50.0; Mena Dione 2.0; Retinol palmitate 100.000 IU; Cholecalciferol 500.000 IU. [2]Mineral premix (as g/kg premix): $CaHPO_4.2H_2O$ 727.7775; $MgSO_4$. H_2O 127.5 ; Kcal50.0 ; Na Cl 60 ; Fe $SO_4.7H_2O$ 25; $ZnSO_4$. $7H_2O$ 505; $MnSO_4.4H_2O$ 2.53 ; Cu SO_4 5 0785 ; $CoSO_4$ $7H_2O$ 0.4775 ; Ca Lo_3. $6H_2O$ .295 ; Cr CL_3. $6H_2O$ 0.1275. [3]Nitrogen free extract (NFE) = 100-(CP+EE+Ash); [4]Gross energy (GE) was calculated as 5.65, 9.45 and 4.2 Kcal/g for CP, EE, and NFE, respectively according to Hepher et al., 1983.

Table 3. Essential amino acids composition (expressed as percentage of diets) of the control and test diets fed to *Oreochromis niloticus* and its requirements.

	D1	D2+AA	D3+AA	D4+AA	D5	D6	D7	Requirements*
Arginine	2.08±0.07[a]	1.48±0.06[d]	1.66±0.04[c]	1.85±0.04[b]	1.39±0.07[d]	1.77±0.05[bc]	1.83±0.04[b]	1.33
Histidine	0.97±0.05[a]	0.53±0.04[d]	0.63±0.04[c]	0.83±0.03[b]	0.49±0.08[d]	0.62±0.04[c]	0.76±0.02[b]	0.54
Isoleucine	1.46±0.03[a]	0.92±0.06[c]	1.18±0.07[b]	1.38±0.06[a]	0.99±0.03[c]	1.14±0.07[b]	1.45±0.06[a]	0.99
Leucine	2.46±0.06[a]	1.67±0.10[d]	2.08±0.04[b]	2.50±0.14[a]	1.74±0.07[d]	1.93±0.04[c]	2.49±0.07[a]	1.09
Lysine	2.45±0.04[a]	2.28±0.02[bc]	2.35±0.03[b]	2.46±0.05[a]	2.20±0.05[c]	2.30±0.02[b]	2.35±0.01[b]	1.63
Methionine	0.74±0.01[a]	0.72±0.02[a]	0.73±0.03[a]	0.72±0.02[a]	0.63±0.01[c]	0.65±0.02[b]	0.67±0.04[b]	1.02
Phenylalanine	1.41±0.04[b]	0.92±0.09[e]	1.24±0.04[c]	1.59±0.10[a]	1.08±0.04[d]	1.10±0.07[d]	1.55±0.08[a]	1.82
Threonine	1.56±0.07[a]	1.16±0.09[c]	1.30±0.08[b]	1.38±0.04[b]	1.07±0.06[c]	1.14±0.08[c]	1.28±0.04[b]	1.15
Valine	1.75±0.06[b]	1.15±0.08[e]	1.51±0.10[c]	1.96±0.11[a]	1.06±0.05[e]	1.34±0.07[d]	1.64±0.09[bc]	1.09
Tryptophan	ND	ND	ND	ND	ND	ND	ND	

*From Santiago and Lovell (1988).

3. Statistical Analysis

The data were analysed statistically by using one-way analysis of variance (ANOVA) technique and the means were compared using the Duncan's Multiple Range Test (DMRT) as described by Snedecor and Cochran (1989).

4. Results

4.1. Biochemical composition of diets

The experimental diets contained different levels of protein ranging between 20-30% with and without amino acid supplementation (Table 2). The dietary AAs shown in Table 3 indicate that almost all the AAs were highest in D1 except Lysine in D4 and D7 which were slightly higher than D1; Phenylalanine was higher again in D4 and D7; and Valine in D4, which contained a high amount of fishmeal with significant differences (P>0.05) compared to other diets. Diets containing plant source protein and AAs (D2, D3, and D4) showed slightly higher levels of some amino acids than the other three diets (D5, D6, and D7), especially of lysine and methionine, which showed significantly higher values. However, most of the dietary amino acid contents were affected in diets excluding AAs.

4.2. Growth performance

The growth performance and feed utilization results for the *O. niloticus* fed on the seven diets are presented in Table 4. No mortality was recorded during the study period. A significant difference (P<0.05) was observed for the final average body weight and length for fish fed with D1 (control diet), D2, D3, D5, and D7. The fish fed with diet D4 and D7 exhibited non-significant difference with each other. The highest mean body weight was recorded as 42.46, 39.97 and 38.33g in fish fed the fishmeal-based control diet (D1), and those with the highest levels of dietary protein (D4 and D7), respectively, compared with the other four diets (D2, D3, D5, and D6) that contained 20 and 25% of protein with and without AAs supplementation (Table 4). These fish were weighed at 30.64, 35.59, 27.45 and 29.42g respectively. Following the same pattern, the results of final body length ranged between 13.32 and 11.46cm (Table 4). The specific growth rate (SGR%) results further substantiated this trends, with high SGR for D1, D4, and D7, that is, 1.42, 1.37 and 1.42% respectively. SGR values for D2, D3, D5, and D6 were 1.05, 1.19, 0.88, and 1.10%, respectively, with significant differences (P<0.05) between all groups except D5 which fed 20% protein without AAs (Table 4). Weight gain also was significantly higher in D1, D4, and D7 compared with other groups (Table 4).

4.3. Feed consumption and feed utilization

The experimental feeds (D1, D3, D4, and D7) were well accepted by *O. niloticus*, except fish fed with diets D2, D5 and D6, which contained 20% protein+ AAs, and 20 and 25% protein without amino acid, respectively. These diets resulted in a total feed intake per fish of 45.50, 39.98 and 39.03g, which were significantly lower (P<0.05) compared to 48.21, 47.76, 48.34 and 48.00g for feeds D1, D3, D4, and D7, respectively (Table 4). Feed intake for *O. niloticus* fed on the D1 (control diet) having the highest amount of fishmeal, D4 and D7 which contained 30% of protein with and without AAs supplementation performed better compared to other diets. The best FCR values were 1.63, 1.77, and 1.80 for D1, D4, and D7, compared to 2.53, 2.12, 2.78, and 2.20 for D2, D3, D5, and D6, respectively. The protein efficiency ratio (PER) was noticeably different between treatments and followed the similar pattern, where the fish fed with D1 had better PER (1.93) followed by D3, D4, and D7 (1.86, 1.90, and 1.85, respectively) with insignificant differences between the groups. Protein productive values (PPV %) values also showed a decrease when fish were fed diets containing 20%+AAs, and 20, 25, and 30% protein without AA supplementation in group fed with D1, D3, D4 with a significant difference (P<0.05) between treatments (Table 4).

4.4. Fish body composition

The initial and final proximate composition of the fish fed with the experimental diets is presented in Table 5. The final proximate composition in *O. niloticus* exhibited

Table 4. Weight increase, feed consumption, nutritive utilization of *Oreochromis niloticus* fed experimental diets (mean ±SD *n=3*).

	D1 (control)	D2 (20%CPV+AA)	D3 (25%CP+AA)	D4 (30%CP+AA)	D5 (20%CP)	D6 (25%CP)	D7 (30% CP)
Mean initial weight (g)	12.94±2.16	12.65±1.12	13.06±1.18	12.66±0.93	13.07±0.75	11.65±1.49	11.64±1.37
Mean final weight (g)	42.46±3.98^{a}	30.64±1.71^{c}	35.59±1.58^{b}	39.97±1.65^{ab}	27.45±1.18^{c}	29.42±1.16^{c}	38.33±1.33^{ab}
Mean initial length (cm)	9.40±0.71	9.01±0.38	9.32±0.31	9.29±0.30	9.33±0.28	8.82±0.36	8.75±0.38
Mean final length (cm)	13.32±1.93^{a}	11.66±1.28^{c}	12.45±1.39^{b}	12.92±1.64^{ab}	11.58±0.92^{c}	11.46±1.21^{c}	12.19±1.67^{bc}
Weight gain (g)	29.53±0.55^{a}	17.99±0.06^{d}	22.53±0.61^{c}	27.31±1.57^{ab}	14.38±0.62^{e}	17.77±0.15^{d}	26.69±0.14^{b}
SGR%[1]	1.42±0.13^{a}	1.05±0.13^{ab}	1.19±0.15^{ab}	1.37±0.14^{a}	0.88±0.13^{b}	1.10±0.16^{ab}	1.42±0.17^{a}
Percentage of WG %	228.23±2.85^{a}	142.17±4.31^{c}	172.55±4.29^{b}	215.79±4.02^{a}	110.00±6.28^{d}	152.57±0.41^{bc}	229.28±1.30^{a}
FI g/fish	48.21±1.68^{a}	45.50±1.71^{b}	47.76±1.73^{a}	48.34±1.39^{a}	39.98±1.33^{c}	39.03±1.16^{c}	48.00±1.08^{a}
FCR[2]	1.63±0.18^{c}	2.53±0.27^{ab}	2.12±0.27^{bc}	1.77±0.17^{c}	2.78±0.25^{a}	2.20±0.48^{bc}	1.80±0.13^{c}
PER[3]	1.93±0.35^{a}	1.81±0.30^{a}	1.86±0.35^{a}	1.90±0.27^{a}	1.65±0.30^{a}	1.77±0.35^{a}	1.85±0.27^{a}
PPV (%)[4]	24.54±1.53^{a}	17.81±1.20^{b}	23.58±1.00^{a}	25.68±1.62^{a}	8.81±1.53^{d}	12.68±1.70^{c}	16.25±1.18^{b}

Values in the same row with the same superscript (a-d) are not significantly different (P >0.05). [1]SGR: [Ln final body weight (g) - Ln initial body weight (g)]/experimental period (days) ×100. [2]FCR: feed intake (g)/body weight gain (g). [3]PER: body weight gain (g)/protein intake (g). [4]PPV (%): retained protein (g)/total protein intake (g) × 100.

Table 5. Body composition of Nile tilapia fed diets with and without amino acids supplementation.

Parameters	Initial	D1 (control)	D2 (20%CP +AA)	D3 (25%CP +AA)	D4 (30%CP +AA)	D5 (20%CP)	D6 (25%CP)	D7 (30% CP)
Moisture	77.53±0.72	74.51±0.63^{c}	74.25±0.45^{c}	75.23±0.63^{bc}	74.57±0.71^{c}	76.72±0.83^{ab}	78.20±0.58^{a}	76.00±0.60^{bc}
Protein	12.63±0.58	16.40±0.55^{a}	14.40±0.20^{b}	15.49±0.30^{a}	16.32±0.48^{a}	13.40±0.14^{b}	13.90±0.59^{b}	14.97±0.20^{a}
Lipid	3.62±0.13	4.90±0.65a	2.99±0.52^{bc}	3.61±0.65^{abc}	2.85±0.62^{c}	4.39±0.52^{abc}	3.88±0.59^{abc}	4.66±0.44^{ab}
Ash	3.47±0.11	4.27±0.13^{ab}	3.46±0.13^{c}	4.03±0.08^{b}	4.02±0.10^{b}	4.12±0.10^{ab}	4.26±0.07^{ab}	4.25±0.10^{ab}

Values in the same row with the same superscript (a-c) are not significantly different (P>0.05). Values expressed as mean ±SE (n=3).

significant (P<0.05) variation in different constituents for different experimental diets. Fish fed with the fishmeal-based control diet D1, and diets D3, D4, and D7 showed a significant elevation in carcass protein with a protein content of 16.40, 15.49, 16.32, and 14.97 respectively compared with other the D2, D5 and D6 diets with values of 14.40, 13.40 and 13.90, respectively. Whole body lipid content showed significant differences between treatment, with the significantly highest lipid content observed in fish fed D1, D5, and D7 compared to other treatments. A significantly higher ash content occurred in fish fed with the diets D1, D5, D6, and D7 (P<0.05), it was lower in diets D2, D3 and D4 respectively. In contrast, fish fed diets with amino acids supplementation showed a decrease in moisture, lipid and ash contents (Table 5).

4.5. Economic evaluation

The results in Table 6 provide an economic evaluation of the experimental diets, including feed costs, costs per one kg weight gain, and the ratio compared to the control diet. The costs of 1kg of the control diet and other tested diets were 1.23, 0.53, 0.69, 0.87, 0.52, 0.67, and 0.86 US Dollar for D1, D2, D3, D4, D5, D6, and D7, respectively. These results indicated that a reduction in the use of fishmeal in tilapia diets reduced the price of 1kg of those diets to 43.09, 56.10, 70.73, 42.28, 54.47, and 69.92% of the control diet. The costs required to produce one kg of weight gain were the highest for the control diet and gradually decreased to 66.66, 72.64 and 73.63 percent for D2, D3, and D4 (i.e. the diets containing AA supplementation) and 72.14, 73.13, and 77.11% for D5, D6, and D7 that did not have AA supplementation (Table 6). The minimum cost of production was found in fish fed with D2.

5. Discussion

The results of this study show that the different protein levels of the experimental feeds noticeably affected the growth performance and feed utilization of Nile tilapia. Most of the AAs determined in the diets were relatively higher than the minimum requirement for AAs in Nile tilapia (Santiago and Lovell, 1988) except methionine and phenylalanine. The findings of this study indicated that the best dietary protein requirement for *O. niloticus* depends

Table 6. Feed costs (US$) for producing one kg weight by fish fed the experimental diets.

	D1	D2+AA	D3+AA	D4+AA	D5	D6	D7
feed cost (US$/Kg)	1.23	0.53	0.69	0.87	0.52	0.67	0.86
Relative to control %	100	43.09	56.10	70.73	42.28	54.47	69.92
Decrease in feed costs %	0.00	56.91	43.90	29.27	57.72	45.53	30.08
FCR	1.63	2.53	2.12	1.77	2.78	2.20	1.80
Feed cost (US$/Kg WG*)	2.01	1.34	1.46	1.48	1.45	1.47	1.55
Relative to control %	100	66.66	72.64	73.63	72.14	73.13	77.11

The price of 1kg ingredients used was 9.2 Saudi Riyals (SR) for fish meal,1.6 soybean, 0.7 for wheat bran, 0.8 for Wheat meal, 0.75 for yellow corn, 12.00 for corn oil, 2.60 for methionine, 1.5 for lysine and 10.00 for vitamin and minerals mix according to market price at the time of study (2014). All prices were converted to US$ which equal 3.75 SR. *Feed costs/1kg weight gain= FCR × costs of kg feed.

on fish weight. In this study, the initial weight of the fish was 12g, meaning that our data was in agreement with Khattab et al. (2000) who reported the best dietary protein level for three *O. niloticus* strains of the similar weight group and noted that the optimum level of dietary protein ranged between 27 and 37%. The results of this study observed that plant protein sources are unable to replace 3g/100g of diets (see Table 2) which equated to about 10% of the total protein fishmeal in diets, especially D2 and D5 that contained less protein than the requirement (20% with or without AAs) for *O. niloticus* (El-Sayed, 2006). Growth and feed utilization were, also, reduced in fish fed with diets D3 and D6, which include (10g/100g diet) in which fishmeal was replaced with vegetable protein sources these might be the ration of replaced is high about 75% of total protein from plant ingredients, these diets contain AAs lower than the minimum requirements of this fish especially without methionine in D6 (Table 2). The addition of AAs enhances the growth and feed utilization for fish fed D3 which has the same protein level but with AAs supplementation. The present findings were in accordance with results illustrated by Santigosa et al. (2008) who observed that substitution of fish meal by vegetable proteins led to a reduction in fish weight in *Oncorhynchus mykiss* and sea bream *Sparusaurata*. *O. niloticus* fed with diets D4 and D7 (30% protein) with and without AA supplementation, showed excellent final body weight with no significant difference when compared to the control. The current study, therefore, serves to recommend that supplemented amino acids might not be efficiently utilized by Nile tilapia fed diets with low protein levels and high amount of plant protein sources.

The decrease in fishmeal content from 40g/100g to 3g/100g in the diets by progressively increasing the extent of replacement with mixed plant protein in gilthead sea bream and tilapia diets reduced the efficiency of digestive enzymes and effected on growth performances (Kokou et al., 2016; Abdel-Warrith et al., 2013). In this study the experimental diets showed a significant effect on the growth performance of Nile tilapia. These outcomes are in line with results reported by Pratoomyot et al. (2010) who observed that when the fishmeal content of the diet was reduced from 25% to 5% there was a decrease in growth performance and thus in body weight among Atlantic salmon *Salmo salar*. Moreover, in our study, growth parameters such as SGR were enhanced by increasing protein level and AA supplementation in the dietThese results were in agreement with results obtained by Pratoomyot et al. (2010) who reported that SGR for *Salmo salar* fed diets containing 18, 11 and 5% of fishmeal decreased compared to control diet (25% fishmeal). The tilapia fed the control 32% fishmeal and 30% with and without AAs for D1, D4, and D7, respectively showed a better weight gain. Our findings were in accord with the findings of Pratoomyot et al. (2008, 2010).

Methionine and lysine addition did not improve the increase in fish weight and length for diets containing 20% protein compared to other two diets with 25 and 30% protein. The amount of amino acids like methionine supplementation in D2 was insufficient (lower than the requirements, as listed in Table 3). This may, in turn, be due to D2 and D5 having low amounts of fishmeal which effect on the amino acids content in diet composition. This was in line with findings of Pratoomyot et al. (2010) who found that increasing alternatives to fishmeal in the form of plant protein in diets for *Salmo salar* caused a decrease growth performance that was due to reduced palatability. This is also compatible with other previous studies (Davies and Morris 1997; Kaushik et al., 2004; Espe et al., 2006; Abdel-Warrith et al., 2013).

The growth performance of *O. niloticus* fed diets containing AAs with a protein level of 25% declined in relation to any of the 30% protein diets. Our study was incompatible with this previous data (Yamamoto et al., 2005). although the individual AA requirements were sufficient except for methionine, which was lower than requirements for Nile tilapia test diets. The methionine concentration in the experimental diets ranged between 0.63%– 0.74% of the feed, being lower in diet D1, D2, D3 and D4 (control and the other three diets supplemented by methionine), but still less than the requirements for all test diets and lower than 1.02% of protein. These results were in agreement with findings of Koch et al. (2016) with respect to the same species of fish. Diógenes et al. (2016) reported that the contents of methionine in the best growth performing substitutent diet was slightly higher than the optimum methionine level for *O. niloticus* (Botaro et al., 2007; Michelato et al., 2013, 2016). On the other hand, the concentration of methionine was lower than this requirement in most of the other test diets.

Variations in protein utilization parameters, such as PER and PPV were affected by the treatments. These parameters were significantly (P>0.05) affected by protein content and AA supplementation in the diets. Protein metabolism increased with increase in dietary protein levels and AA supplementation. It might be because a major part of weight increase is related to the protein retention, and protein increases establish equilibrium between protein anabolism and catabolism (Diogenes et al.,2016). Abdel-Tawwab et al. (2010) reported that protein metabolism, including PER, PPV, and PGR, were significantly (P>0.05) affected by protein levels in the diets. Moreover, feed efficiency was significantly affected by protein content and AA supplementation, FCR improved with fish fed diets containing high protein levels and AAs supplementation as previously shown in respect to the Nile tilapia (Abdelghany, 2000; Khattab et al., 2000).

The proximate composition of the carcasses of the fish was significantly affected by dietary protein level, and AA supplementation of the fish fed diets containing high amounts of protein showed high values of protein in their body composition. This was in line with results obtained by Abdel-Tawwab et al. (2010) who demonstrated that Nile tilapia fed a 25%-CP diet had lower protein values and higher lipid content than fish fed 35%- or 45%-CP diets. This might be related to the high feed consumption, feed utilization, and the high nutrient digestibility as the accumulated nutrients increased in the experimental diets. Furuya et al. (2004) mentioned that dietary amino acid supplementation caused an increase in protein and decreased the lipid in the fish body. The variations in lysine requirement within and among species have been observed in several publications. The variations in amino acids supplementation may be a result of nutrient utilization, different basal diets, different feeding levels and environmental conditions, the age of fish and species or strains.

In this study, all diets other than the control diet were different from commercial diets in terms of cost because it's containing less amount of fishmeal. Therefore, the growth performance for tilapia was significantly lower than the control except for D4 and D7, which were insignificantly different in terms of growth performance and feed utilization compared to the control, which saves about 30% of feed costs (US$) for producing one kg of additional weight. Furthermore, the amino acid supplementation improved the economic evaluation in tilapia diets. Most studies have evaluated fishmeal substitution in tilapia feeds from biological and nutritional standpoints. A very little attention has been paid to the cost analysis of these dietary sources but the few investigations in this area have indicated that the unusual or plant protein sources were more economical compared with fishmeal.

6. Conclusion

It can be concluded that *O. niloticus* are able to utilize plant protein-based diets with mixed sources (soybean, yellow corn, wheat meal and wheat bran) that covered about 60% of total protein (D4 and D7) with and without amino acids supplementation. Growth and feed utilization were much lower than in the fish fed the control diet except for the D4 and D7 groups which contained 30% protein, with and without methionine and lysine supplementation, respectively. The economic evaluation conducted in this study saved about 30% of feed costs for producing one kg weight. Future studies should, therefore, consider the use of these ingredients with supplementation of high amounts of amino acids.

Acknowledgements

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for the funding of this research through the Research Group Project No. RGP-VPP-304.

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