**Ideal protein concept and its application in practical diets for Nile tilapia *Oreochromis niloticus***

by

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## ABSTRACT

Protein is considered the most important dietary component as it comprises a significant proportion of whole body dry matter of fish. As it has high cost per unit, provision of feed meeting an exact requirement for protein is a prerequisite for efficient and economical fish production. To do this, the diet has to be formulated properly with regard to amino acid (AA) balance. Like other fish species, research on the balance of indispensable AA (IAA) and dispensable AA (DAA) for promoting maximum growth and protein deposition on Nile tilapia *Oreochromis niloticus* has been limited and inconsistent. Considering the importance of meeting AA requirement, this research sought to optimize AA balance of the diets offered to Nile tilapia by applying and validating the use of the ideal protein concept.

The first study was conducted to evaluate the production performance of Nile tilapia fed graded levels of protein with or without crystalline IAA supplemented in practical diets. Reducing the levels of intact protein from 32 to 24% without IAA supplementation resulted in a significant reduction in growth of Nile tilapia. The supplementation of crystalline IAA to meet AA requirements at two intact protein levels of 29.7 and 27.2% helped the fish to reach comparable growth rates and protein utilization efficiencies with fish fed the reference diet (32% intact protein). Further reduction of intact protein levels to 24.7 and 22.2% of diet, however, induced growth depression of fish which could result from a deficiency of nonspecific nitrogen as a source of energy or limitation of daily IAA intake.

The following study was conducted to further optimize AA balance of diets for Nile tilapia. The IAA profiles of diets in this study were enhanced to IAA profile of the diet which supported the best performance of fish in the first study. This diet was after that supplemented with DAA at 4% to evaluate the role of DAA in the low protein diet. The results obtained after ten weeks indicated that the supplementation of DAA in the low protein diet is significant to overcome limitations of nonspecific nitrogen. Enhancing IAAs to IAA profile of the diet, which supported the best performance of fish in the first study without DAA supplements, could not help the fish to reach comparable weight gain to fish fed the diet with DAA supplements. The potentially limiting IAA (arginine, threonine, valine, tryptophan and isoleucine) in addition to lysine, methionine and threonine in the ingredient matrix was also confirmed. These IAAs were individually deleted from the IAA profile of the diet with enhanced IAA and DAA supplements. Results illustrated that with the exception of valine, the deletion of the other crystalline IAA supplements (tryptophan, arginine, threonine and isoleucine) did not cause any deleterious effects on growth performance and protein utilization efficiency of fish. Therefore, in addition to lysine, methionine and threonine, valine is limiting in our ingredient matrix and the supplementation of this IAA is necessary to meet the requirements of fish.

Even though tryptophan is not limiting in our ingredient matrix, the reported requirement of this IAA is inconsistent so the requirement was confirmed. The tryptophan requirement of juvenile Nile tilapia was confirmed at 0.31% (0.25 - 0.37%), 0.33% (0.26 - 0.39%), 0.25% (0.24 - 0.25%) and 0.27% (0.25 - 0.31%) of the diet for optimal growth, tryptophan deposition, feed efficiency and apparent net protein deposition (95% of maximum value), respectively.

The final study was conducted to confirm the role of DAA in low and moderate protein diets and evaluate the limitation of daily IAA intake in diets with high inclusion of crystalline AA (CAA) supplemented in the diets. Intact protein was kept constant at 22.2% for all of the diets and CAA was then added to adjust IAA profiles of feed to reach 100, 110 and 120% of the respective requirement of Nile tilapia (NRC, 2011) with or without DAA supplementation. Results confirmed that DAA plays an important role in meeting the nitrogen requirement of fish. Growth performance of fish fed diets without DAA supplementation was not comparable to fish fed diets supplemented with DAA in spite of IAA supplements up to 120% NRC requirement. The results of this study also indicated the inferior growth of fish fed diets with IAA supplements at 100% NRC requirement, which could result from limitation of daily IAA intake. The effect of feeding regimes on the efficacy of CAA utilization was also evaluated in which the feed offered to fish fed 100% IAA without DAA supplements and 110% IAA with 4% DAA supplements was paired for two and four feedings per day. Results indicated that fish can be fed successfully two times per day regardless of high inclusion levels of CAA.

Based on the data obtained from this study, it can be concluded that ideal protein concept can be applied in formulating the diets for Nile tilapia to optimize AA profiles of the diets. Well-balanced IAA profiles can be used to reduce the intact protein levels of feed without causing impaired growth of fish. However, if low intact protein levels are to be formulated, the supplementation of DAA might be required to satisfy the nitrogen requirement of fish. In our ingredient matrix, in addition to lysine, methionine and threonine, valine is limiting and the supplementation of this IAA is necessary to satisfy the requirements of fish.

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# Chapter I

## INTRODUCTION

Since the late 1980s, fisheries production has been relatively static (FAO, 2016). Expansion of aquaculture production with more intensive practices is necessary to meet the future demand for seafood and fisheries products. Such production will greatly depend on the utilization of precision feed formulations applied to produce well-balanced feeds at cost-effective prices (NRC, 2011). According to Alltech (2016), global aquaculture has shown a growing tendency of intensification of production, depending highly on the use of nutritionally-complete feed. In 2015, about 35.47 mmt of manufactured feed were produced for aquaculture (Alltech, 2016). As nutrition plays a key role in the success of intensive aquaculture, additional research efforts on nutritional requirements as well as nutrient levels and availability in feed ingredients are needed to provide a context for understanding both biological and economic performance.

Protein is considered the most important dietary component as it comprises a significant proportion of whole body dry matter of fish. As it has high cost per unit, provision of feed meeting an exact requirement for protein is a prerequisite for efficient and economical fish production. To do this, a diet has to be formulated properly with regard to AA balance. A dietary requirement for protein is essentially a requirement of the indispensable AA (IAA) to meet the need for protein synthesis and growth and sufficient dispensable AA (DAA) or nitrogen to enable fish to synthesize IAA.

Basically, the term IAA and DAA are widely used to classify the importance of AA in fish. Cowey and Walton (1989) distinguished IAA and DAA by using a deletion method in which

individual AAs were deleted from diets and growth performance was analyzed accordingly. While the deletion of DAA did not cause any deleterious growth, thereby indicating that they can be synthesized by animals, IAA omission would significantly reduce animal growth performance. It is clear from all available evidence published to date that 10 IAAs cannot be synthesized by fish and, therefore are necessary to be provided in the diet including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (taurine has been defined as essential or conditionally essential in some fish species). Along with these ten IAAs, alanine, aspartic acid, asparagine, glutamic acid, glutamine, proline, and serine are considered DAA since fish can synthesize them from simple precursors. Cysteine and tyrosine are synthesized in the body from methionine and phenylalanine, respectively, and considered semi-essential AA.

Fishmeal was once a dominant protein source of farmed fish feed due to its adequate AA profile, high digestibility and palatability. Given the increased demand of aquafeeds and the relatively static state of the world's fishmeal production, global fish meal supplies are clearly inadequate to meet the demand of the feed industry. According to NRC (2011), the overall inclusion levels of fishmeal in aquafeeds have reduced by approximately half over the last decade. Research efforts have also been devoted to replacing fishmeal with other alternative ingredients from less expensive and more sustainable sources. In reviewing the literature, many studies have shown that economical feeds can be formulated successfully with very high levels of plant-based protein ingredients as long as the feed formulation meets all the nutritional requirements of the animal (Chebbaki et al., 2010; De Francesco et al., 2007; Gomez-Requeni et al., 2004; Gonzales et al., 2007; Kaushik et al., 2004; Kissil and Lupatsch., 2004; Lee et al., 2002; Lunger et al., 2007; Riche and Williams, 2011; Sanchez-Lozano et al., 2009; Silva et al., 2009; Slawski et al., 2012;

Slawski et al., 2013; Thompson et al., 2012; Trushenski., 2011).

While the AA composition of fishmeal closely corresponds to the requirement of fish, most plant proteins are considered nutritionally unbalanced in terms of IAA. Hence, a replacement of fish meal with plant-based protein sources has a possible risk of creating a deficiency or an imbalance of dietary digestible AA (NRC, 2011). Therefore, supplementation of limiting IAAs has become imperative to promote higher performance of fish (Alam et al., 2011; Deng et al., 2006; Fournier et al., 2004; Nguyen and Davis, 2016; Watanabe et al., 2001). Quite often, the utilization of crystalline AA (CAA) is applied to overcome the deficiency of essential AA in high plant-based protein diets. CAAs have been used to meet IAA requirements of fish and considered key components of cost effective fish feed formulations to offset the high cost of fish meal and increase utilization of more economical protein sources with "imperfect" IAA profiles.

Generally, the ideal protein concept is useful as a guideline to establish an IAA profile in formulating diets of fish. Especially with low and moderate protein levels, the AA proportions need to be precisely adjusted to avoid deficiencies. The application of ideal protein concept in this case makes it possible to maximize AA utilization and lower feed costs by promoting the deposition of AAs. Effective utilization of protein by minimizing catabolism is also one of the key concepts to reduce nitrogen waste outputs from animals. According to Chatvijitkul et al. (2015), up to 65-70% of nitrogen and phosphorus applied in feed cannot be ingested by animals, thereby contributing a significant portion of the total nutrient loading to aquaculture systems. Minimizing the catabolism of proteins and promoting the deposition of AAs leads to a reduction of nitrogen loading on the environment without causing deleterious effects on growth performance of fish (Anderson and De Silva, 1997; Cho, 1992,1994; Johnsen et al., 1993; Kissil and Lupatsch, 1992).

Over the past century, numerous studies have been dedicated to the evaluation of formulating diets with sufficient IAAs to meet the needs for maximal growth and optimal health of fish (Espe and Lied, 1994; Espe et al., 2006; Murai et al., 1987; Pérez-Jiménez, 2014; Rollin, 1999; Rollin et al, 2003; Williams et al., 2001; Yu and Zhang, 2012; Zarate et al., 1999). The DAA is, however, getting less consideration as it was thought that they were all synthesized sufficiently in the body for maximizing growth. Even though DAA can be synthesized from precursors, their supplementation in the diets can minimize the need for fish to synthesize them (NRC, 2011). Also, the importance of balance IAA: and DAA has been documented in several fish species (Cowey, 1995; Green et al., 2002; Peres and Oliva-Teles, 2006; Gaye-Siessegger et al., 2007; Silva et al., 2009; Vilhelmsson et al., 2004). Furthermore, the supplementation of some DAAs (e.g., glycine, glutamate, glutamine, aspartate) could help the fish to improve performance via their critical roles in maintaining digestive function, enhancing hepatic function, signaling cell, reducing stress and eliminating peroxide (Abboudi et al., 2009; Cheng et al., 2012; Gómez-Requeni et al., 2003; Hughes, 1985; Kim et al., 1991; Larsson et al., 2014; Mambrini and Kaushik, 1994; Pereira et al., 2017; Pohlenz et al., 2012a, 2012b, 2012c; Schuhmacher et al., 1995; Wu et al., 2015).

Tilapia is a primary food fish species cultured in the world. According to Fitzsimmons (2016), global tilapia production was estimated at about 5.6 million metric tons for 2015. Like other fish species, research on the balance of IAA and DAA for promoting maximum growth and protein deposition in Nile tilapia has been limited and inconsistent. Santiago and Lovell (1988) first defined the IAA requirements for growth of Nile tilapia *Oreochromis. niloticus* with values reported for lysine (1.43%), arginine (1.18%), methionine (0.75%), histidine (0.48%), isoleucine

(0.87%), leucine (0.95%), valine (0.78%), phenylalanine (1.05%), threonine (1.05%), tryptophan (0.28%). Several studies (Figueiredo-Silva et al., Gan et al., 2015; He et al., 2015; 2015; Nguyen and Davis, 2016; Yue et al., 2013) have been conducted after that showing a huge variation in optimal values of IAAs for Nile tilapia compared to those reported by Lovell (1988), which are often used as recommended levels of IAAs in formulating diets for Nile tilapia. Also, limited data exist on the influences of DAA as protein sparing in practical diets for Nile tilapia (Furuya et al., 2004; Mambrini and Kaushik, 1994). Given the debate over the importance of meeting AA requirements, this research sought to further optimize AA balance of the diets offered to Nile tilapia by applying and validating the use of the ideal protein concept. Moreover, the supplementation of DAA to spare the use of relatively expensive IAA was also taken into consideration in revising the ideal protein concept.

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# Chapter II

# EFFICACY OF REDUCED PROTEIN DIETS AND THE EFFECTS OF INDISPENSABLE AMINO ACID SUPPLEMENTS FOR NILE TILAPIA *Oreochromis niloticus*


## Abstract

The study was conducted to evaluate the production performance of Nile tilapia *Oreochromis niloticus* fed graded levels of protein with or without indispensable AA (IAA) supplemented in practical diets. A control diet containing 32% intact protein was modified to meet the respective requirements of IAA based upon NRC recommendations for Nile tilapia. From this diet, two series of diets were formulated to confirm the efficacy of crystalline AA (CAA) used as supplements in the practical diets of fish by applying "ideal protein concept". In the first series of diets, intact protein was reduced gradually from 32 to 24% of the diet without IAA supplementation. In the second series of diets, the same dietary protein levels were formulated; however, the IAA profiles of these diets were modified to meet the respective requirement of Nile tilapia (NRC, 2011). Five commercially-available AAs, including methionine, lysine, tryptophan, histidine and valine, were supplemented if necessary to attain the targeted AA profile. The supplementation of all deficient IAAs was obtained in the last diet to evaluate if there is further benefit of using IAAs other than commercial AAs to balance the AA profiles of diets. Four groups of Nile tilapia (12.7 ± 0.2 g) were fed each diet four times daily to apparent satiation. The results obtained after eight weeks indicated that fish fed diets with IAA supplementation had significantly

higher weight gain compared to fish fed diets without IAA supplementation. Fish fed intact protein levels above 27.2% with the supplementation of IAA up to the reported requirement (NRC, 2011) showed comparable performance to fish fed the control diet. The reduction of intact protein further than that, however, resulted in reduced growth rate (thermal-unit growth coefficient) of fish even though the IAA profiles of these diets met the reported requirement of tilapia for optimal growth (NRC, 2011). Based on the data obtained from this study, it can be concluded that ideal protein concept can be applied in formulating the diets for Nile tilapia to optimize AA profile of the diets. Well-balanced IAA profile can be used to reduce the intact protein levels of feed (32 to 27.2%) without causing impaired growth of fish.

## 1. Introduction

Protein constitutes 50 to 60% of feed costs for aquaculture production and is considered the single most important component of aquatic feeds, especially for carnivorous and marine fish with higher protein requirements (Wilson, 2002). Provision of feed meeting an exact requirement for protein is a prerequisite for efficient and economical fish production. To do this, the diet has to be formulated properly with regard to AA balance as well as digestible energy to protein ratio (NRC, 2011).

Formerly, fish meal was the major protein source of aquatic feeds, constituting up to 60% of fish diets. As feed demand has increased, fish meal as a limited resource has also increased in price. According to Shepherd and Jackson (2013), the fishmeal and soybean meal price ratio increased from 2:1 to 4:1 from 1990 to 2010. Therefore, the topic of using ingredients from less expensive and more sustainable sources as alternatives to fish meal has been discussed during the last decade. It is assumed that the utilization of by-products from agriculture and plant-based products has the possibility of reducing feed costs and increasing profits in modern farming operations. Indeed, recent studies have indicated that economical feeds can be formulated successfully with very high levels of plant-based protein ingredients as long as the feed formulation is meeting all the nutritional requirements of the animal (Riche and Williams, 2011; Slawski et al., 2012; Slawski et al., 2013; Thompson et al., 2012).

Concurrently, the utilization of supplemental AA has expanded in the animal feed industry. This is due to the progress in biotechnology, which allows firms to reduce manufacturing cost of crystalline AA (CAA) production. CAAs have been used to meet indispensable AA (IAA) requirements of the fish and are considered key components of cost effective fish feed formulations

to offset the high cost of fish meal and increase utilization of more economical protein sources with "imperfect" IAA profiles. The supplementation of CAA following the "ideal protein concept" helped fish to increase feeding efficiency while reducing pressure and reliance on feed resources (Cheng et al., 2003, Gaylord and Barrows, 2009; Viola and Lahav, 1991; Wilson and Poe 1985).

Assuming that there is a relationship between the first limiting AA and protein level of feed, ideal protein concept points that increasing protein level results in reduced utilization efficiency of the first limiting AA (NRC, 2011). At higher levels of protein or higher protein:energy ratios, only one part of protein will be used to build new tissues and the remainder will be catabolized to supply energy (NRC, 2011). In contrast, failure to furnish an adequate protein will result in reduced growth and poor feed conversion. The animals will withdraw protein from their less vital tissues to maintain the function of more vital ones (Lim and Webster, 2006).

Tilapia, like other fish, require continuous supplies of indispensable and dispensable AAs rather than protein for maintenance, growth, and other physiological functions. There have been several studies related to protein and its utilization in Nile tilapia reporting various optimal protein and AA levels for different stages of fish (Abdelghany, 2000; Abdelghany, 2003; Abdel-Tawwab et al., 2009; Al-Hafedh, 1999; El-Sayed et al., 1991; Khattab et al., 2000; Siddiqui et al, 1988; Wee and Tuan, 1988). As the ideal protein concept has been well adapted in formulation of cost-effective manufactured feeds, the application of its utilization in practical environments is critical for the development of the aquaculture feed industry. The possibility of reducing the dietary protein content by using the ideal protein concept to balance AA content in Nile tilapia diets is, however, reported by limited research (Furuya et al., 2004; Furuya et al., 2005; Botaro et al., 2007). Given the successful use of ideal protein concept in formulating low protein diets, this research is

to evaluate the efficacy of CAA supplements in practical diets of Nile tilapia with the indication of how far CAA could be used to lower the protein content of fish feed.

## 2. Materials and Method

### *2.1. Experimental diets and feeding trial*

Formulations for the ten experimental diets are presented in Table 1. A control diet containing 32% intact protein was modified to meet the respective requirement of IAAs based on NRC recommendations for Nile tilapia. In the first series of diets, soybean meal, fish meal and corn protein concentrate were gradually reduced from the control diet to attain graded levels of protein ranging from 32% to 24% of the diet. In the second series of diets, the same dietary protein levels were formulated; however, the IAA profiles of feed in these diets were modified to meet the respective requirements of Nile tilapia (NRC, 2011) except for those of histidine and methionine. Optimal histidine and methionine values were obtained from suggested numbers of Santiago and Lovell (1988) and Nguyen and Davis (2009), respectively. Five commercially-available AAs, including methionine, lysine, tryptophan, histidine and valine, were supplemented if necessary to attain the targeted AA profile. Estimated intact protein levels used in this series of diets were 29.7%, 27.2%, 24.7% and 22.2%, respectively. The same method was applied in the last diet using lowest intact protein level with the supplementation of all deficient IAA.

The test diets were prepared at the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn, AL, USA). Pre-ground feed ingredients and oil were placed in a food mixer (Hobart Corporation, Troy, Ohio, USA) for 15 minutes. Hot water was then added at 30% to the mixture in order to attain an appropriate consistency for pelleting. Diets were then extruded through a 4-mm diameter die in a meat grinder,

air dried at < 50°C to a moisture less than 11%, and stored in the freezer at -20°C until used. A sample of each feed was collected and analyzed for proximate composition (g 100 $g^{-1}$ as is) and

**Table 1** Ingredient compositions (g 100 $g^{-1}$ as-is) of ten experimental diets[a] offered to juvenile Nile tilapia (12.7 ± 0.2 g) over an eight week growth period.

| Ingredient | 32CP | 30CP | 28CP | 26CP | 24CP | 30AAR | 28AAR | 26AAR | 24AAR | 24AAR+ |
|---|---|---|---|---|---|---|---|---|---|---|
| Menhaden fishmeal[b] | 6.00 | 5.40 | 4.80 | 4.20 | 3.60 | 5.40 | 4.80 | 4.20 | 3.60 | 3.60 |
| Soybean meal[c] | 42.58 | 38.32 | 34.06 | 29.81 | 25.55 | 38.32 | 34.06 | 29.81 | 25.55 | 25.55 |
| CPC[d] | 6.20 | 6.68 | 7.16 | 7.63 | 8.11 | 6.28 | 6.15 | 6.01 | 5.83 | 5.78 |
| Menhaden fish oil[e] | 5.37 | 5.52 | 5.67 | 5.82 | 5.97 | 5.52 | 5.69 | 5.85 | 6.01 | 6.01 |
| Lecithin[f] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Corn Starch[g] | 10.75 | 14.78 | 18.81 | 22.84 | 26.87 | 14.87 | 19.01 | 23.15 | 27.31 | 27.32 |
| Whole wheat[h] | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| Mineral premix[i] | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin premix[j] | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 |
| Choline chloride[k] | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Stay C[l] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| CaP-dibasic[m] | 1.50 | 1.70 | 1.90 | 2.10 | 2.30 | 1.70 | 1.90 | 2.10 | 2.30 | 2.30 |
| Lysine[n] | | | | | | 0.19 | 0.39 | 0.58 | 0.78 | 0.78 |
| Methionine[n] | | | | | | | 0.02 | 0.06 | 0.10 | 0.10 |
| Threonine[n] | | | | | | 0.04 | 0.13 | 0.23 | 0.32 | 0.32 |
| Tryptophan[n] | | | | | | | | | 0.03 | 0.03 |
| Isoleucine[g] | | | | | | | | | | 0.04 |
| Valine[n] | | | | | | 0.07 | 0.19 | 0.32 | 0.44 | 0.44 |
| Cysteine[g] | | | | | | 0.01 | 0.06 | 0.09 | 0.13 | 0.13 |

[a] Diet designations: 32CP, 30CP, 28CP, 26CP, 24CP = 32%, 30%, 28%, 26%, 24% crude protein from intact sources; 30AAR = 29.69% crude protein from intact sources plus supplemental Lys, Thr, Val and Cys; 28AAR = 27.22% crude protein from intact sources plus supplemental Lys, Met, Thr, Val and Cys; 26AAR = 24.73% crude protein from intact sources plus supplemental Lys, Met, Thr, Val and Cys; 24AAR = 22.22% crude protein from intact sources plus supplemental Lys, Met, Thr, Trp, Val and Cys; 24AAR+ = 22.22% crude protein from intact sources plus supplemental Lys, Met, Thr, Trp, Val, Iso and Cys.

[b] Omega Protein Inc., Houston, Texas, USA.

[c] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA.

[d] Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.

[e] Omega Protein Inc., Reedville, VA, USA.

[f] The Solae Company, St. Louis, MO, USA.

[g] MP Biochemicals Inc., Solon, OH, USA.

[h] Bob's Red Mill Natural Foods, Milwaukie, OR, USA.
[i] Trace mineral (g/100g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.250; Furrous sulfate, 4.000; Magnesium sulfate anhydrous, 13.862; Manganous sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate hepahydrate, 13.193; Alpha-cellulose, 67.964.
[j] Vitamin (g/kg premix): Thiamin HCl, 0.44; Riboflavin, 0.63; Pyridoxine HCl, 0.91; DL pantothenic acid, 1.72; Nicotinic acid, 4.58; Biotin, 0.21; Folic acid, 0.55; Inositol, 21.05; Menadione sodium bisulfite, 0.89; Vitamin A acetate, 0.68; Vitamin $D_3$, 0.12; dL-alpha-tocoperol acetate, 12.63; Alpha-cellulose, 955.59.
[k] Amresco Inc., Solon, Ohio, USA.
[l] Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products., Parsippany, NJ, USA.
[m] Alfa Aesar, Ward Hill, MA, USA.
[n] Ajinomoto Heartland Inc., Chicago, IL, USA.

AA profile (g 100 $g^{-1}$ as is) following AOAC (1995) procedures by the Experiment Station Chemical Laboratories, University of Missouri, Columbia, USA (Table 2).

*2.2. Culture methods*

The trial was conducted at the E.W. Shell Fisheries Center, Auburn, Alabama. Nile tilapia fry spawned at this center were stocked in the nursery tank until the beginning of the trial. After acclimating, juvenile Nile tilapia (12.7 ± 0.2 g) were randomly stocked into thirty-six rectangular 50-L aquaria of a 3,800-L indoor recirculation system at 15 fish per aquarium. Each of the ten treatments was assigned to four randomly chosen tanks. Samples of fish from the initial stocking were retained for later protein retention analysis.

Water temperature was maintained at around 28˚C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA) and dissolved oxygen was maintained near saturation using air stones. Dissolved oxygen (DO) and water temperature were measured twice per day using YSI 650 multi-parameter instrument (YSI, Yellow Springs, Ohio), while pH, total ammonia nitrogen (TAN) and nitrite-nitrogen were measured twice per week. During the experimental period, DO, temperature, salinity, pH, TAN, and nitrite were maintained within acceptable ranges for tilapia at 6.63 ± 0.54 mg/L, 28.28 ± 0.67°C, 3.94 ± 1.58 ppt, 7.10 ± 0.52, 0.21 ± 0.14 mg/L, 0.24 ± 0.15 mg/L, respectively.

Diets were offered to fish at 5-6% body weight daily according to fish size. Test diets were applied four times per day at 08:00, 11:00, 13:00 and 16:00 h for an eight-week growth period. Fish were weighed every week for the first two weeks and every other week thereafter. Daily feed rations were adjusted each week based on growth and observation of the feeding response. At the

end of the growth trial, fish were counted and group weighed to determine weight gain, survival, and feed conversion ratio. Four fish were randomly collected from every aquarium and frozen at

**Table 2** Analyzed proximate composition and amino acid profile of the experimental diets[a] (g 100 $g^{-1}$ as-is) fed to juvenile Nile tilapia (12.7 ± 0.2 g) over an eight week growth period.

| Composition | 32CP | 30CP | 28CP | 26CP | 24CP | 30AAR | 28AAR | 26AAR | 24AAR | 24AAR |
|---|---|---|---|---|---|---|---|---|---|---|
| Crude Protein | 33.08 | 30.99 | 29.26 | 27.85 | 25.35 | 31.54 | 28.68 | 26.81 | 24.63 | 24.73 |
| Moisture | 7.50 | 8.61 | 7.00 | 6.44 | 6.84 | 7.04 | 8.50 | 8.18 | 8.14 | 8.65 |
| Crude Fat | 9.02 | 8.95 | 9.02 | 9.33 | 8.92 | 9.11 | 9.08 | 9.28 | 8.99 | 9.42 |
| Crude Fiber | 2.98 | 2.90 | 2.79 | 2.70 | 2.49 | 3.03 | 2.83 | 2.72 | 2.54 | 2.50 |
| Ash | 5.68 | 5.39 | 5.24 | 4.95 | 4.81 | 5.65 | 5.15 | 4.95 | 4.68 | 4.63 |
| **IAA** | | | | | | | | | | |
| Arginine | 1.94 | 1.76 | 1.65 | 1.54 | 1.36 | 1.84 | 1.60 | 1.47 | 1.28 | 1.29 |
| Histidine | 0.79 | 0.72 | 0.70 | 0.64 | 0.59 | 0.74 | 0.65 | 0.61 | 0.54 | 0.53 |
| Isoleucine | 1.42 | 1.31 | 1.27 | 1.19 | 1.10 | 1.36 | 1.19 | 1.12 | 0.98 | 0.98 |
| Leucine | 2.91 | 2.77 | 2.78 | 2.62 | 2.54 | 2.78 | 2.50 | 2.38 | 2.14 | 2.12 |
| Lysine | 1.77 | 1.58 | 1.50 | 1.37 | 1.21 | 1.82 | 1.73 | 1.77 | 1.78 | 1.77 |
| Methionine | 0.56 | 0.53 | 0.52 | 0.47 | 0.46 | 0.53 | 0.49 | 0.51 | 0.50 | 0.49 |
| Phenylalanine | 1.64 | 1.53 | 1.50 | 1.40 | 1.32 | 1.55 | 1.38 | 1.30 | 1.16 | 1.16 |
| Threonine | 1.18 | 1.08 | 1.05 | 0.96 | 0.88 | 1.14 | 1.09 | 1.12 | 1.10 | 1.10 |
| Tryptophan | 0.43 | 0.41 | 0.39 | 0.33 | 0.34 | 0.39 | 0.35 | 0.32 | 0.30 | 0.31 |
| Valine | 1.55 | 1.42 | 1.38 | 1.30 | 1.20 | 1.54 | 1.47 | 1.51 | 1.53 | 1.47 |
| **DAA** | | | | | | | | | | |
| Alanine | 1.66 | 1.58 | 1.57 | 1.47 | 1.42 | 1.59 | 1.43 | 1.34 | 1.20 | 1.17 |
| Cysteine | 0.45 | 0.42 | 0.41 | 0.38 | 0.37 | 0.43 | 0.42 | 0.44 | 0.44 | 0.41 |
| Glutamic Acid | 6.53 | 6.08 | 5.92 | 5.59 | 5.27 | 6.11 | 5.57 | 5.26 | 4.78 | 4.67 |
| Glycine | 1.41 | 1.31 | 1.24 | 1.14 | 1.05 | 1.34 | 1.20 | 1.09 | 0.95 | 0.93 |
| Proline | 2.02 | 1.92 | 1.90 | 1.80 | 1.72 | 1.92 | 1.76 | 1.67 | 1.53 | 1.50 |
| Serine | 1.47 | 1.33 | 1.24 | 1.15 | 1.04 | 1.27 | 1.18 | 1.11 | 1.01 | 1.00 |
| Taurine | 0.18 | 0.18 | 0.18 | 0.19 | 0.19 | 0.18 | 0.18 | 0.18 | 0.19 | 0.18 |
| Tyrosine | 1.12 | 1.06 | 1.00 | 0.99 | 0.92 | 1.07 | 0.96 | 0.90 | 0.82 | 0.81 |

[a] Diets were analyzed by the Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri.

20$^{o}$C for later biochemical analysis. These whole-body fish samples were homogenized and subdivided for analysis. A subsample was sent to Midwest Laboratories (Omaha, NE, USA) for conducting dry matter, crude protein, crude lipid, and ash analyses. Another sub-sample was freeze-dried and then sent to Ajinomoto Heartland Inc., (Chicago, IL, USA) for AA analysis.

Growth performance, apparent net protein retention, and feed conversion ratio were computed using the following calculations:

a) Thermal-unit growth coefficient (TGC) = (final weight$^{1/3}$ - initial weight$^{1/3}$) / (temperature × day) × 100.

b) Apparent net protein retention (ANPR, %) = (final weight × final protein content) - (initial weight × initial protein content) × 100 / protein intake.

*c)* Feed conversion ratio (FCR) = dry feed intake / weight gain.

*2.3. Statistical Analysis*

All data were subjected to a one-way analysis of variance to determine significant differences ($P < 0.05$) among the treatments, which was followed by Tukey's multiple comparison test to distinguish significant differences among treatment means. The analysis of covariance ANCOVA was used to compare the two regression lines of tilapia responses to the use of crystalline versus intact protein. All the data were analyzed using SAS (V9.4. SAS Institute, Cary, North Carolina, USA).

## 3. Results

There were significant differences ($P < 0.05$) among mean final weight (FW), thermal-unit growth coefficient (TGC), feed conversion ratio (FCR), apparent net protein retention (ANPR) and lipid deposition of fish receiving various dietary protein contents (Table 3). In both series of diets with and without IAA supplements, fish fed higher protein levels had higher mean FW, TGC and lower mean ANPR, FCR and lipid deposition. However, no significant differences were observed in crude protein (13.90 - 14.80%), moisture (71.43 - 73.62%) and ash (3.32 - 5.14%) of whole body fish samples (Table 3) among fish fed various dietary treatments. Few differences were observed regarding the AA composition of whole-body tissues (Table 4) mainly between fish fed the lowest protein level at 24% without IAA supplements and fish fed 30% protein with IAA supplements (Table 4).

Analysis of covariance of mean TGC, FCR, and ANPR of tilapia receiving various dietary treatments is presented in Table 5. The combined effects of protein levels and sources (with and without CAA supplementation) were observed on mean TGC, FCR and ANPR. Generally, tilapia fed CAA supplemented diets showed better responses for TGC, FCR and ANPR than those of fish fed diets without CAA supplementation with the adjusted mean values were 0.135 and 0.130 and 1.09 and 1.14, 42.99 and 39.65%, respectively. The Y intercepts of the two regression lines for TGC, FCR, ANPR against dietary protein levels were significantly different ($P < 0.05$) between fish fed diets with and without CAA supplementation (Fig. 1, Fig. 2 and Fig. 3).

The supplementation of five commercially-available IAAs to meet the respective requirements of IAA in two intact protein levels of 29.7 and 27.2% helped the fish to attain comparable growth rates (TGC) to that of fish fed the control diet. The reduction of intact protein

further than that (24.7 and 22.2%), however, resulted in reduced mean growth rates (thermal-unit growth coefficient) and increased mean FCRs of fish even though the IAA profiles of these diets met the reported requirement of tilapia for optimal growth (NRC, 2011).

**Table 3** Mean response of Nile tilapia (12.7 ± 0.2 g) fed diets containing different dietary protein levels with or without crystalline amino acid supplements over an eight-week growth period.

| Diet | Initial weight | Final weight | Thermal-unit growth coefficient | Feed conversion ratio | Apparent net protein retention | Survival |
|---|---|---|---|---|---|---|
| 32CP | 12.67 | 93.54$^{a}$ | 0.140$^{a}$ | 1.04$^{c}$ | 37.94$^{cd}$ | 93.33 |
| 30CP | 12.72 | 87.67$^{bc}$ | 0.133$^{bc}$ | 1.10$^{abc}$ | 37.76$^{d}$ | 96.67 |
| 28CP | 12.76 | 85.58$^{c}$ | 0.131$^{c}$ | 1.12$^{ab}$ | 38.88$^{cd}$ | 96.67 |
| 26CP | 12.92 | 85.09$^{c}$ | 0.130$^{c}$ | 1.16$^{a}$ | 39.20$^{bcd}$ | 96.67 |
| 24CP | 12.77 | 84.01$^{c}$ | 0.129$^{c}$ | 1.15$^{a}$ | 41.71$^{abcd}$ | 94.88 |
| 30AAR | 12.79 | 95.03$^{a}$ | 0.141$^{a}$ | 1.08$^{bc}$ | 39.12$^{cd}$ | 96.67 |
| 28AAR | 12.74 | 90.86$^{ab}$ | 0.136$^{ab}$ | 1.05$^{c}$ | 43.02$^{abcd}$ | 93.33 |
| 26AAR | 12.75 | 87.37$^{bc}$ | 0.133$^{bc}$ | 1.12$^{abc}$ | 43.55$^{abc}$ | 96.67 |
| 24AAR | 12.73 | 86.39$^{c}$ | 0.132$^{bc}$ | 1.11$^{abc}$ | 44.86$^{ab}$ | 96.67 |
| 24AAR+ | 12.63 | 84.99$^{c}$ | 0.130$^{c}$ | 1.13$^{ab}$ | 45.43$^{a}$ | 94.88 |
| *P*-value | 0.9287 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.9501 |
| PSE[a] | 0.1215 | 0.8982 | 0.0011 | 0.0151 | 1.1766 | 2.4021 |

Means (n = 4) in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by Tukey's multiple range test.

[a] PSE: Pooled standard error.

**Table 4** Whole body proximate composition[a] and amino acid (AA) composition[b] (g 100 $g^{-1}$ as-is) of juvenile Nile tilapia (12.7 ± 0.2 g) fed diets containing different dietary protein levels with or without crystalline amino acid supplements for eight weeks.

| Composition | 32CP | 30CP | 28CP | 26CP | 24CP | 30AAR | 28AAR | 26AAR | 24AAR | 24AAR+ | *P*-value | PSE[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Crude protein | 14.75 | 14.78 | 14.43 | 14.28 | 13.90 | 14.38 | 14.80 | 14.43 | 14.28 | 14.63 | 0.8312 | 0.3832 |
| Moisture | 73.62 | 73.43 | 72.81 | 72.69 | 71.57 | 73.21 | 73.07 | 72.79 | 72.83 | 71.43 | 0.0489 | 0.4842 |
| Lipids | 8.13$^{b}$ | 8.95$^{b}$ | 9.62$^{ab}$ | 9.57$^{ab}$ | 11.54$^{a}$ | 9.21$^{b}$ | 9.12$^{b}$ | 9.41$^{ab}$ | 10.11$^{ab}$ | 10.27$^{ab}$ | 0.0080 | 0.5103 |
| Ash | 3.42 | 3.70 | 4.48 | 5.14 | 4.06 | 3.39 | 3.98 | 3.32 | 3.65 | 3.97 | 0.4347 | 0.5512 |
| **IAA** | | | | | | | | | | | | |
| Arginine | 0.91 | 0.91 | 0.89 | 0.88 | 0.86 | 0.91 | 0.93 | 0.89 | 0.89 | 0.91 | 0.5601 | 0.0204 |
| Histidine | 0.35$^{ab}$ | 0.34$^{ab}$ | 0.33$^{ab}$ | 0.32$^{ab}$ | 0.30$^{b}$ | 0.36$^{a}$ | 0.34$^{ab}$ | 0.35$^{ab}$ | 0.33$^{ab}$ | 0.33$^{ab}$ | 0.0286 | 0.0104 |
| Isoleucine | 0.57$^{a}$ | 0.54$^{ab}$ | 0.54$^{ab}$ | 0.52$^{ab}$ | 0.50$^{b}$ | 0.56$^{a}$ | 0.56$^{ab}$ | 0.55$^{ab}$ | 0.53$^{ab}$ | 0.55$^{ab}$ | 0.0277 | 0.0114 |
| Leucine | 1.02 | 1.00 | 0.99 | 0.96 | 0.93 | 1.02 | 1.02 | 1.01 | 0.98 | 1.00 | 0.0350 | 0.0198 |
| Lysine | 1.10 | 1.09 | 1.07 | 1.01 | 0.97 | 1.12 | 1.09 | 1.11 | 1.07 | 1.07 | 0.1517 | 0.0370 |
| Methionine | 0.36$^{ab}$ | 0.35$^{ab}$ | 0.35$^{ab}$ | 0.34$^{ab}$ | 0.33$^{b}$ | 0.36$^{ab}$ | 0.37$^{a}$ | 0.37$^{a}$ | 0.35$^{ab}$ | 0.36$^{ab}$ | 0.0283 | 0.0072 |
| Phenylalanine | 0.60 | 0.59 | 0.59 | 0.57 | 0.54 | 0.60 | 0.60 | 0.60 | 0.58 | 0.60 | 0.1282 | 0.0140 |
| Threonine | 0.61 | 0.60 | 0.59 | 0.58 | 0.56 | 0.61 | 0.61 | 0.60 | 0.59 | 0.61 | 0.1258 | 0.0128 |
| Tryptophan | 0.14 | 0.13 | 0.14 | 0.13 | 0.13 | 0.14 | 0.14 | 0.13 | 0.13 | 0.13 | 0.4348 | 0.0039 |
| Valine | 0.63 | 0.62 | 0.62 | 0.59 | 0.58 | 0.63 | 0.63 | 0.63 | 0.60 | 0.62 | 0.0793 | 0.0123 |
| **DAA** | | | | | | | | | | | | |
| Alanine | 0.96 | 0.95 | 0.94 | 0.92 | 0.91 | 0.96 | 0.97 | 0.93 | 0.93 | 0.96 | 0.6558 | 0.0206 |
| Aspartic Acid | 1.35 | 1.32 | 1.31 | 1.26 | 1.23 | 1.34 | 1.35 | 1.33 | 1.29 | 1.33 | 0.0601 | 0.0271 |
| Cysteine | 0.13$^{ab}$ | 0.13$^{ab}$ | 0.13$^{ab}$ | 0.13$^{ab}$ | 0.12$^{b}$ | 0.14$^{a}$ | 0.14$^{a}$ | 0.14$^{a}$ | 0.13$^{ab}$ | 0.14$^{a}$ | 0.0245 | 0.0031 |
| Glutamic Acid | 1.93 | 1.90 | 1.88 | 1.83 | 1.78 | 1.92 | 1.94 | 1.90 | 1.86 | 1.91 | 0.1754 | 0.0413 |
| Glycine | 1.19 | 1.22 | 1.18 | 1.18 | 1.20 | 1.20 | 1.21 | 1.12 | 1.18 | 1.21 | 0.7980 | 0.0379 |
| Proline | 0.75 | 0.76 | 0.73 | 0.71 | 0.72 | 0.76 | 0.75 | 0.70 | 0.71 | 0.75 | 0.6026 | 0.0243 |
| Serine | 0.59 | 0.59 | 0.58 | 0.56 | 0.55 | 0.59 | 0.59 | 0.58 | 0.56 | 0.59 | 0.4054 | 0.0134 |
| Tyrosine | 0.35$^{a}$ | 0.33$^{ab}$ | 0.33$^{ab}$ | 0.32$^{ab}$ | 0.30$^{b}$ | 0.34$^{ab}$ | 0.34$^{ab}$ | 0.34$^{ab}$ | 0.32$^{ab}$ | 0.34$^{ab}$ | 0.0120 | 0.0081 |

Means (n = 4) in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by Tukey's multiple range test.

[a] Diets were analyzed at Midwest Laboratories (Omaha, NE, USA).

[b] Diets were analyzed at Ajinomoto Heartland Inc, (Chicago, IL, USA).
[c] PSE: Pooled standard error

**Table 5** Analysis of covariance output of Nile tilapia fed diets containing different dietary protein levels with or without crystalline amino acid supplements over an eight-week growth period.

| Adjusted mean least-squares means | | | |
|---|---|---|---|
| Lysine source | Thermal-unit growth coefficient | Feed conversion ratio | Apparent net protein retention (%) |
| Without IAA | $0.130^{b}$ | $1.14^{a}$ | $39.65^{b}$ |
| With IAA | $0.135^{a}$ | $1.09^{b}$ | $42.99^{a}$ |
| Slope *P* value | 0.0521 | 0.7669 | 0.4041 |
| Intercept *P* value | <0.0001 | 0.0002 | <0.0001 |

Means (n = 4) in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by Tukey's multiple range test.

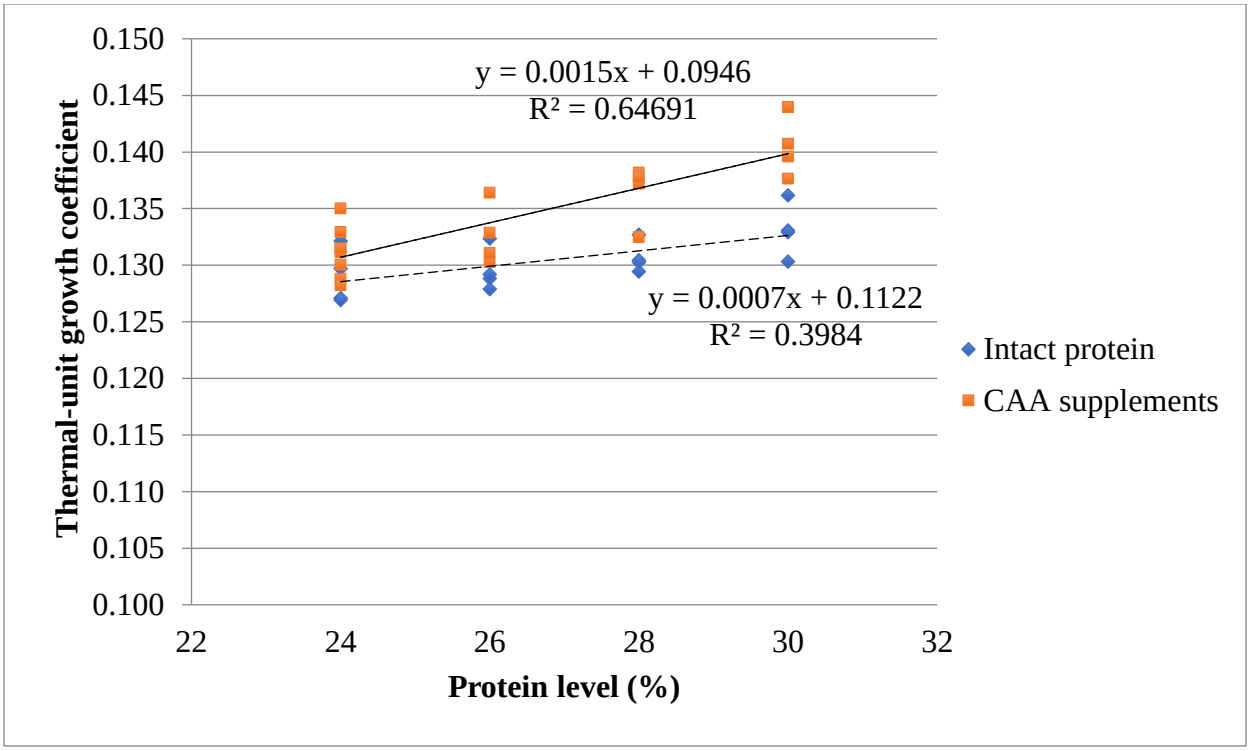


Fig. 1. Regression of mean thermal-unit growth coefficient of Nile tilapia against dietary protein levels in both sets of diets with and without CAA supplements. Solid line represents diets containing crystalline amino acid supplements; dashed line is diets with intact protein only.

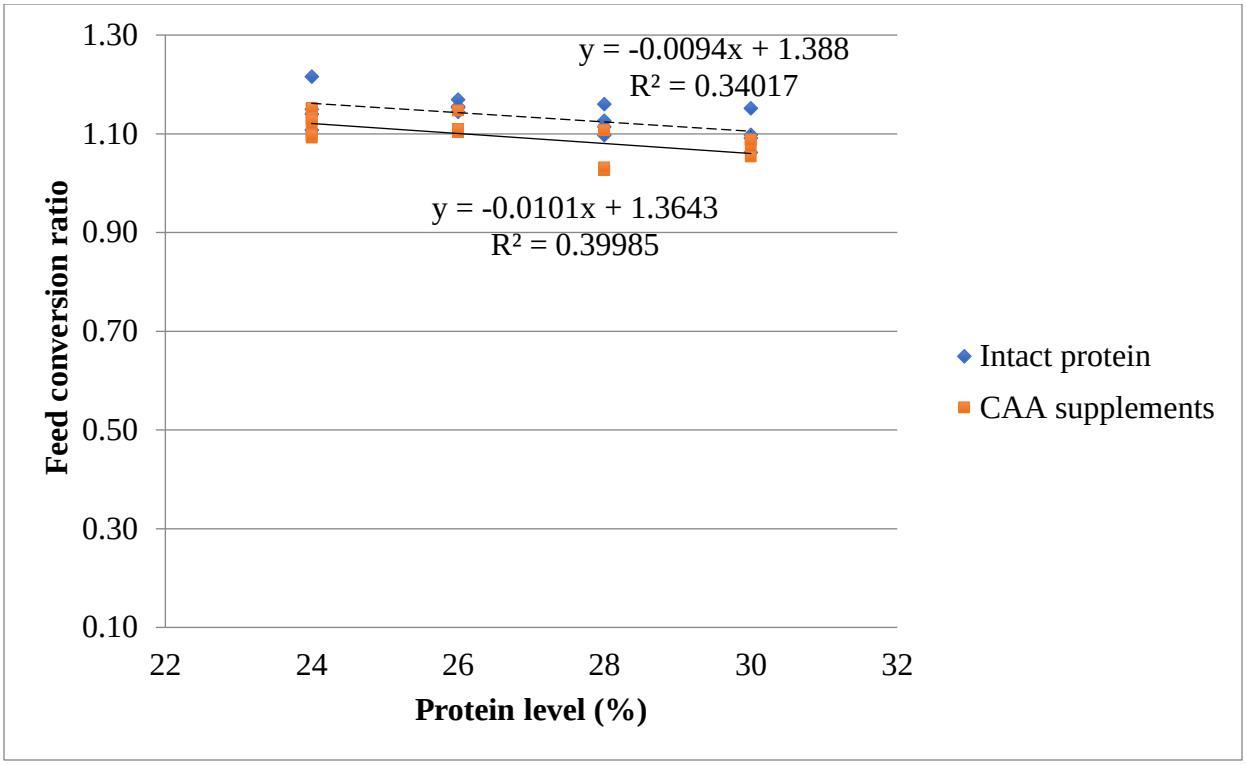


Fig. 2. Regression of mean feed conversion ratio of Nile tilapia against dietary protein levels in both sets of diets with and without CAA supplements. Solid line represents diets containing crystalline amino acid supplements; dashed line is diets with intact protein only.

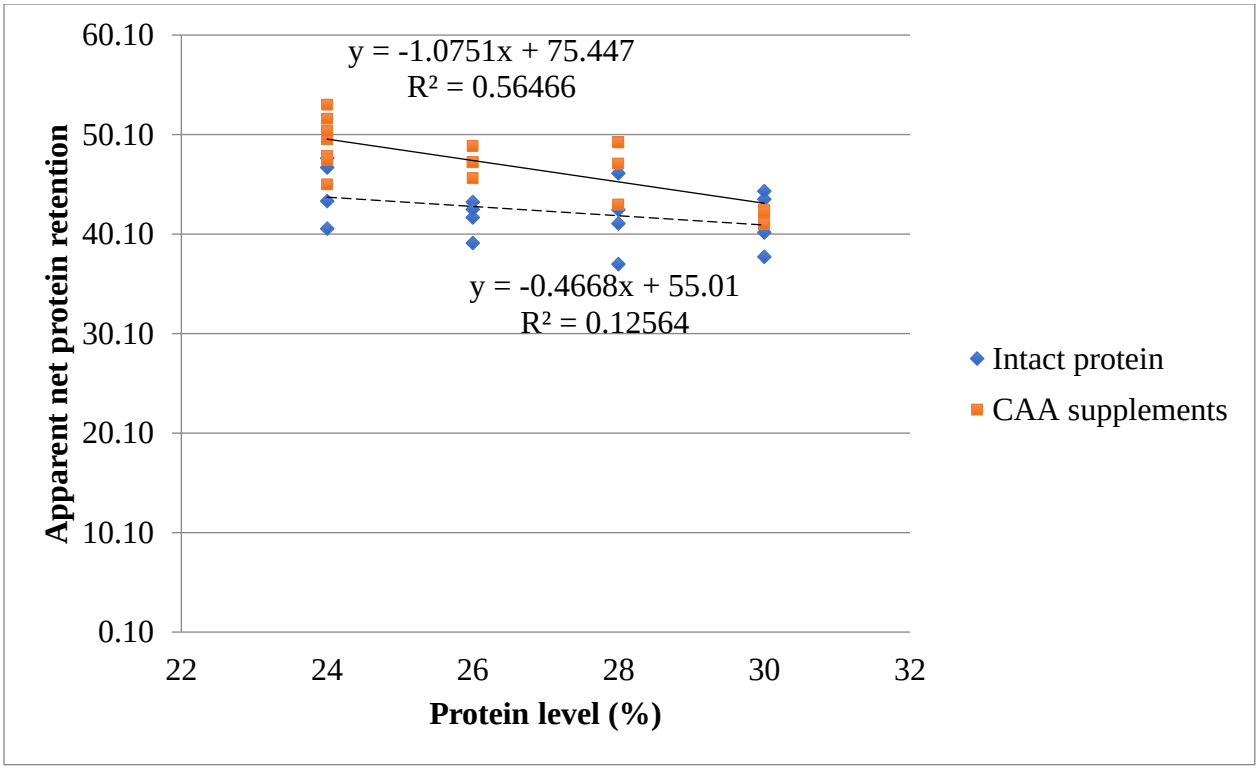


Fig. 3. Regression of mean apparent net protein retention of Nile tilapia against dietary protein levels in both sets of diets with and without CAA supplements. Solid line represents diets containing crystalline amino acid supplements; dashed line is diets with intact protein only.

## 4. Discussion

In this study, the reference profile of all IAAs was built to meet the respective requirements of NRC (2011) except methionine and histidine, which were obtained from recommended numbers by Santiago and Lovell (1988) and Nguyen and Davis (2009), respectively. According to NRC (2011), histidine and methionine requirements of Nile tilapia are 1.00% and 0.75%, respectively. However, in reviewing the literature, histidine requirement at 0.48% (Santiago and Lovell, 1988) and methionine requirement at 0.49% (Nguyen and Davis, 2009) are more reasonable and used as references for our study.

Results of this study demonstrated the well-known effect of dietary protein influencing the growth performance of the fish as expressed by higher mean final weights and corresponding growth rates (TGC). Abdelghany (2000) observed the same trend in which Nile tilapia fed diets with protein below 30% had significantly lower weight gain compared to those of fish fed diets with higher dietary protein levels. Tawwab et al. (2010) also confirmed that fish growth was correlated with dietary protein levels. In their study, higher growth rates of Nile tilapia were obtained in fish fed diets with higher levels of dietary crude protein while fish fed lower protein levels exhibited lower growth rates. The decrease in growth performance of Nile tilapia in our study could be due to the imbalance of IAA profiles of diets resulting from decreasing protein levels. Indeed, in diet containing 30% intact protein, IAA including lysine, threonine and valine became limiting compared to the reported numbers of NRC (2011). Methionine was the fourth limiting AA in moderate protein diets including 28 and 26% intact protein and isoleucine was the fifth limiting AA if the intact protein of feed was reduced to 24% of diet. The same patterns were observed on the feed utilization efficiency of fish in which fish fed diets with higher protein levels

had lower FCR in both sets of diets. The FCR of this study ranging from 1.04 to 1.16 matched previously published ranges for Nile tilapia (Siddiqui et al., 1988; Al-Hafedh, 1999; Abdelghany, 2000; Khattab et al., 2000; Tawwab et al., 2010).

Proximate composition of whole-body tissues was not significantly influenced by dietary protein level except for lipid content of fish., even though fish fed with lower crude protein diets exhibited lower protein content. Significantly higher lipid content was observed in fish fed lower protein diet compared to fish fed diets with higher protein content. Al-Hafedh (1999), Khattab et al. (2000) and Wee and Tuan (1988) showed that protein and lipid content of fish were significantly affected by protein levels. Abdel-Tawwab et al. (2006), Fauconneau (1984), Smith, (1981) and Soivio et al. (1989) explained that the variation of protein and lipid deposition on fish body might result from differences in their synthesis. Conversely, Loum (2013) showed no significant differences in protein content of Nile tilapia fed on five protein levels from 21 to 45%, as compared to the initial fish. The results of their study, however, demonstrated that higher lipid content was observed in response to increasing dietary protein levels from 21 to 45%, and fish fed the highest protein levels had highest carcass lipid content. The discrepancies of results might come from differences in experimental design. In their study, the energy levels yielding from carbohydrates were kept constant among experiments. Therefore, when maximum protein deposition was reached, additional energy intake from excess protein enhanced lipid deposition. Whereas in our study, corn starch was used to balance the diets with reduced protein levels. The reduction of protein from 32 – 24% resulted in an increased use of corn starch from 10.75 to 26.87%. High levels used of corn starch or high carbohydrate levels resulted in increases in body fat deposition (Brauge et al., 1994; Hemre et al., 2002; Kaushik et al., 1989; Shimeno et al., 1993).

The reduced levels of protein in the experimental diets affected the efficiency of protein utilization of fish, which have been observed previously by Ahmad et al. (2004), Bahnasawy (2009), Jauncey (1982), Kheir (1997); Shiau and Huang (1989) and Wee and Tuan (1988). Reduction of dietary protein also resulted in reduction of whole body AA profile. The AA profiles (IAA and DAA) of fish fed diets with high dietary protein were higher compared to those of fish fed diets with low and moderate protein levels in the first series of diets without IAA supplementation. When reducing the dietary protein to 24%, fish had significantly lower deposition AAs, especially isoleucine, histidine and methionine, compared to those of fish fed with other diets. These differences might result from variation of AA profiles of diets offered to fish. According to NRC (2011), AA depositions of whole body usually represents 25 to 50% of total AA consumed by animals (NRC, 2011). The AA profiles of fish are partly represented the AA profiles of feed that were gradually reduced in diets containing low and moderate dietary protein. The AA profiles of whole body fish, however, were lower than the AA profiles of feed, which represented 40-80% AA profile of feed (g 100 $g^{-1}$ as-is). This result indicated that the estimation of IAA requirement based on whole body AA profile might underestimate AA requirements of fish

Investigations into the efficacy of CAAs as supplements on growth performance of Nile tilapia were conducted in the second series of diets. According to the results of this study, supplementation of CAAs in the formulated diets is essential to improve the growth performance of Nile tilapia. Growth rate (TGC) and ANPR of Nile tilapia fed diets with CAA supplements were significantly higher compared to those of fish fed diets without CAA supplementation. Fish fed intact protein levels above 27.2% with the supplementation of IAA up to the reported requirement

(NRC, 2011) showed comparable performance to fish fed the control diet. This result is close to the results obtained by Nguyen and Davis (2016), which indicated that crystalline lysine supplementation had the same effect to that of Nile tilapia fed intact lysine from high lysine corn protein concentrate. Similarly, earlier studies conducted on Nile tilapia showed that CAA had comparable effectiveness to intact protein in meeting essential AA requirements of fish (Furuya and Furuya, 2010; Kumar et al., 2012; Lim et al., 2007; Yu et al., 2012). Evidence from fish other than Nile tilapia also confirms the efficacy of CAA supplements. Fournier et al (2004) indicated that turbot, *Psetta maxima*, could use up to 80% plant protein ingredients as fish meal replacement without deleterious effects on growth performance considering that CAAs were added to reflect the AA profile of a reference fish meal based diet. Silva et al. (2009) reported that the marine fish *Senegal sole* could be grown effectively on diets devoid of fish meal provided the dietary AA composition was appropriately formulated. Furthermore, the use of 98% of dietary protein as plant meals did not reduce somatic growth or nitrogen utilization of sea bass grown over a 12-week period (Kaushik et al., 2004). Contrary to the results of this present study, Gaye-Siessegger (2007) indicated that the utilization of free AA by tilapia was poor and the reasons could be because of rapid uptake of AA into the plasma of fish compared to fish fed intact protein. The results of that study were in agreement with other studies conducted on species, such as sea bass and salmon (Dabrowski et al., 2010; El Haroun and Bureau, 2006; Hauler et al., 2007; Liu et al., 2002; Sveier et al., 2001; Yamada et al., 1981). The discrepancy in results of these studies might be interrelated with dietary ingredients that slowly digested proteins might inferior the utilization efficiency of CAA (Ambardekar, 2007). The faster absorbed AA could result in greater proportions of AAs being used for catabolism instead of protein synthesis (Zarate et al., 1999). Leaching of CAA from

the diets prior to consumption is also one of reasons for impairing growth in some species, which have a slow eating habit like shrimp (Fox et al., 2006). Another added benefit of including crystalline IAAs in the diets is the improvement of protein retention. According to the results of the present study, Nile tilapia fed crystalline IAA supplemented diets had better efficiency of protein utilization. Similar results obtained in trout showed that fish fed diets with higher levels of IAA supplements had higher nitrogen retention of 50 – 52% compared to the control group at 35–36% (Yamamoto, 2005).

Excess of dietary protein containing an imbalanced AA profile is unexpected in formulating aquatic feed. Gatlin (2007) stated that the balance of amino profile is the main key contributing to the efficiency of feed regardless of the protein sources used as ingredients. The results of this study indicated that well-balanced IAA profile can be used to reduce the intact protein levels of feed (32 - 27.2%) without causing impaired growth of fish. Similarly, Botaro et al. (2007) indicated no significant differences on growth, carcass yield and fillet chemical composition of juvenile Nile tilapia (4.40 ± 0.9 g) fed reduced levels of protein from 30 to 27.5% with the supplementation of CAA to maintain AA levels according to the ideal protein. Righetti et al. (2011) also demonstrated the possibility of reducing dietary protein from 26.74 to 24.53% for Nile tilapia (100 to 500 g phase) by applying ideal protein concept. The same patterns were observed on grass carp (Gan et al., 2012) showing that dietary crude protein of practical diets could be reduced from 32 to 30% under the supplementation of lysine and methionine while Viola and Lahav (1991) previously reported that up to 5% (from 30 to 25%) of crude protein could be reduced using lysine supplemented diets for the grass carp. Cheng et al. (2003) also indicated that the reduction of dietary crude protein from 42 to 37% did not affect growth of rainbow trout, provided

that lysine was supplemented at above 1.8% while crystalline methionine, threonine and tryptophan were added in these diets to the same levels of 42% protein diet. Gaylord and Barrows (2009) reported that the protein level in trout diets could be reduced by about 5.1% from 46 to 40.9% crude protein (analyzed values) with the supplementation of three IAAs including methionine, lysine and threonine based on an ideal protein basis. Reducing the level of intact protein from 32 to 24.73% and below while maintaining the level of IAAs at the requirement (NCR, 2011), however, induced a growth reduction in Nile tilapia. A portion of this growth depression appears to be due to limitations of DAA as nitrogen source of protein. Deng (2009) indicated that the supplementation of IAA including lysine, methionine, threonine, tryptophan, leucine, isoleucine and valine to low protein diets could not help piglets to restore protein synthesis or whole-body growth effectively compared to that of piglets eating balanced protein diets. Dabrowski and Guderley (2002) also demonstrated that supplementation of DAA at specific levels is needed to satisfy the nitrogen requirement of animals as IAA might not be used as efficiently as DAA for DAA synthesis.

## 5. Conclusion

Based on the data obtained from this study, it can be concluded that ideal protein concept can be applied in formulating the diets for Nile tilapia to optimize AA profile of the diets. Well-balanced IAA profile can be used to reduce the intact protein levels (32 to 27.2%) of tilapia feeds without causing impaired growth of fish. The reduction of intact protein levels to 24.7 and 22.2% of diet, however, induced growth depression of fish which could result from a deficiency of nonspecific nitrogen as a source of energy or limitation of daily IAA intake.

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# Chapter III

## OPTIMIZING AMINO ACID BALANCE IN DIETS FOR NILE TILAPIA

### *Oreochromis niloticus*


## Abstract

A ten-week growth trial was conducted to evaluate AA balance of diets for Nile tilapia. Nine diets with different AA profiles were formulated to evaluate the response of this species to the shifts of dietary AA profiles. A diet containing 30% crude protein (29.69% intact protein and 0.31% crystalline AA), supporting the highest growth rate of fish in the previous work, was used as the reference diet (30AAR). The basal diet (24AAR) was assigned to meet the respective requirement of indispensable AA (IAA) based on NRC recommendations for Nile tilapia. This diet contained 24% protein in which 22.2% was derived from intact protein and 1.8% from crystalline AA (CAA). All of the IAAs in the 24AAR diet were then enhanced to the IAA levels of 30AAR diet to confirm potential limitation of IAAs. To elucidate the role of dispensable AA (DAA), they were incorporated at 4% of the diet, bringing the crude protein to 30% of the diet (24AAE+N). Finally, to confirm possible limitations of IAAs, tryptophan (Trp), isoleucine (Iso), arginine (Arg), histidine (His) and valine (Val), were individually deleted from diet 24AAE+N. A total of 780 fish were randomly stocked into 36 aquaria with 20 fish per/tank. Fish were fed based on percent body weight four times per day for a 10-week period. The results obtained after ten weeks indicated that supplementation of DAA had positive effects on growth performance of Nile tilapia. Enhancing of IAA to 30AAR diet levels did help the fish to attain better growth; the achievement of fish fed this diet, however, was not comparable to fish fed the reference diet or diet with DAA

supplementation. With the exception of valine, the deletion of selected IAAs (Trp, Arg, Iso and His) did not influence the growth response of Nile tilapia. Fish fed diet with Val deletion had significantly lower growth rate (thermal-unit growth coefficient), apparent net protein retention (ANPR) and higher feed conversion ratio (FCR) compared to those of fish fed the reference diet. Whole body proximate composition (crude protein, moisture, lipids, ash) of Nile tilapia was not affected by various dietary treatments. In contrast, body condition indexes of fish were significantly affected by the shift of AA profiles of diets. Fish fed diets without Trp and Val supplements had significantly higher mean intraperitoneal fat (IPF) than that of fish maintained on the reference diet, while fish fed diet 24AAR had significantly higher mean hepatosomatic index (HSI) compared to fish fed other diets. Few differences were observed regarding the AA composition of whole-body tissues with the exception of fish fed diet 24AAR, which had significantly lower levels of arginine, histidine, lysine, phenylalanine, threonine, alanine, aspartic acid, glutamic acid, serine compared to fish fed 30AAR diet. In summary, the presence of supplemental DAAs in the low protein diet is needed to overcome limitations of nonspecific nitrogen and optimize protein efficiency. In our ingredient matrix, Val is likely limiting and the supplementation of this IAA is needed to improve the performance of fish.

## 1. Introduction

Over the last decade, global aquaculture industry has shown a growing tendency of intensification of production to satisfy the demand for seafood and fisheries products (FAO, 2016). Continued growth of aquaculture production will depend largely on precision feed formulations used to produce well-balanced feeds at cost-effective prices that maximize fish growth and health while minimizing environmental effects (NRC, 2011). Because of this, information on nutritional requirements as well as nutrient levels and availability in feed ingredients are critical to provide a context for understanding both biological and economic performance.

Protein is considered the most important dietary component as it comprises a significant proportion of whole body dry matter of fish. A continuous supply of protein is needed to meet the needs for maintenance and growth of animals throughout their life. Generally, protein can be provided in the feed by using mixtures of protein sources that may complement one another in terms of AA composition. To be efficient, the diet should have a protein content that meets but does not exceeds the animal's protein requirement, a well-balanced AA profile and high digestibility (NRC, 2011).

The "ideal protein concept" has been widely studied and applied in formulating practical diets for terrestrial and aquatic animals to produce a balanced feed protein. In general, this concept was built on the hypothesis that feed used for farming practices needs to provide an exact balance of AAs to meet the needs for protein synthesis of cultured species (NRC, 2011). Formulating low and moderate protein diets based on ideal protein concept has resulted in improved efficiency of protein utilization and minimal nitrogenous wastes (Botaro et al., 2007; Furuya et al., 2004; Furuya and Fruruya, 2010; Righetti et al., 2011).

Despite being the objective of a large number of published studies, a precise AA pattern for Nile tilapia has not yet been established. Discrepancies in reports have been observed in the optimum levels of IAAs and the way to express their requirement for several fish species, including Nile tilapia. This variability may result from different experimental conditions and the wide range of mathematical and statistical approaches applied to estimate requirements. Santiago and Lovell (1988) conducted the first experiment to estimate the IAA requirements of Nile tilapia, using 28% whole egg protein as the standard AA profile. From that time, several studies have been conducted to estimate the quantitative requirement of ten IAAs of Nile tilapia employing different methods. Most of the studies have determined requirements using conventional growth response assays in which ideal AA profiles were based on IAAs of some reference protein, such as whole hen's egg protein, whole body protein or fish meal (Furuya et al., 2004; Gomez-Requeni, 2003, Gaye-Siessegger, 2007) with inconsistent results in the reported IAAs requirement.

In stark contrast to the IAA, DAA have received less consideration by nutritionists. Although the DAA can be adequately synthesized by fish, their presences in the diets have nutritional significance because the need for fish to synthesize them is reduced (Webster and Lim, 2002). Tilapia, like other fish, do not have a true protein requirement, but need a well-balanced mixture of indispensable and dispensable AAs (Shiau, 2002). Although this concept is presented in many nutrition books, the relationship is seldom defined. In our previous work, the utilization of low protein diets with supplemental IAAs to meet the reported requirement of Nile tilapia based on recommendations of NRC (2011) led to growth suppression of this species. This growth depression might have resulted from the deficiency of nonspecific nitrogen as a source of energy or limitation of IAA intake. Therefore, the objective of this study was to evaluate AA balance in

formulating low protein diets. The supplementation of DAA to spare the use of expensive IAA was taken into consideration in revising the ideal protein concept. The potentially limiting IAAs (Trp, Arg, Iso, His and Val) in addition to lysine, methionine and threonine in the ingredient matrix was also tested.

## 2. Materials and Method

### *2.1. Experimental design and diets*

Nine experimental diets were formulated to contain different AA profiles (Table 1). Diet 30AAR, supporting the highest performance of Nile tilapia in the previous work, was used as a reference diet. This diet contained 30% protein in which 29.69% derived from intact protein and 0.31% CAA (lysine, threonine and valine). Intact protein ingredients were then gradually decreased to produce the basal diet contained 22.20% of intact protein originating primarily from 3.60% fishmeal, 25.55% soybean meal, 5.83% corn protein concentrate and 25.00% whole wheat as protein sources. CAA was after that provided at 1.78% to this diet to assign the IAAs profile meeting the respective requirements of NRC (2011), except methionine and histidine, whose optimal values were obtained from suggested numbers by Santiago and Lovell (1988) and Nguyen and Davis (2009), respectively. The IAA profiles of diets were then enhanced to IAA profile of the diet which supported the best performance of fish in the first study (24AAE) with the analyzed crude protein at 26.66% (Table 2). To elucidate the role of dispensable AA (DAA), they were incorporated at 4% of the diet, bringing the crude protein to 30% of the diet (24AAE+N). The DAA were used with ratios built up to simulate the AA content of a 28% whole egg protein (Nguyen and Davis, 2009). Aspartic acid was used as a standard and other DAA were calculated accordingly. Finally, to confirm possible limitations of IAAs, tryptophan (Trp), isoleucine (Iso), arginine (Arg), histidine (His) and valine (Val) were individually deleted from diet 24AAE+N (Table 2).

**Table 1** Calculated amino acid profile of the basal diet, the reference diet 30AAR and NRC requirement (g 100 $g^{-1}$ as-is).

| Amino acid profile | Basal diet | 30AAR | NRC[a] |
|---|---|---|---|
| Arginine | 1.30 | 1.80 | 1.20 |
| Histidine | 0.56 | 0.75 | 0.48 |
| Isoleucine | 0.95 | 1.29 | 1.00 |
| Leucine | 2.07 | 2.66 | 1.90 |
| Lysine | 1.60 | 1.60 | 1.60 |
| Methionine | 0.49 | 0.51 | 0.49 |
| Methionine + cysteine | 0.94 | 0.94 | 0.94 |
| Phenylalanine | 1.14 | 1.51 | 1.10 |
| Phenylalanine + tyrosine | 1.97 | 2.59 | 1.60 |
| Threonine | 1.10 | 1.10 | 1.10 |
| Tryptophan | 0.30 | 0.37 | 0.30 |
| Valine | 1.50 | 1.50 | 1.50 |

[a] All of the AA were from NRC (2011) except histidine, methionine and methionine + cysteine values which were from Santiago and Lovell (1989) and Nguyen and Davis (2009), respectively.

**Table** 2 Ingredient compositions (g 100 $g^{-1}$ as-is) of ten experimental diets[a] offered to juvenile Nile tilapia (7.4 ± 0.1 g) culture in a recirculating system over a ten-week period.

| Ingredient | 30AAR | 24AAR | 24AAE | 24AA+N | -Trp | -Iso | -Arg | -His | -Val |
|---|---|---|---|---|---|---|---|---|---|
| Menhaden fishmeal[b] | 5.40 | 3.60 | 3.60 | 3.60 | 3.60 | 3.60 | 3.60 | 3.60 | 3.60 |
| Soybean meal[c] | 38.32 | 25.55 | 25.55 | 25.55 | 25.55 | 25.55 | 25.55 | 25.55 | 25.55 |
| CPC[d] | 6.28 | 5.83 | 5.83 | 5.83 | 5.83 | 5.83 | 5.83 | 5.83 | 5.83 |
| Menhaden fish oil[e] | 5.53 | 6.01 | 6.01 | 6.01 | 6.01 | 6.01 | 6.01 | 6.01 | 6.01 |
| Lecithin[f] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Corn Starch[g] | 14.86 | 27.31 | 24.60 | 21.12 | 21.22 | 21.46 | 21.63 | 21.31 | 21.56 |
| Whole wheat[h] | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| Mineral premix[i] | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin premix[j] | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 |
| Choline chloride[k] | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Stay C[l] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| CaP-dibasic[m] | 1.70 | 2.30 | 2.30 | 2.30 | 2.30 | 2.30 | 2.30 | 2.30 | 2.30 |
| Arginine[g] | | | 0.51 | 0.51 | 0.51 | 0.51 | | 0.51 | 0.51 |
| Histidine[g] | | | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | | 0.19 |
| Isoleucine[g] | | | 0.34 | 0.34 | 0.34 | | 0.34 | 0.34 | 0.34 |
| Leucine[g] | | | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| Lysine 78.8%[n] | 0.19 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 |
| Methionine[n] | | 0.10 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 |
| Phenylalanine[g] | | | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 |
| Threonine[g] | 0.04 | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 |
| Tryptophan[n] | | 0.03 | 0.10 | 0.10 | | 0.10 | 0.10 | 0.10 | 0.10 |
| Valine[n] | 0.07 | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 | 0.44 | |
| Alanine[g] | | | | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 | 0.43 |
| Aspartic Acid[g] | | | | 0.79 | 0.79 | 0.79 | 0.79 | 0.79 | 0.79 |
| Cysteine[g] | 0.01 | 0.13 | | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 |
| Glutamic Acid[g] | | | | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 |
| Glycine[g] | | | | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 | 0.27 |
| Proline[g] | | | | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 |
| Serine[g] | | | | 0.58 | 0.58 | 0.58 | 0.58 | 0.58 | 0.58 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Tyrosine[g] | 0.51 | 0.51 | 0.51 | 0.51 | 0.51 | 0.51 | 0.51 |

[a] Diet designations: 30AAR = 29.69% crude protein from intact sources plus supplemental IAAs to the reference requirement (NRC); 24AAR = 22.22% crude protein from intact sources plus supplemental IAAs to the reference requirement (NRC); 24AAE = 22.22% crude protein from intact sources plus supplemental IAAs to IAA levels of 30AAR diet; 24AAE+N = 22.22% crude protein from intact sources plus supplemental IAAs to IAA levels of 30AAR diet plus DAA; –Trp = 24AAE+N subtract Trp; -Iso = 24AAE+N subtract Iso; -Arg: = 24AAE+N subtract Iso; -Arg = 24AAE+N subtract Arg; -His = 24AAE+N subtract His; -Val = 24AAE+N subtract Val.

[b] Omega Protein Inc., Houston, Texas, USA.

[c] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA

[d] Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA

[e] Omega Protein Inc., Reedville, VA, USA.

[f] The Solae Company, St. Louis, MO, USA.

[g] MP Biochemicals Inc., Solon, OH, USA.

[h] Bob's Red Mill Natural Foods, Milwaukie, OR, USA.

[i] Trace mineral (g/100g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.250; Furrous sulfate, 4.000; Magnesium sulfate anhydrous, 13.862; Manganous sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate hepahydrate, 13.193; Alpha-cellulose, 67.964.

[j] Vitamin (g/kg premix): Thiamin HCl, 0.44; Riboflavin, 0.63; Pyridoxine HCl, 0.91; DL pantothenic acid, 1.72; Nicotinic acid, 4.58; Biotin, 0.21; Folic acid, 0.55; Inositol, 21.05; Menadione sodium bisulfite, 0.89; Vitamin A acetate, 0.68; Vitamin $D_3$, 0.12; dL-alpha-tocoperol acetate, 12.63; Alpha-cellulose, 955.59.

[k] Amresco Inc., Solon, Ohio, USA.

[l] Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[m] Alfa Aesar, Ward Hill, MA, USA.

[n] Ajinomoto Heartland Inc., Chicago, IL, USA.

The test diets were prepared in the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn, AL, USA). Pre-ground feed ingredients and oil were placed in a food mixer (Hobart Corporation, Troy, Ohio, USA) for 15 minutes. Hot water was then added to the mixture in order to attain an appropriate consistency for pelleting. Diets were then extruded through a 4-mm diameter die in a meat grinder, air dried at < 50° C to a moisture less than 11%, and stored in the freezer at -20°C until used. A sample of each feed was collected and analyzed for proximate composition by Midwest Laboratories (Omaha, NE, USA) and AA compositions by Ajinomoto Heartland Inc, (Chicago, IL, USA) (Table 3).

*2.2. Culture methods*

The trial was conducted at the E.W. Shell Fisheries Center, Auburn, Alabama. Nile tilapia fry spawned at this center were stocked into the nursery tank until the beginning of trial. After acclimating, juvenile Nile tilapia (7.4 ± 0.1 g) were randomly stocked into thirty-six rectangular 50-L aquaria of a 3,800-L indoor recirculation system at 20 fish per aquarium. Each of the nine treatments was assigned to four randomly chosen tanks. Samples of fish from the initial stocking were retained for later protein retention analysis.

Water quality (DO, salinity, temperature) was monitored daily. Water temperature was maintained at around 28˚C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA) and dissolved oxygen was maintained near saturation using air blower. Dissolved oxygen (DO) and water temperature were measured twice per day using YSI 650 multi-parameter instrument (YSI, Yellow Springs, Ohio) while pH, total ammonia nitrogen (TAN) and nitrite-nitrogen were measured twice per week. Photoperiod was set at 14 h light and 10 h dark. During the experimental period, DO, temperature, salinity, pH, TAN, and nitrite were maintained

**Table 3** Analyzed proximate composition[a] and amino acid profile[b] of the experimental diets (g 100 $g^{-1}$ as-is) fed to juvenile Nile tilapia (7.4 ± 0.1 g) over a ten week period.

| Composition | 30AAR | 24AAR | 24AAE | 24AAE+N | -Trp | -Iso | -Arg | -His | -Val |
|---|---|---|---|---|---|---|---|---|---|
| Crude protein | 29.83 | 24.03 | 26.96 | 29.05 | 29.11 | 28.63 | 28.45 | 29.15 | 29.34 |
| Moisture | 9.43 | 9.36 | 8.54 | 9.80 | 8.60 | 9.07 | 7.72 | 7.77 | 7.38 |
| Crude Fat | 7.57 | 8.62 | 8.84 | 9.64 | 8.88 | 9.61 | 9.50 | 9.52 | 9.51 |
| Crude Fiber | 2.66 | 2.12 | 1.89 | 1.87 | 2.05 | 2.08 | 2.11 | 2.11 | 2.09 |
| Ash | 5.38 | 4.59 | 4.69 | 4.54 | 4.64 | 4.64 | 4.62 | 4.70 | 4.68 |
| **IAA** | | | | | | | | | |
| Arginine | 1.70 | 1.25 | 1.66 | 1.67 | 1.67 | 1.65 | 1.23 | 1.70 | 1.67 |
| Histidine | 0.69 | 0.52 | 0.68 | 0.70 | 0.70 | 0.69 | 0.71 | 0.54 | 0.72 |
| Isoleucine | 1.25 | 0.95 | 1.18 | 1.21 | 1.31 | 0.94 | 1.33 | 1.29 | 1.24 |
| Leucine | 2.61 | 2.06 | 2.66 | 2.61 | 2.68 | 2.64 | 2.70 | 2.71 | 2.75 |
| Lysine | 1.70 | 1.73 | 1.72 | 1.74 | 1.73 | 1.70 | 1.75 | 1.78 | 1.77 |
| Methionine | 0.51 | 0.47 | 0.49 | 0.49 | 0.50 | 0.48 | 0.51 | 0.52 | 0.50 |
| Phenylalanine | 1.46 | 1.13 | 1.51 | 1.44 | 1.51 | 1.55 | 1.51 | 1.52 | 1.51 |
| Taurine | 0.20 | 0.21 | 0.21 | 0.19 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Threonine | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 |
| Tryptophan | 0.38 | 0.34 | 0.40 | 0.39 | 0.33 | 0.44 | 0.45 | 0.46 | 0.45 |
| Valine | 1.43 | 1.45 | 1.41 | 1.43 | 1.43 | 1.45 | 1.50 | 1.51 | 1.08 |
| **DAA** | | | | | | | | | |
| Alanine | 1.49 | 1.17 | 1.16 | 1.56 | 1.57 | 1.56 | 1.60 | 1.62 | 1.65 |
| Aspartic Acid | 2.59 | 1.88 | 1.87 | 2.66 | 2.61 | 2.61 | 2.64 | 2.71 | 2.72 |
| Cysteine | 0.42 | 0.44 | 0.31 | 0.42 | 0.42 | 0.42 | 0.42 | 0.43 | 0.44 |
| Glutamic Acid | 5.80 | 4.52 | 4.50 | 5.44 | 5.46 | 5.42 | 5.54 | 5.61 | 5.66 |
| Glycine | 1.25 | 0.95 | 0.94 | 1.20 | 1.18 | 1.16 | 1.21 | 1.24 | 1.23 |
| Proline | 1.72 | 1.43 | 1.37 | 1.70 | 1.63 | 1.65 | 1.65 | 1.70 | 1.72 |
| Serine | 1.30 | 0.92 | 0.92 | 1.35 | 1.32 | 1.39 | 1.26 | 1.24 | 1.33 |
| Tyrosine | 1.01 | 0.81 | 1.27 | 1.23 | 1.27 | 1.27 | 1.22 | 1.23 | 1.20 |

[a] Diets were analyzed at Midwest Laboratories (Omaha, NE, USA).

[b] Diets were analyzed at Ajinomoto Heartland Inc, (Chicago, IL, USA).

within acceptable ranges for Nile tilapia at 5.62 ± 0.63 mg/L, 28.26 ± 1.31°C, 1.08 ± 0.97 ppt, 7.30 ± 0.31, 0.16 ± 0.05 mg/L, 0.06 ± 0.04 mg/L.

Diets were offered to fish at 6-5% body weight daily four times per day at 08:00, 11:00 13:00, and 16:00 h according to fish size. Fish were bulk-weighed and enumerated every week in the first two weeks and every other week thereafter. Daily feed rations were calculated and adjusted weekly based on percent body weight after each weighing and for intermittent weeks we assumed a 30% increase in weight gain across all treatments. Upon termination of the experiment, fish were counted and group weighed. Four fish per aquarium were randomly sampled after being euthanized with tricaine methanesulphonate to measure condition index including individual weight and length, hepatosomatic index (HSI), and intraperitoneal fat (IPF) ratio. These whole-body fish samples were frozen at -20$^{o}$C and then were ground homogenously before a subsample was sent to Midwest Laboratories (Omaha, NE, USA) for dry matter, crude protein, crude lipid, and ash analysis. The left over whole-body fish samples were freeze dried and sent to Ajinomoto Heartland Inc., (Chicago, IL, USA), for AA analysis. Subsequently, thermal-unit growth coefficient (TGC), apparent net protein retention, feed efficiency ratio, feed conversion ratio, condition factor, hepatosomatic index and intraperitoneal fat were determined using the following calculations:

a) Thermal-unit growth coefficient (TGC) = (final weigh$^{1/3}$ - initial weight$^{1/3}$) / (temperature × day) × 100.
b) Apparent net protein retention (ANPR, %) = (final weight × final protein content) - (initial weight × initial protein content) × 100 / protein intake.
c) Feed efficiency ratio (FE) = weight gain/ dry feed intake.

d) Feed conversion ratio (FCR) = dry feed intake / weight gain.

e) Condition factor (K, 100 × g/cm$^3$) = 100 × individual body weight / (individual body length$^3$).

f) Hepatosomatic index (HSI, %) = (liver weight/fish weight) × 100.

g) Intraperitoneal fat (IPF, %) = (IPF weight/fish weight) × 100.

*2.3. Statistical Analysis*

All data were subjected to a one-way analysis of variance to determine significant differences ($P < 0.05$) among the treatments, which was followed by Tukey's multiple comparison test to distinguish significant differences among treatment means. Dunnett's test was performed to determine significant difference between the growth performance of fish fed the reference diet and other diets. All the data were analyzed using SAS (V9.4. SAS Institute, Cary, North Carolina, USA).

## 3. Results

Mean performances of Nile tilapia *Oreochromis niloticus* offered diets containing different AA profiles are presented in Table 4. The experimental groups did not differ significantly from each other with respect to survival which ranged from 98.75 – 100%. There were significant differences ($P < 0.05$) among mean final weight (FW), growth rate (TGC), feed conversion ratio (FCR), and apparent net protein retention (ANPR) of fish receiving various dietary treatments. Results from Dunnett's test (Table 5) showed that the reference diet produced a faster growth rate (TGC; 0.145) than did that of fish offered diet 24AAR (0.137), 24AAE (0.140) and –Val (0.139). Mean FCRs of fish fed diet –Val (1.12) and 24AAR (1.10) were also significantly higher compared to those of fish fed the other diets. The supplementation of IAA up to the IAA levels of the reference diet 30AAR helped the fish to attain a better TGC (0.140) compared to fish fed 24AAR diet even though no significant differences were observed between fish fed these diets compared to those of fish fed diets with IAAs supplemented up to the NRC requirement (0.137). The fish fed diets with DAA supplementation, however, showed significantly higher mean TGC (0.146) and lower FCR (1.06) compared to those of fish fed diets with IAAs supplemented up to the requirement. The fish fed these diets have comparable growth performance and feed utilization efficiency to fish fed the reference diet. The results of this experiment also demonstrated that the deletion of tryptophan, isoleucine, arginine and histidine from the diets did not significantly affect the mean growth performance of fish. Fish fed these diets had comparable mean FW, TGC, FCR and ANPR to those of fish fed the reference diet 30AAR, which ranged from 113.54 – 116.48 g, 0.144 – 0.147, 1.06 – 1.07 and 41.28 – 42.66%, respectively. Fish fed diet -Val, however, had

lower mean FW (106.07), TGC (0.139), ANPR (38.79) and higher FCR (1.12) compared to those of fish fed other diets.

**Table 4** Mean response of juvenile Nile tilapia (7.4 ± 0.1 g) fed diets containing different amino acid profiles over a ten week period performed by Tukey's test.

| Diet | Initial weight | Final weight | Thermal-unit growth coefficient | Feed conversion ratio | Apparent net protein retention | Survival |
|---|---|---|---|---|---|---|
| 30AAR | 7.88 | 114.68$^{a}$ | 0.145$^{ab}$ | 1.07$^{b}$ | 41.28$^{ab}$ | 98.75 |
| 24AAR | 7.95 | 104.20$^{b}$ | 0.137$^{d}$ | 1.10$^{ab}$ | 43.81$^{a}$ | 100.00 |
| 24AAE | 7.99 | 108.09$^{b}$ | 0.140$^{bcd}$ | 1.08$^{b}$ | 42.17$^{ab}$ | 100.00 |
| 24AAE+N | 7.97 | 115.93$^{a}$ | 0.146$^{a}$ | 1.06$^{b}$ | 41.97$^{ab}$ | 100.00 |
| -Trp | 7.89 | 113.76$^{a}$ | 0.144$^{abc}$ | 1.06$^{b}$ | 41.76$^{ab}$ | 100.00 |
| -Iso | 7.87 | 113.54$^{a}$ | 0.144$^{abc}$ | 1.06$^{b}$ | 41.90$^{ab}$ | 100.00 |
| -Arg | 7.91 | 115.38$^{a}$ | 0.145$^{ab}$ | 1.06$^{b}$ | 42.66$^{ab}$ | 100.00 |
| -His | 7.83 | 116.48$^{a}$ | 0.147$^{a}$ | 1.05$^{b}$ | 41.99$^{ab}$ | 100.00 |
| -Val | 7.84 | 106.07$^{b}$ | 0.139$^{cd}$ | 1.12$^{a}$ | 38.79$^{b}$ | 98.75 |
| *P*-value | 0.6928 | <0.0001 | <0.0001 | <0.0001 | 0.1066 | 0.5493 |
| PSE[a] | 0.0689 | 1.5838 | 0.0012 | 0.0009 | 5.6960 | 0.5893 |

Means (n = 4) in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by Tukey's multiple range test.

[a] PSE: Pooled standard error.

**Table 5** Mean response of juvenile Nile tilapia (7.4 ± 0.1 g) fed diets containing different amino acid profiles over a ten week period performed by Dunnett's test [a].

| Diet | Final weight | Thermal-unit growth coefficient | Feed conversion ratio | Apparent net protein retention |
|---|---|---|---|---|
| 30AAR | 114.68 | 0.145 | 1.07 | 41.28 |
| 24AAR | 104.20* | 0.137* | 1.10* | 43.81 |
| 24AAE | 108.09* | 0.140* | 1.08 | 42.17 |
| 24AAE+N | 115.93 | 0.146 | 1.06 | 41.97 |
| -Trp | 113.76 | 0.144 | 1.06 | 41.76 |
| -Iso | 113.54 | 0.144 | 1.06 | 41.90 |
| -Arg | 115.38 | 0.145 | 1.06 | 42.66 |
| -His | 116.48 | 0.147 | 1.05 | 41.99 |
| -Val | 106.07* | 0.139* | 1.12* | 38.79 |

* Means (n = 4) are different from the reference diet ($\alpha$=0.05).

Mean whole body moisture, crude protein, lipids and ash contents of tilapia fed the experimental diets are summarized in Table 6. Mean values for body moisture (65.23 - 70.95%), crude protein (14.86 - 16.08%), lipids (9.70 - 11.70%) and ash (3.32 - 6.49%) were not significantly influenced by the shift in the AA profiles of the diets. Nevertheless, the condition indexes (his and IPF) were significantly affected by various AA profiles (Table 6). Fish fed diet –Val and –Trp have significantly higher mean IPF at 3.46% and 3.11% compared to fish fed the reference diet (2.26%). Fish fed diet 24AAR had significantly higher HSI (2.93%) compared to those of fish fed other diets which ranged from 1.99% to 2.40%.

In the present study, few significant differences were observed regarding the AA composition of whole-body tissues (Table 7) except fish fed diet 24AA, which had significantly lower mean levels of arginine, histidine, lysine, phenylalanine, threonine, alanine, aspartic acid, glutamic acid, and serine compared to those of fish fed 30AAR diet.

**Table 6** Proximate analyses[a] (g 100 $g^{-1}$ as-is) of juvenile Nile tilapia (7.4 ± 0.1 g) fed diets containing different amino acid profiles over a ten-week period.

| Composition | 30AAR | 24AAR | 24AAE | 24AAE+N | -Trp | -Iso | -Arg | -His | -Val | *P*-value | PSE[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Crude protein | 16.08 | 14.86 | 15.78 | 15.37 | 15.87 | 15.74 | 15.73 | 15.72 | 15.91 | 0.0802 | 0.2515 |
| Moisture | 69.08 | 67.80 | 69.58 | 70.95 | 65.23 | 69.80 | 69.28 | 69.80 | 69.65 | 0.0870 | 1.1653 |
| Lipids | 9.90 | 11.04 | 11.48 | 9.71 | 11.70 | 10.37 | 9.70 | 10.07 | 11.63 | 0.1673 | 0.6624 |
| Ash | 4.83 | 5.15 | 3.62 | 3.32 | 6.49 | 4.54 | 4.36 | 3.99 | 3.99 | 0.1739 | 0.7523 |
| IPF | $2.26^{c}$ | $2.97^{abc}$ | $2.65^{abc}$ | $2.93^{bc}$ | $3.46^{a}$ | $2.94^{abc}$ | $2.86^{abc}$ | $2.79^{abc}$ | $3.11^{ab}$ | 0.0018 | 0.1567 |
| HSI | $2.05^{b}$ | $2.93^{a}$ | $2.05^{ab}$ | $2.35^{b}$ | $2.16^{b}$ | $2.40^{ab}$ | $2.02^{b}$ | $2.14^{b}$ | $1.99^{b}$ | 0.0076 | 0.1609 |

Means (n = 4) in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by Tukey's multiple range test.

[a] Diets were analyzed at Midwest Laboratories (Omaha, NE, USA).

[b] PSE: Pooled standard error.

**Table 7** Whole-body amino acid (AA) composition[a] (g 100 $g^{-1}$ as-is) of juvenile Nile tilapia (7.4 ± 0.1 g) fed diets containing different amino acid profiles over a ten-week period.

| Composition | 30AAR | 24AAR | 24AAE | 24AA+N | -Trp | -Iso | -Arg | -His | -Val | *P*-value | PSE[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **IAA** | | | | | | | | | | | |
| Arginine | 0.97[a] | 0.87[b] | 0.94[ab] | 0.97[a] | 0.96[ab] | 0.96[a] | 0.98[a] | 0.97[a] | 0.98[a] | 0.0043 | 0.0174 |
| Histidine | 0.37[a] | 0.33[b] | 0.37[a] | 0.36[ab] | 0.36[ab] | 0.36[ab] | 0.37[a] | 0.36[ab] | 0.37[a] | 0.0254 | 0.0082 |
| Isoleucine | 0.63 | 0.58 | 0.58 | 0.61 | 0.60 | 0.59 | 0.61 | 0.60 | 0.60 | 0.4326 | 0.0153 |
| Leucine | 1.13 | 1.02 | 1.07 | 1.10 | 1.08 | 1.08 | 1.10 | 1.10 | 1.09 | 0.1615 | 0.0241 |
| Lysine | 1.22[a] | 1.06[b] | 1.14[ab] | 1.16[ab] | 1.16[ab] | 1.16[ab] | 1.18[a] | 1.18[a] | 1.18[a] | 0.0116 | 0.0248 |
| Methionine | 0.41 | 0.37 | 0.38 | 0.40 | 0.39 | 0.39 | 0.40 | 0.40 | 0.39 | 0.2728 | 0.0110 |
| Phenylalanine | 0.64[a] | 0.57[b] | 0.61[ab] | 0.62[a] | 0.62[ab] | 0.61[ab] | 0.62[a] | 0.62[ab] | 0.62[ab] | 0.0136 | 0.0109 |
| Threonine | 0.67[a] | 0.60[b] | 0.64[ab] | 0.65[ab] | 0.65[ab] | 0.65[ab] | 0.66[ab] | 0.66[ab] | 0.65[ab] | 0.0294 | 0.0125 |
| Tryptophan | 0.14 | 0.13 | 0.14 | 0.15 | 0.13 | 0.15 | 0.14 | 0.14 | 0.14 | 0.9298 | 0.0085 |
| Valine | 0.71 | 0.65 | 0.67 | 0.69 | 0.68 | 0.67 | 0.69 | 0.69 | 0.68 | 0.2080 | 0.0148 |
| **DAA** | | | | | | | | | | | |
| Alanine | 1.03[a] | 0.94[b] | 0.99[ab] | 1.01[ab] | 1.02[ab] | 1.02[ab] | 1.02[ab] | 1.02[ab] | 1.03[a] | 0.0327 | 0.0172 |
| Aspartic Acid | 1.51[a] | 1.33[b] | 1.42[ab] | 1.45[ab] | 1.44[ab] | 1.44[ab] | 1.46[ab] | 1.46[ab] | 1.45[ab] | 0.0277 | 0.0285 |
| Cysteine | 0.14 | 0.13 | 0.13 | 0.13 | 0.13 | 0.12 | 0.13 | 0.13 | 0.13 | 0.7303 | 0.0054 |
| Glutamic Acid | 2.16[a] | 1.91[b] | 2.04[ab] | 2.08[ab] | 2.06[ab] | 2.08[ab] | 2.09[ab] | 2.10[ab] | 2.08[ab] | 0.0496 | 0.0434 |
| Glycine | 1.23 | 1.16 | 1.23 | 1.24 | 1.29 | 1.28 | 1.26 | 1.28 | 1.31 | 0.1577 | 0.0344 |
| Proline | 0.79 | 0.74 | 0.80 | 0.79 | 0.80 | 0.78 | 0.81 | 0.78 | 0.83 | 0.3545 | 0.0242 |
| Serine | 0.64[a] | 0.57[b] | 0.62[ab] | 0.64[a] | 0.62[ab] | 0.63[ab] | 0.63[a] | 0.63[ab] | 0.63[ab] | 0.0163 | 0.0116 |
| Tyrosine | 0.40 | 0.36 | 0.37 | 0.38 | 0.38 | 0.39 | 0.38 | 0.39 | 0.38 | 0.3703 | 0.0120 |

Means (n = 4) in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by Tukey's multiple range test.

[a] Diets were analyzed at Ajinomoto Heartland Inc, (Chicago, IL, USA)

[b] PSE: Pooled standard error.

## 4. Discussion

A balanced AA profile required for growth and maintenance of animals is one of the most important factors which determines the protein quality of a feed. For years, numerous studies have investigated IAA requirements to attain maximum growth rate and protein retention of Nile tilapia (Diogenes, 2017; Figueiredo-Silva et al., 2015; Furuya et al., 2015; Gan et al., 2015; He et al., 2015; Michelato et al., 2015, 2016, 2017; Silva et al., 2012; Yue et al., 2013; Zaminhan et al., 2017). However, dietary dispensable AA levels and ingredient sources should also be taken into account (Boisen et al., 2000; Heger, 2003). Based on the results of this study, fish fed diets with DAA supplementation had significantly higher mean growth rate (TGC), ANPR and lower FCR compared to those of fish fed diets supplemented with IAA but without DAA supplements, indicating the importance of meeting nitrogen requirement in formulating a low protein diet. Similarly, Gaye-Siessegger et al., (2007) indicated the importance of dietary DAA and DAA composition for the growth performance of Nile tilapia. In their study, fish fed diet with DAA supplements exhibited an increase in body mass gain; whereas, fish fed diets supplemented with IAA precursor and glutamate lost body mass. According to Abboudi et al. (2009), Atlantic salmon, *Salmo salar*, fry fed the DAA protein free diet gained higher metabolic efficiency than fish fed the diet without DDA supplementation. Enhancing IAAs to IAA profile of the diet which supported the best performance of fish in the first study without DAA supplements could not help the fish to reach comparable weight gain to those in fish fed the diets with DAA supplements. The excess of arginine, histidine, isoleucine, leucine, methionine, phenylalanine and tryptophan in diet 24AAE had no effect on growth performance of Nile tilapia, illustrating that excessive IAAs (AA catabolized for energy) may not be used by Nile tilapia to spare all of the deficient DAA in the

low protein diet. Similarly, Dabrowski and Guderley (2002) also demonstrated that supplementation of DAA at specific levels is critical to satisfy the nitrogen requirement of animals as IAA might not be used as efficiently as DAA for DAA synthesis.

The potential deficiency of Trp, Iso, Arg, His and Val was evaluated through individual deletion of these AA from diet 24AAE+N. No deleterious effects were observed on growth rates of fish fed diets without Trp, Iso, Arg, and His supplements. Fish fed these diets have comparable FW, TGC, FCR and ANPR to fish fed the reference diet. The possible explanation for this is the fact that these IAA levels in our ingredient matrix were adequate to promote optimal fish growth. Even though Trp, Iso, Arg and His were subtracted from the diet with IAAs resembling the reference diet (with DAA supplements), the analyzed concentrations of these IAAs were all above the levels reported by NRC (2011) and Santiago and Lovell (1988) at 0.33%, 0.94%, 1.23% and 0.54%, respectively. Analyzed value of Trp (0.32%) in –Trp diet was close to the requirement recently reported by Zaminhan et al. (2017) in which Trp requirement of Nile tilapia (38.2 ± 0.09 g) was estimated at 0.29, 0.31, 0.31, and 0.29 % for optimal weight gain, feed conversion ratio, protein efficiency ratio and fish uniformity. Also in our study, the analyzed value of Iso (0.94%) in -Iso diet was higher than the requirement reported by Neu et al. (2017) and Santiago and Lovell (1988) at 0.7% and 0.87%, respectively. Regarding His, Santiago and Lovell (1988) estimated the His requirement of Nile tilapia (75 g) at 0.48% for optimum growth of fish, which is lower than His levels of the –His diet in our study (0.54%). However, in a recent study conducted by Michelato et al. (2017), His requirement of Nile tilapia was defined at 0.89%. In their study, diets containing graded levels of histidine (0.42, 0.54, 0.71, 0.89, 0.98 and 1.15% of dry diet) were fed to juveniles Nile tilapia (4.84 ± 0.04 g). Since practical diets were used as experimental diets

instead of purified ingredients, the lowest analyzed His was 0.42% of the diet. As this His level was above lower values evaluated by Santiago and Lovell (1988) at 0.20%, this might have resulted in an overestimation of the requirement of His. In the present study, deletion of Arg resulted in reduced inclusion level of Arg from 1.7% in the reference diet to 1.23% in –Arg diet. This value of Arg is relatively close to the optimal level of Arg (1.18%) for maximizing growth performance of Nile tilapia reported by Santiago and Lovell et al., (1988). Recently, Neu et al. (2016) conducted the study to evaluate the growth performance of juvenile Nile tilapia (2.95 ± 0.79 g) fed increasing levels of Arg at 0.95, 1.10, 1.25, 1.40, and 1.55% and concluded that a diet containing 1.36% of arginine (with 1.53% lysine in diet) met the requirements of fish. This study also demonstrated the optimal arginine:lysine for maximum weight gain at 0.89:1, which is higher than the ratio described by Santiago and Lovell (1988) and our analyzed value at 0.82 and 0.70%, respectively. Feeding an excess of Arg has been reported to have no added benefit on growth performance of Nile tilapia (Pereira et al., 2017). Fish fed the diet contained 2.01% Arg did not have significant difference in weight gain compared to those of fish fed diets with 3.56% and 4.38% arginine. Overall, in our ingredient matrix, Trp, His, Arg, Iso is likely adequate for growth of Nile tilapia.

Fish fed the diet –Val had, however, lower mean FW (106.07 g), TGC (0.139), ANPR (38.79%) and higher FCR (1.12) compared to those of fish fed the reference diet. The subtraction of Val from the 24AAE+N induced the analyzed value of Val at 1.08% which was relatively lower compared to the recommended number (1.50%) reported by NRC (2011). This value is, however, above the estimated requirement of Santiago and Lovell (1988) who suggested an optimal Val for growth of Nile tilapia (50g) at 0.87%. Recently, Xiao et al. (2017) conducted an experiment to

determine Val requirement of Nile tilapia (6.48 ± 0.06 g). In their study, fish were fed six graded levels of dietary valine (0.41, 0.72, 0.99, 1.27, and 1.56 %). Broken-line regression analysis was used to fit the relationship between weight gain, protein retention efficiency against dietary Val levels in which the optimal Val levels were estimated to be 1.15 and 1.27, respectively. This level is above the Val level of our 24AAR diet. NRC (2011) also suggested the safety margin for Val with higher reported value at 1.5%. Thus, in our ingredient matrix Val is likely limited and the supplementation of this IAA is needed to improve the performance of fish.

Generally, body protein represents between 25 to 55% of total AAs consumed by fish and protein deposition is often used as an indicator of the dietary IAA profile required by the animal (NRC, 2011). The shift of AA profiles of the feed in the present study did not affect whole body composition of fish with the exception of fish fed the 24AAR diet. Even though the crude protein of fish were not separated enough to see the significant difference among treatments, fish fed 24AAR diet had numerically lower crude protein levels compared to fish fed other diets. The same pattern was observed on AA composition of whole-body tissues with the lowest inclusion levels of IAA was obtained on fish fed 24AAR diet with significantly lower arginine, histidine, lysine, phenylalanine, threonine, alanine, aspartic acid, glutamic acid and serine compared to fish fed 30AAR diet. The possible reason for protein deposition reduced is that limitation of DAA depressed protein synthesis rates in muscle. While there is a great dearth of information about the role of DAA on protein synthesis of fish, the importance of balance of dietary IAA and DAA on nitrogen retention of European sea bass (*Dicentrarchus labrax*) (Peres and Oliva-Teles, 2006), rainbow trout (*Oncorhynchus mykiss*) (Green et al., 2002, Schuhmacher et al., 1995) and gilthead sea bream (Gómez-Requeni et al., 2003) have been documented.

Even though fish fed –Val diet exhibited significant lower weight gain compared to fish fed the reference diet, no significant difference in whole body AA composition of fish fed –Val diet was detected. Similarly, Xiao et al. (2017) observed no significant changes in analyzed AA composition of muscle of Nile tilapia fed diets deficient in Val. Nevertheless, the condition indexes, HSI and IPF, were affected in fish fed diets with various AA profiles. Fish fed diet –Val and –Trp have significantly higher IPF at 3.46% and 3.11% compared to fish fed the reference diet (2.26%) while fish fed diet 24AAR have significantly higher HSI (2.93%) compared to those of fish fed other diets, ranging from 1.99 to 2.40%. These higher IPF and HSI reflect imbalanced diets as the accumulation of energy in the liver and viscera. Similarly, Botaro et al. (2005) stated that Nile tilapia 4.40 ± 0.9 g fed the incremental levels of digestible protein from 25.5 to 30% had reduced HSI from 1.74 to 1.53%.

## 5. Conclusion

Results indicated that the presence of DAA in the lower protein diet is needed to overcome limitations of nonspecific nitrogen and optimize the protein efficiency. Growth performance of fish fed diets without DAA supplementation was not comparable to those of fish fed diets supplemented with DAA in spite of their IAA supplements up to IAA profile of the reference diet. In our ingredient matrix, Val is likely limiting and the supplementation of this IAA is needed to improve the performance of fish.

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## Chapter IV

## TRYPTOPHAN REQUIREMENT IN SEMI-PURIFIED DIETS OF JUVENILE NILE TILAPIA *Oreochromis niloticus*


### Abstract

To further optimize practical diets with respect to IAA requirements, this study was conducted to confirm and refine the tryptophan requirement, which is a potentially limiting AA in our matrix of ingredients. Twelve iso-nitrogenous and isoenergetic diets containing 30% protein and 8% lipid were formulated to meet the nutritional requirement of juvenile Nile tilapia (*Oreochromis niloticus*) with the exception of tryptophan. The basal diet (24AAR) deficient in tryptophan was assigned to meet all other known nutrient requirements of the Nile tilapia (NRC, 2011). This diet contained 24% protein in which 22.22% was derived from intact protein and 1.78% from crystalline AA (CAA). L-tryptophan was then added to a basal diet at 0.04% increments to produce tryptophan levels ranging from 0.21 to 0.61% of the diet. Diet 30AAR, which supported the best performance of Nile tilapia in previous work, was used as a reference diet. Each diet was fed to three replicate groups of juvenile Nile tilapia (7.9 ± 0.1 g) in a recirculation system for eight weeks. Saturation kinetic model, broken line models with linear or quadratic ascending portions, were used to evaluate dose-response relationships of thermal-unit growth coefficient, apparent net protein retention, tryptophan retention against dietary tryptophan. Akaike weights were calculated and used for model selection in addition to the model's overall $R^2$. The tryptophan requirement of juvenile Nile tilapia was estimated at 0.31% (0.25 - 0.37%), 0.33%

(0.26 - 0.39%), 0.25% (0.24 - 0.25%), 0.27% (0.25 - 0.31%) of the diet for optimum growth, tryptophan deposition, feed efficiency, and apparent net protein deposition (95% of maximum value), respectively.

## 1. Introduction

From the last decade, global aquaculture industry has shown a growing tendency of intensification of production, depending highly on the use of nutritionally-complete feed. In 2015, about 35.47 mmt of manufactured feed was produced for aquaculture (Alltech, 2016). Fishmeal was once a dominant protein source of farmed fish feed due mainly to its high protein content, adequate AA profile, high protein digestibility and high palatability. Given the relatively static state of fishmeal production, an increased demand for aquafeeds is putting enormous pressure on the use of fish meal with the overall inclusion level in aquafeeds currently less than 10%. In 2015, more than 3 mmt (over 4.73 mmt) of fishmeal production went to aquaculture (www.seafish.org). The utilization of alternative ingredients of fish meal is, therefore, needed to produce efficient and economical feedstuffs with more sustainable.

The use of alternative protein sources as fish meal replacement in feed, however, requires much more consideration in formulation and accurate estimates of dietary requirements for essential nutrients. With the exception of proteins from rendered and concentrated products, the use of cheap and available plant and animal protein sources tends to be deficient in some IAAs. Davis (2015) compiled the list of limiting IAAs of selected alternative ingredients of fish meal based on recommended mean values for IAA inclusion in diets for Atlantic salmon, rainbow trout, common carp, tilapia and catfish from NRC (2011) and illustrated that most of them have imbalanced IAA profiles in one or more IAA. While lysine and methionine are usually the first and second limiting AAs, arginine, threonine, histidine and tryptophan may also be limited in several feed stuffs. Lysine, methionine, arginine, and leucine requirements have been well studied and requirements verified. Estimates for other essential AAs like threonine, isoleucine,

phenylalanine, valine, histidine and tryptophan are available from the results of only a few studies, which are often not in agreement. In order to ensure the best information on each AA, a rigorous evaluation must be done to establish essential AA requirements are indeed correct.

Tryptophan (Trp) is an essential AA, containing an α-amino group, an α-carboxylic acid group, and a side chain indole. Being a biochemical precursor for several compounds, including serotine and a neurotransmitter, Trp is involved in various biologic functions. In addition to protein synthesis, key functions include regulating feed intake and controlling the immune response (Wen et al., 2014). A deficiency of tryptophan has been found to cause spinal deformities and disruption of mineral metabolism in certain salmonids, including rainbow trout *Salmo gairdneri* (Walton et al., 1984), sockeye salmon *Oncorhynchus nerka* (Halver and Shanks, 1960), and chum salmon *Oncorhynchus keta* (Akiyama et al., 1986). A review of literature also indicated the added benefit of supplementation of L-Trp to mitigate thermal, crowding and handling stress of rohu *Labeo rohita* (Kumar at al., 2014), *Cirrhinus mrigala* fingerlings (Tejpal et al., 2009) and brown trout *Salmo trutta* (Hoglund et al., 2007). Furthermore, supplementation of Trp helped to suppress aggression and cannibalism in juvenile Atlantic cod *Gadus morhua*, juvenile grouper *Epinephelus coioides*, rainbow trout *Oncorhynchus mykiss* and freshwater crayfish *Astacus leptodactylus* Eschscholtz (Harlioglu et al., 2014; Höglund et al., 2005; Hseu et al., 2003; Lepage et al., 2002, 2005; Winberg et al., 2001).

Tilapia is the second main group of fish cultured in the world, with nearly 5.6 mmt produced in 2015 (Fitzsimmons, 2016). Similar to other species of cultured animals, alternatives to high-cost protein supplements in formulated diets for Nile tilapia are desirable to reduce feed costs and increase profits in modern farming operations. Recently, Zaminhan et al., (2017)

indicated Trp was limited in practical corn and soybean meal based diet of Nile tilapia. In their study, the supplementation of Trp was helpful to improve the growth performance of fish fed deficient Trp diets. Given the important function of Trp and the potential deficiency of this IAA in our ingredient matrix for Nile tilapia, the objective of this study was to confirm and refine the tryptophan requirement to further optimize practical diets with respect to IAA.

## 2. Materials and Method

### *2.1. Experimental design and diets*

Twelve diets were formulated to determine Trp requirement for tilapia (Table 1). As a reference, diet 30AAR, was included as it showed the best performance in previous trial identifying limiting IAAs. It contained 30% protein in which 29.69% derived from intact protein and 0.31% crystalline AA (lysine, threonine and valine). To this diet, intact protein ingredients (fish meal, soybean meal, corn protein concentrate) were reduced by 50% with the addition of 7.5% gelatin to produce a basal diet, deficient in Trp (0.21%), but meeting all other known nutrient requirements of the Nile tilapia (NRC, 2011). L-tryptophan was then added to the ten remaining diets at 0.04% increment to produce levels ranging from 0.21 to 0.61% of the diet. The DAA premix was included in the diets to make sure the feed has sufficient AA as nitrogen source for protein synthesis of Nile tilapia. The DAA were used with ratios built up to simulate the AA content of a 28% whole egg protein (Nguyen and Davis, 2009). Aspartic acid was used as a standard and other DAA were calculated accordingly.

The test diets were prepared in the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn, AL, USA). Pre-ground feed ingredients and oil were placed in a food mixer (Hobart Corporation, Troy, Ohio, USA) for 15 minutes. Cold water was used instead of hot water to reduce gelling of feed due to the high level of gelatin in these diets. Diets were then extruded through a 4-mm diameter die in a meat grinder, air dried at < 50° C to a moisture less than 11%, and stored in the freezer at -20° C until used. A sample of each feed was collected and analyzed for proximate composition by Midwest

Laboratories (Omaha, NE, USA) and AA compositions by Ajinomoto Heartland Inc., (Chicago, IL, USA) (Table 2).

**Table 1** Ingredient compositions (g 100 $g^{-1}$ as-is) of twelve experimental diets with increasing levels of tryptophan offered to juvenile Nile tilapia (7.4 ± 0.1 g) over an 8 week period**.**

| Composition | Referenc | Basa | D 1 | D 2 | D 3 | D 4 | D 5 | D 6 | D 7 | D 8 | D 9 | D 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fishmeal[a] | 5.40 | 2.70 | 2.70 | 2.70 | 2.70 | 2.70 | 2.70 | 2.70 | 2.70 | 2.70 | 2.70 | 2.70 |
| Soybean meal[b] | 38.32 | 19.1 | 19.16 | 19.16 | 19.16 | 19.16 | 19.16 | 19.16 | 19.16 | 19.16 | 19.16 | 19.16 |
| CPC[c] | 6.28 | 3.14 | 3.14 | 3.14 | 3.14 | 3.14 | 3.14 | 3.14 | 3.14 | 3.14 | 3.14 | 3.14 |
| Gelatin[d] | | 7.40 | 7.40 | 7.40 | 7.40 | 7.40 | 7.40 | 7.40 | 7.40 | 7.40 | 7.40 | 7.40 |
| Fish oil[e] | 5.53 | 6.31 | 6.31 | 6.31 | 6.31 | 6.31 | 6.31 | 6.31 | 6.31 | 6.31 | 6.31 | 6.31 |
| Lecithin[f] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Corn Starch[g] | 14.86 | 24.8 | 24.80 | 24.80 | 24.80 | 24.80 | 24.80 | 24.80 | 24.80 | 24.80 | 24.80 | 24.80 |
| Whole wheat[h] | 25.00 | 25.0 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| Mineral | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 |
| Choline | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Stay C[l] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| CaP-dibasic[m] | 1.70 | 2.64 | 2.64 | 2.64 | 2.64 | 2.64 | 2.64 | 2.64 | 2.64 | 2.64 | 2.64 | 2.64 |
| Histidine[g] | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Isoleucine[g] | | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 |
| Leucine[g] | | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 |
| Lysine 78.8%[n] | 0.19 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 |
| Methionine[n] | | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 |
| Phenylalanine[g] | | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |
| Threonine[n] | 0.04 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 | 0.39 |
| Tryptophan[n] | | | 0.04 | 0.08 | 0.12 | 0.16 | 0.20 | 0.24 | 0.28 | 0.32 | 0.36 | 0.40 |
| Valine[n] | 0.07 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 |
| Alanine[g] | | 0.47 | 0.47 | 0.46 | 0.46 | 0.45 | 0.45 | 0.44 | 0.44 | 0.43 | 0.43 | 0.42 |
| Aspartic Acid[g] | | 0.85 | 0.85 | 0.84 | 0.83 | 0.82 | 0.81 | 0.80 | 0.79 | 0.78 | 0.77 | 0.76 |
| Cysteine[g] | 0.01 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 | 0.19 |
| Glutamic Acid[g] | | 1.28 | 1.27 | 1.25 | 1.24 | 1.23 | 1.21 | 1.20 | 1.18 | 1.17 | 1.16 | 1.14 |
| Glycine[g] | | 0.30 | 0.29 | 0.29 | 0.29 | 0.28 | 0.28 | 0.28 | 0.27 | 0.27 | 0.27 | 0.26 |
| Proline[g] | | 0.14 | 0.14 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.12 | 0.12 | 0.12 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Serine[g] | 0.63 | 0.62 | 0.62 | 0.61 | 0.60 | 0.59 | 0.59 | 0.58 | 0.57 | 0.57 | 0.56 |

[a] Omega Protein Inc., Houston, Texas, USA
[b] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA
[c] Empyreal 75®, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA
[d] My spice sage®, Yonkers, NY, USA
[e] Omega Protein Inc., Reedville, VA, USA.
[f] The Solae Company, St. Louis, MO, USA.
[g] MP Biochemicals Inc., Solon, OH, USA.
[h] Bob's Red Mill Natural Foods, Milwaukie, OR, USA.
[i] Trace mineral (g/100g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.250; Furrous sulfate, 4.000; Magnesium sulfate anhydrous, 13.862; Manganous sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate hepahydrate, 13.193; Alpha-cellulose, 67.964.
[j] Vitamin (g/kg premix): Thiamin HCl, 0.44; Riboflavin, 0.63; Pyridoxine HCl, 0.91; DL pantothenic acid, 1.72; Nicotinic acid, 4.58; Biotin, 0.21; Folic acid, 0.55; Inositol, 21.05; Menadione sodium bisulfite, 0.89; Vitamin A acetate, 0.68; Vitamin $D_3$, 0.12; dL-alpha-tocoperol acetate, 12.63; Alpha-cellulose, 955.59.
[k] Amresco Inc., Solon, Ohio, USA.
[l] Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products., Parsippany, NJ, USA.
[m] Alfa Aesar, Ward Hill, MA, USA.
[n] Ajinomoto Heartland Inc, Chicago, IL, USA.

**Table 2** Analyzed proximate composition[a] and amino acid profile[b] of the experimental diets (g 100 $g^{-1}$ as-is) fed to tilapia (7.4 ± 0.1 g) over an eight week period.

| Composition | Reference | Basal | D 1 | D 2 | D 3 | D 4 | D 5 | D 6 | D 7 | D 8 | D 9 | D 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Crude Protein | 29.80 | 29.80 | 29.70 | 30.20 | 30.30 | 30.00 | 29.20 | 29.50 | 29.60 | 29.80 | 28.90 | 29.30 |
| Moisture | 10.04 | 10.26 | 7.84 | 7.74 | 10.38 | 8.12 | 11.72 | 11.17 | 9.25 | 8.68 | 8.90 | 8.23 |
| Crude Fat | 7.89 | 7.97 | 8.49 | 8.16 | 8.03 | 8.72 | 7.90 | 8.42 | 7.95 | 8.58 | 8.04 | 8.24 |
| Crude Fiber | 4.61 | 3.30 | 3.43 | 3.14 | 3.07 | 3.21 | 2.34 | 2.36 | 2.71 | 2.75 | 2.56 | 2.94 |
| Ash | 5.15 | 4.03 | 4.40 | 4.15 | 4.04 | 4.33 | 4.11 | 4.14 | 4.16 | 4.09 | 4.08 | 4.06 |
| Alanine | 1.56 | 1.97 | 2.02 | 2.03 | 1.98 | 1.99 | 1.93 | 1.92 | 1.91 | 1.89 | 1.86 | 1.89 |
| Arginine | 1.77 | 1.53 | 1.59 | 1.58 | 1.54 | 1.62 | 1.53 | 1.50 | 1.51 | 1.47 | 1.46 | 1.51 |
| Aspartic Acid | 2.73 | 2.71 | 2.80 | 2.76 | 2.64 | 2.70 | 2.63 | 2.58 | 2.59 | 2.56 | 2.52 | 2.63 |
| Cysteine | 0.47 | 0.48 | 0.48 | 0.48 | 0.46 | 0.47 | 0.44 | 0.42 | 0.42 | 0.43 | 0.42 | 0.43 |
| Glutamic Acid | 5.89 | 5.53 | 5.62 | 5.65 | 5.41 | 5.53 | 5.33 | 5.25 | 5.29 | 5.27 | 5.15 | 5.28 |
| Glycine | 1.30 | 2.74 | 2.84 | 2.76 | 2.84 | 2.81 | 2.68 | 2.71 | 2.71 | 2.61 | 2.63 | 2.65 |
| Histidine | 0.77 | 0.53 | 0.54 | 0.55 | 0.47 | 0.49 | 0.48 | 0.45 | 0.46 | 0.46 | 0.46 | 0.48 |
| Isoleucine | 1.24 | 0.95 | 1.06 | 1.00 | 1.02 | 0.99 | 0.98 | 0.99 | 0.97 | 1.01 | 0.99 | 0.97 |
| Leucine | 2.65 | 1.94 | 1.97 | 1.99 | 1.94 | 1.98 | 1.94 | 1.91 | 1.91 | 1.91 | 1.89 | 1.93 |
| Lysine | 1.88 | 1.73 | 1.78 | 1.77 | 1.74 | 1.76 | 1.71 | 1.74 | 1.76 | 1.75 | 1.75 | 1.73 |
| Methionine | 0.51 | 0.49 | 0.51 | 0.51 | 0.49 | 0.50 | 0.48 | 0.49 | 0.49 | 0.49 | 0.49 | 0.50 |
| Phenylalanine | 1.58 | 1.12 | 1.16 | 1.16 | 1.08 | 1.10 | 1.09 | 1.05 | 1.04 | 1.03 | 1.01 | 1.04 |
| Proline | 1.97 | 2.35 | 2.42 | 2.43 | 2.37 | 2.40 | 2.28 | 2.28 | 2.28 | 2.27 | 2.25 | 2.21 |
| Serine | 1.45 | 1.61 | 1.68 | 1.66 | 1.63 | 1.63 | 1.58 | 1.54 | 1.54 | 1.53 | 1.51 | 1.58 |
| Threonine | 1.11 | 1.10 | 1.11 | 1.12 | 1.07 | 1.08 | 1.09 | 1.05 | 1.05 | 1.04 | 1.03 | 1.07 |
| Tryptophan | 0.33 | 0.19 | 0.23 | 0.25 | 0.31 | 0.33 | 0.38 | 0.42 | 0.45 | 0.51 | 0.53 | 0.55 |
| Tyrosine | 0.88 | 0.55 | 0.55 | 0.55 | 0.53 | 0.56 | 0.54 | 0.53 | 0.53 | 0.52 | 0.50 | 0.53 |
| Valine | 1.40 | 1.47 | 1.51 | 1.50 | 1.47 | 1.48 | 1.46 | 1.43 | 1.45 | 1.45 | 1.43 | 1.45 |

[a] Diets were analyzed at Midwest Laboratories (Omaha, NE, USA).

[b] Diets were analyzed at Ajinomoto Heartland Inc, (Chicago, IL, USA).

*2.2 Culture methods*

The trial was conducted at the E.W. Shell Fisheries Center, Auburn, Alabama. Nile tilapia fry spawned at this center were stocked in the nursery tank and maintained on a commercial diet until the beginning of the trial. After acclimating, juvenile Nile tilapia (7.4 ± 0.1 g) were randomly stocked at 20 fish per aquarium into thirty six rectangular 50-L aquaria, which are part of a 3,800-L indoor recirculation system. Each of the twelve treatments were randomly assigned to three replicate groups of juvenile Nile tilapia in a recirculation system for eight weeks. Samples of fish from the initial stocking were retained for later biochemical analysis. Water temperature was maintained at around 28˚C using a submerged 3,600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA) and dissolved oxygen was maintained near saturation using air blower. Dissolved oxygen (DO) and water temperature were measured twice per day using YSI 650 multi-parameter instrument (YSI, Yellow Springs, Ohio) while pH, total ammonia nitrogen (TAN) and nitrite-nitrogen were measured twice per week. Photoperiod was set at 14 h light and 10 h dark. During the experimental period, DO, temperature, salinity, pH, TAN, and nitrite were maintained within acceptable ranges for tilapia at 5.92 ± 0.68 mg/L, 27.62 ± 0.79°C, 1.47 ± 1.27 ppt, 6.90 ± 0.44, 0.26 ± 0.04 mg/L, 0.18 ± 0.33 mg/L respectively.

Diets were offered to fish at 6-5% body weight daily according to fish size. Test diets were applied four times daily at 08:00, 11:00, 13:00, and 16:00 h for an eight-week growth period. Fish were weighed every two weeks. Daily feed rations were adjusted each week based on growth and observation of the feeding response. At the end of the growth trial, fish were counted and group weighed to determine weight gain, survival, and feed conversion ratio. Four fish per aquarium were randomly sampled after being euthanized with tricaine methanesulphonate to

measure condition index including individual weight and length, hepatosomatic index (HSI), intraperitoneal fat (IPF) ratio. These whole-body fish samples were frozen at -20$^{o}$C and then were ground homogenously before a subsample was sent to Midwest Laboratories (Omaha, NE, USA) for dry matter, crude protein, crude lipid, and ash analyses. The left over whole-body fish samples were freeze dried and sent to Ajinomoto Heartland Inc., (Chicago, IL, USA), for AA analysis. Subsequently, thermal-unit growth coefficient (TGC), apparent net protein retention (ANPR), feed efficiency ratio (FE), tryptophan deposition (TD), tryptophan deposition efficiency (TDE), condition factor (K), hepatosomatic index (HSI) and intraperitoneal fat (IPF) were determined using the following calculations:

a) Thermal-unit growth coefficient (TGC) = (final weigh$^{1/3}$ - initial weight$^{1/3}$) / (temperature × day) × 100.
b) Apparent net protein retention (ANPR, %) = (final weight × final protein content) - (initial weight × initial protein content) × 100 / protein intake.
c) Feed efficiency ratio (FE) = weight gain/ dry feed intake.
d) Tryptophan deposition (TD, g) = $Trp_{final}$ × final weight − $Trp_{initial}$ × initial weight.
e) Tryptophan deposition efficiency (TDE, %) = ($Trp_{final}$ × final weight − $Trp_{initial}$ × initial weight) × 100 / Trp intake.
f) Condition factor (K, 100 × g/cm$^3$) = 100 × individual body weight / (individual body length$^3$).
g) Hepatosomatic index (HSI, %) = (liver weight/fish weight) × 100.
h) Intraperitoneal fat (IPF ratio, %) = (Intraperitoneal fat weight/fish weight) × 100.

*2.3. Statistical Analysis*

Average initial weight (IW), final body weight (FW), thermal-unit growth coefficient (TGC), survival, apparent net protein retention (ANPR), Trp deposition (TD), Trp depositition efficiency (TDE) were analyzed by one-way ANOVA, followed by Tukey's multiple comparison test when applicable ($\alpha = 0.05$). TGC, ANPR, FE, TD were fitted against analyzed dietary Trp to estimate the Trp requirement using saturation kinetic model (SKM) (a), broken line models with linear (BLM) (b) or quadratic ascending portions (BQM) (c). BLM defines the requirement as the abscissa of the breaking point between a linear ascending portion of the response line and a linear portion (plateau) while breaking point between quadratic ascending portion of the response curve and a linear portion (plateau) used for BQM. SKM is a nonlinear, continuous model in which the requirement is defined as the level resulting in 95% of the maximum observed response (Mercer et al. 1989; Hernandez-Llamas 2009). Confidence intervals (CIs) were determined at the 95% level using the SAS *nlin* procedure for the BLM and BQM and by bootstrapping for SKM (Efron and Tibshirani 1998; Hansen et al. 1999). The residual sums of square, the second-order Akaike information criterion (AICc), Akaike weights were calculated and used for model selection in addition to the model's overall $R^2$ (Anderson et al., 2000; Arnold, 2010; Salze et al., 2017).

$$Y = \frac{i*k+Y_{max}*X^n}{k+X^n} \text{ (a)}$$

$$Y = L + U * (R - X) \text{ (b)}$$

$$Y = L + U * (R - X)^2 \text{ (c)}$$

Where *Y* is the measured response (TGC, FE, ANPR, TD), X is the dependent variable (dietary Trp), *i* is the intercept, *k* is a coefficient, *n* is the kinetic order, L is the ordinate, R is the abscissa of the breakpoint, U and V are the slopes for the line.

## 3. Results

Over the 8-week trial, fish growth ranged from 829 to 983% of initial body weight. Positive growth response of Nile tilapia was obtained in accordance with the increase of dietary Trp in which lowest TGC values were observed in fish fed the reference diet with 0.19% Trp (0.141) and fish fed the reference diet containing 0.33% had highest TGC (0.152) (Table 3). Fish fed diets with Trp supplement above 0.25% had significantly higher mean TGC ($P < 0.05$) than values reported for the other diets with added Trp below 0.25%. Even though fish fed reference diet contained 0.33% Trp had the highest mean FW, TGC and lowest FCR, no significant differences among fish in this group and those fed diets with Trp supplemented above 0.25% were observed. The same pattern was found when analyzing the mean FCR, ANPR, TD and TDE deposition efficiency, ranging from 1.04 to 1.19, 43.27 to 49.74%, 0.082 to 0.106, 22.76 to 49.26%, respectively.

Whole body moisture (69.47 - 74.04%), crude protein (13.13 - 15.30%), lipids (8.86 - 11.09%), ash (2.91 - 4.23%), IPF (1.94 - 2.74%) and HSI (1.99 - 2.39%) contents of tilapia fed different dietary Trp levels are summarized in Table 4. These variables were not significantly influenced ($P < 0.05$) by various dietary Trp supplements. The same pattern was observed on whole-body essential AA values, which are summarized in Table 5.

The fitting results of the BLM, BQM and SKM to the growth rate (TGC), ANPR, FE and TD against dietary Trp are presented in Table 6. The residual sums of square, the second-order Akaike information criterion (AICc), Akaike weights, are $R^2$ reflected that BLM was the best model for fitting TGC. While $R^2$ values (0.88) were very close among the three models, the BLM had highest Akaike weight (0.541) and lowest total SSE (0.000088) compared to SKM which Akaike weight (0.441) and total SSE (0.000092). BQM had smallest Akaike weight (0.018) and

**Table 3** Mean response of Nile tilapia (7.4 ± 0.1 g) fed diets with increasing levels of tryptophan over an eight-week period.

| Diet | Initial weight | Final weight | Thermal-unit growth coefficient | Feed conversion ratio | Feed intake | Apparent net protein retention | Tryptophan deposition | Tryptophan deposition efficiency | Survival |
|---|---|---|---|---|---|---|---|---|---|
| Reference | 7.49 | 79.52$^{a}$ | 0.152$^{a}$ | 1.08$^{b}$ | 77.78 | 48.61$^{a}$ | 0.106$^{a}$ | 38.07$^{b}$ | 100.00 |
| Basal | 7.45 | 70.34$^{c}$ | 0.141$^{c}$ | 1.17$^{a}$ | 73.83 | 43.27$^{b}$ | 0.082$^{b}$ | 48.54$^{a}$ | 100.00 |
| D 1 | 7.42 | 71.65$^{bc}$ | 0.142$^{c}$ | 1.19$^{a}$ | 76.35 | 43.68$^{b}$ | 0.092$^{ab}$ | 49.26$^{a}$ | 100.00 |
| D 2 | 7.39 | 73.74$^{b}$ | 0.145$^{bc}$ | 1.09$^{b}$ | 72.28 | 46.30$^{ab}$ | 0.090$^{ab}$ | 45.78$^{a}$ | 98.33 |
| D 3 | 7.40 | 77.53$^{a}$ | 0.150$^{ab}$ | 1.04$^{b}$ | 72.90 | 49.55$^{a}$ | 0.101$^{a}$ | 41.47$^{b}$ | 100.00 |
| D 4 | 7.39 | 78.04$^{a}$ | 0.150$^{ab}$ | 1.06$^{b}$ | 75.11 | 48.30$^{a}$ | 0.106$^{a}$ | 39.93$^{b}$ | 100.00 |
| D 5 | 7.42 | 77.65$^{a}$ | 0.150$^{ab}$ | 1.06$^{b}$ | 74.47 | 49.68$^{a}$ | 0.101$^{a}$ | 32.53$^{c}$ | 100.00 |
| D 6 | 7.42 | 77.46$^{a}$ | 0.150$^{ab}$ | 1.06$^{b}$ | 74.55 | 49.47$^{a}$ | 0.098$^{ab}$ | 28.30$^{d}$ | 100.00 |
| D 7 | 7.54 | 77.21$^{a}$ | 0.149$^{ab}$ | 1.07$^{b}$ | 74.72 | 49.20$^{a}$ | 0.104$^{a}$ | 28.28$^{d}$ | 100.00 |
| D 8 | 7.44 | 77.44$^{a}$ | 0.149$^{ab}$ | 1.06$^{b}$ | 74.20 | 48.39$^{a}$ | 0.104$^{a}$ | 25.66$^{de}$ | 100.00 |
| D 9 | 7.38 | 77.47$^{a}$ | 0.150$^{ab}$ | 1.06$^{b}$ | 74.27 | 49.07$^{a}$ | 0.097$^{ab}$ | 22.95$^{e}$ | 100.00 |
| D10 | 7.51 | 77.58$^{a}$ | 0.149$^{ab}$ | 1.05$^{b}$ | 73.88 | 49.74$^{a}$ | 0.100$^{a}$ | 22.76$^{e}$ | 100.00 |
| *P*-value | 0.6866 | <0.0001 | <0.0001 | <0.0001 | 0.5625 | <0.0001 | <0.0001 | <0.0001 | 0.5096 |
| PSE$^{a}$ | 0.0517 | 0.6878 | 0.0009 | 0.016 | 2.1268 | 1.0093 | 0.0029 | 0.9545 | 0.4256 |

Means (n = 3) in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by Tukey's multiple range test.

[a] PSE: Pooled standard error.

highest total SSE (0.000102), which indicated that this model did not fit well to the TGC data. Based on BLM, Trp requirement of juvenile Nile tilapia was 0.31% (0.25 - 0.37%) of the diet for optimum TGC (Fig. 1). The same patterns were observed on TD of fish in which BLM fitted the best for the response of Nile tilapia against dietary Trp with the highest $R^2$ values (0.76) and Akaike weight (0.833) and lowest total SSE (0.001170). SKM, BQM did not fit well with the data with very low Akaike weight at 0.167 and 0, respectively. BLM indicated the Trp requirement at 0.33% (0.26 - 0.39%) for optimum Trp deposition (Fig. 4). SKM was the best model fitted to FE and ANPR of fish in which the highest $R^2$ values and Akaike weight and smallest total SSE were obtained using this model. Dietary Trp requirement is 0.25% (0.24 - 0.25%) and 0.27% (0.25 - 0.31%) for optimum FE and ANPR (95% of maximum value), respectively (Fig. 2 and Fig.3).

**Table 4** Body condition indexes and whole-body composition[a] (g 100 $g^{-1}$ as-is) of juvenile Nile tilapia (7.4 ± 0.1 g) fed diets with increasing levels of tryptophan over an eight week period.

| Composition | Reference | Basal | D 1 | D 2 | D 3 | D 4 | D 5 | D 6 | D 7 | D 8 | D 9 | D 10 | *P*-value | PSE[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Crude protein | 14.73 | 14.07 | 13.50 | 13.13 | 14.37 | 15.47 | 14.50 | 14.70 | 14.33 | 13.70 | 14.57 | 15.30 | 0.1313 | 0.4549 |
| Moisture | 70.97 | 72.10 | 72.70 | 71.70 | 70.60 | 70.33 | 72.37 | 71.77 | 73.40 | 72.83 | 72.57 | 69.47 | 0.5946 | 1.1003 |
| Lipids | 10.05 | 10.73 | 9.29 | 9.07 | 8.86 | 11.09 | 8.26 | 10.20 | 9.58 | 10.05 | 9.17 | 9.77 | 0.0597 | 0.4787 |
| Ash | 4.09 | 3.64 | 3.70 | 3.35 | 3.85 | 3.30 | 3.58 | 3.61 | 2.99 | 2.90 | 3.28 | 4.23 | 0.8127 | 0.4517 |
| K | 2.25 | 2.34 | 2.36 | 2.28 | 2.32 | 2.43 | 2.25 | 2.16 | 2.19 | 2.14 | 2.27 | 2.24 | 0.8146 | 0.2146 |
| IPF | 2.18 | 2.56 | 2.68 | 2.31 | 2.38 | 1.94 | 2.55 | 2.32 | 2.22 | 2.00 | 2.24 | 2.74 | 0.6034 | 0.4471 |
| HSI | 2.35 | 2.34 | 2.39 | 2.22 | 2.34 | 2.32 | 2.24 | 2.29 | 2.15 | 3.29 | 2.00 | 2.15 | 0.6735 | 0.63801 |

Means (n = 3) in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by Tukey's multiple range test.

[a] Diets were analyzed at Midwest Laboratories (Omaha, NE, USA).

[b] PSE: Pooled standard error.

**Table 5** Amino acid (AA) composition[a] (g 100 $g^{-1}$ as-is) of juvenile Nile tilapia (7.4 ± 0.1 g) fed diets with increasing levels of tryptophan for an eight week period.

| Composition | Reference | Basal | D 1 | D 2 | D 3 | D 4 | D 5 | D 6 | D 7 | D 8 | D 9 | D 10 | *P*-value | PSE[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Indispensable AA | | | | | | | | | | | | | | |
| Arginine | 0.96 | 0.92 | 0.93 | 0.93 | 0.94 | 0.95 | 0.95 | 0.95 | 0.95 | 0.94 | 0.91 | 0.94 | 0.1836 | 0.0190 |
| Histidine | 0.35 | 0.32 | 0.33 | 0.32 | 0.32 | 0.33 | 0.32 | 0.31 | 0.33 | 0.33 | 0.32 | 0.31 | 0.0610 | 0.0074 |
| Isoleucine | 0.59 | 0.58 | 0.58 | 0.58 | 0.58 | 0.59 | 0.57 | 0.57 | 0.59 | 0.59 | 0.58 | 0.56 | 0.4241 | 0.0121 |
| Leucine | 1.06 | 1.04 | 1.05 | 1.04 | 1.05 | 1.07 | 1.04 | 1.03 | 1.08 | 1.05 | 1.04 | 1.02 | 0.2011 | 0.0187 |
| Lysine | 1.11 | 1.07 | 1.09 | 1.08 | 1.08 | 1.10 | 1.08 | 1.05 | 1.10 | 1.09 | 1.07 | 1.04 | 0.2751 | 0.0228 |
| Methionine | 0.38 | 0.36 | 0.37 | 0.37 | 0.37 | 0.38 | 0.37 | 0.36 | 0.38 | 0.37 | 0.37 | 0.36 | 0.1512 | 0.0065 |
| Phenylalanine | 0.60 | 0.58 | 0.59 | 0.59 | 0.60 | 0.60 | 0.59 | 0.59 | 0.60 | 0.60 | 0.58 | 0.58 | 0.2239 | 0.0107 |
| Threonine | 0.63 | 0.61 | 0.62 | 0.62 | 0.62 | 0.63 | 0.62 | 0.62 | 0.64 | 0.62 | 0.61 | 0.61 | 0.0518 | 0.0098 |
| Tryptophan | 0.14 | 0.13 | 0.15 | 0.14 | 0.14 | 0.15 | 0.14 | 0.13 | 0.15 | 0.15 | 0.14 | 0.14 | 0.3997 | 0.0131 |
| Valine | 0.66 | 0.65 | 0.66 | 0.65 | 0.65 | 0.66 | 0.64 | 0.65 | 0.67 | 0.65 | 0.65 | 0.63 | 0.0561 | 0.0032 |
| Dispensable AA | | | | | | | | | | | | | | |
| Alanine | 1.00 | 0.95 | 0.95 | 0.96 | 0.97 | 0.98 | 0.97 | 0.99 | 0.99 | 0.97 | 0.94 | 0.98 | 0.1013 | 0.0197 |
| Aspartic Acid | 1.40 | 1.34 | 1.37 | 1.36 | 1.37 | 1.39 | 1.36 | 1.35 | 1.40 | 1.38 | 1.34 | 1.34 | 0.0727 | 0.0228 |
| Cysteine | 0.14 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.12 | 0.13 | 0.13 | 0.13 | 0.13 | 0.3370 | 0.0039 |
| Glutamic Acid | 1.99 | 1.94 | 1.97 | 1.95 | 1.97 | 2.01 | 1.96 | 1.95 | 2.02 | 1.98 | 1.93 | 1.93 | 0.0953 | 0.0326 |
| Glycine | 1.26 | 1.16 | 1.17 | 1.20 | 1.22 | 1.24 | 1.23 | 1.29 | 1.23 | 1.20 | 1.16 | 1.30 | 0.2727 | 0.0458 |
| Proline | 0.81 | 0.77 | 0.76 | 0.77 | 0.79 | 0.78 | 0.79 | 0.82 | 0.79 | 0.77 | 0.73 | 0.82 | 0.2608 | 0.0254 |
| Serine | 0.62 | 0.63 | 0.63 | 0.64 | 0.64 | 0.65 | 0.63 | 0.64 | 0.65 | 0.62 | 0.62 | 0.63 | 0.0741 | 0.0101 |
| Tyrosine | 0.35 | 0.34 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.34 | 0.36 | 0.35 | 0.35 | 0.34 | 0.0642 | 0.0063 |

Means (n = 3) in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by Tukey's multiple range test.

[a] Diets were analyzed at Ajinomoto Heartland Inc, (Chicago, IL, USA).

[b] PSE: Pooled standard error.

## 4. Discussion

Trp and other IAAs play a central role for body protein synthesis and cellular growth. Deficiency in Trp can affect the utilization of other IAAs, inducing poor protein utilization and growth of fish. As Trp cannot be synthesized by fish, it has to be supplied through their diets in adequate amounts to cover their requirements. Concurrently, Trp can be limited in selected alternative ingredients of fish meal. According to Davis (2015), it is the first limiting AA in high fat meat and bone meal, second limiting AA in maize gluten meal, blood and poultry offal meal, and faba bean (*Vicia faba*) and third limiting AA in lupin (*Lupinus angustifolius*) and feather meal. Therefore, efforts to replace fish meal with other alternative ingredients have a possible risk of creating a Trp deficient diet.

Supplementing a deficient diet with crystalline Trp has been documented to improve growth performance of African catfish *Clarias gariepinus* (Fagbenro and Nwanna, 1999), hybrid striped bass *Morone chrysops* × *M. saxatilis* (Gaylord et al., 2005), rohu *Labeo rohita* (Abidi and Khan, 2010) and Pacific white shrimp *Litopenaeus vannamei* (Sun et al., 2015; Yin et al., 2017). In this study, supplementation of Trp in the diets supported the growth performance of Nile tilapia. TGC of Nile tilapia responded to different levels of Trp supplementation and showed significant improvement compared to the reference diet without added Trp. These results are in agreement with the findings of Santiago and Lovell (1988) who reported that growth rates of Nile tilapia were correlated with dietary Trp levels up to the requirement. In their study, seven graded levels of Trp from 0.05 to 0.45% were fed to 56 g Nile tilapia for eight weeks and added benefit of Trp was observed in fish fed supplemented Trp diets from 0.05 to 0.25% with increasing percent weight

gain from 1843 ± 46% to 2409 ± 50%. Similarly, Zaminhan et al., (2017) indicated that body weight gain of Nile tilapia (38.2 ± 0.09 g) increased from 71.4 g to 91.4 g when they were fed

**Table 6** Quality of fit of the models used to calculate requirement estimates[a]

| | | Estimated requirement | AICc[b] | Akaike weight | 95% CI[c] | $R^2$ | Total SSE[d] |
|---|---|---|---|---|---|---|---|
| Thermal-unit growth coefficient | SKM[e] | 0.25 | -427.52 | 0.441 | 0.23-0.28 | 0.88 | 0.000092 |
| | BQM[f] | 0.32 | -421.09 | 0.018 | 0.29-0.41 | 0.86 | 0.000102 |
| | BLM[g] | **0.31** | **-427.94** | **0.541** | **0.25-0.37** | **0.88** | **0.000088** |
| Feed efficiency | SKM[e] | **0.25** | **-243.45** | **0.975** | **0.24-0.25** | **0.76** | **0.012800** |
| | BQM[f] | 0.32 | -232.34 | 0.004 | 0.29-0.41 | 0.66 | 0.002030 |
| | BLM[g] | 0.31 | -235.84 | 0.021 | 0.25-0.37 | 0.70 | 0.018500 |
| Apparent net protein retention | SKM[e] | **0.27** | **6.93** | **0.896** | **0.25-0.31** | **0.87** | **83.31840** |
| | BQM[f] | 0.38 | 16.98 | 0.006 | 0.31-0.47 | 0.82 | 97.23530 |
| | BLM[g] | 0.31 | 11.37 | 0.098 | 0.28-0.35 | 0.85 | 89.67000 |
| Tryptophan deposition | SKM[e] | 0.27 | -348.48 | 0.167 | 0.25-0.30 | 0.73 | 0.001220 |
| | BQM[f] | 0.36 | -351.69 | 0.000 | 0.25-0.46 | 0.57 | 0.001200 |
| | BLM[g] | **0.33** | **-351.69** | **0.833** | **0.26-0.39** | **0.76** | **0.001170** |

[a] Bold font indicates the best fitting and/or more parsimonious model selected for data interpretation.
[b] Second-order Akaike information criterion.
[c] Confidence interval.
[d] Sum of square error.
[e] Saturation kinetic model.
[f] Broken line models with quadratic ascending portions.
[g] Broken line models with linear ascending portions

incremental levels of Trp from 0.18 to 0.27% of the diet. Farhat and Khan (2014) demonstrated that reduced weight gain of fish fed Trp deficient diets could result from a reduction in feed intake. In his study, sting catfish *Heteropneustes fossilis* fed deficient Trp diet at 0.1 and 0.15% Trp had significantly lower feed intake compared to fish fed diets with sufficient Trp levels at 0.24% to 0.27% of the diet. Ahmed (2012) also observed an increase in feed intake of Indian catfish fed increasing Trp levels up to requirement of fish. However, in our study and another study conducted on Nile tilapia (Zaminhan et al., 2017), no significant difference ($P > 0.05$) was observed in feed intake of fish fed Trp deficient diets and the other diets. As the inclusion levels of Trp varied among diets, daily Trp intake increased in fish fed higher Trp diets. Thus, dietary Trp was the only difference in daily intake, indicating that effects on growth performance were not a reflection of food palatability but associated to dietary Trp levels. Hoglund et al., (2007) illustrated that the effect of dietary manipulations of Trp on feeding behavior is dependent on the stress levels of experimental animals in which feeding behavior affected disturbed fish only while the undisturbed fish did not seem to be affected by dietary Trp levels.

Our data showed that the supplementation of increasing Trp levels up to 0.55% of the diet did not depress growth performance and feed efficiency of Nile tilapia. Nevertheless, Santiago and Lovell (1988) indicated that excess of Trp above 0.25% induced poor weight gain of fish which was also more recently reported by Zaminhan et al., (2017) with excessive Trp at or above 0.27% of the diet. In their studies, reduced growth of fish resulted from reduced feed intake in which fish fed diets with Trp supplemented at or above 0.27% had lower feed intake compared to fish fed diets with Trp supplemented below this level even though no significant difference in feed intake was obtained among treatments. In the present study, increasing dietary Trp levels did not appear

to affect the feed intake of Nile tilapia. No significant differences were observed in fish fed various dietary Trp levels. The same pattern was observed by Fagbenro and Nwanna (1999) on African catfish *Clarias gariepinus* that were fed graded increments of Trp (0.12 - 0.52%) and expected linear increase of mean weight gain as dietary Trp increased up to 0.44% and plateaued after that. Excessive amounts of Trp also had no effect on weight gain of red drum *Sciaenops ocellatus* (Pewitt et al., 2015) and hybrid striped bass *Morone chrysops x M. saxatilis* (Gaylord et al., 2005). Reduced feed intakes of fish fed excessive levels of dietary Trp were, however, observed by Farhat and Khan (2014) in stinging catfish, *Heteropneustes fossilis* (Bloch) in which excess of Trp at 0.35 and 0.39% of the diet caused reduced feed intake of fish compared to fish fed dietary Trp at 0.27 and 0.32% of the diet. According to Tagliamonte et al. (1973), adverse effects of excesses of dietary Trp might result from elevated brain concentrations of the neurotransmitter serotonin due to increased Trp concentrations in the blood of fish fed high inclusion levels of Trp which previous observed by Fernstrom, (1983).

Survival of fish in this study was not affected by dietary Trp levels with overall survival reported from 98 – 100%. A similar trend was observed in barramundi *Lates calcarifer* juveniles (Coloso et al., 2004) in which survival was observed at 100% for all treatments. Ahmed (2012) also observed high survival of Indian catfish, *Heteropneustes fossilis* (Bloch) at 100% in higher concentration of Trp supplemental diets except for fish fed 0.04 and 0.14% Trp diets that had 90 and 95% survival rates, respectively. In contrast, Fagbenro and Nwanna (1999) observed anorexia after 10 days of their experiment in African catfish *Clarias gariepinus* fed diets containing low levels of Trp at 0.12 and 0.20%. Consequently, higher mortality at 80 and 82%, respectively, were obtained on fish fed these two diets while fish fed higher inclusion levels of dietary Trp had

significantly higher survival above 91%. Similarly, Gaylor et al. (2005) experienced high mortality at 40% of fish fed basal diet without Trp supplemented while the surviving fish fed this diet were in poor health.

The BLM model indicated the breakpoint for TGC at 0.31% (0.25 - 0.37%) of the diet. These numbers are relatively close to the value reported by Santiago and Lovell (1998) and Zaminhan et al., (2017) at 0.28% of and 0.29%, respectively, for optimal weight gain of fish. Diogenes et al. (2016), however, indicated the relatively higher Trp requirement at 0.37% to meet the requirement of Nile tilapia. A possible reason for the variation observed in Trp requirements of Nile tilapia among these studies might obtain from different methodological approaches. In the present study and the other studies conducted by Santiago and Lovell (1988) and Zaminhan et al. (2017), the quantification of Trp requirements were based on a dose-response curve in which Trp requirements were obtained at lowest levels of Trp used to maximize weight gain and protein depositions. Even though, different graded levels of Trp were tested and different models were used for analysis, the results were closer compared to the results of Diogenes et al., (2016). To be more specific, Santiago and Lovell (1988) used broken line model to estimate the Trp requirement of Nile tilapia while Zaminhan et al. (2007) used a second-order polynomial regression analysis to fit the relationship between growth rate and protein deposition parameters against dietary Trp levels. Diogenes et al. (2016) determined the optimal Trp and other IAAs for juvenile Nile tilapia using the deletion method in which a dietary IAA profile was calculated from the changes in nitrogen retention in conjunction with reduction of individual IAA. The disadvantage of this method was mentioned in their study since the requirements of IAA for maintenance and utilization efficiency of IAA were assumed similar among different species of fish. IAA

requirements for maintenance may differ among fish (Fournier et al., 2002; Mambrini and Kaushik 1995, Mambrini and Seudre 1995; Rodehutscord et al., 1997).

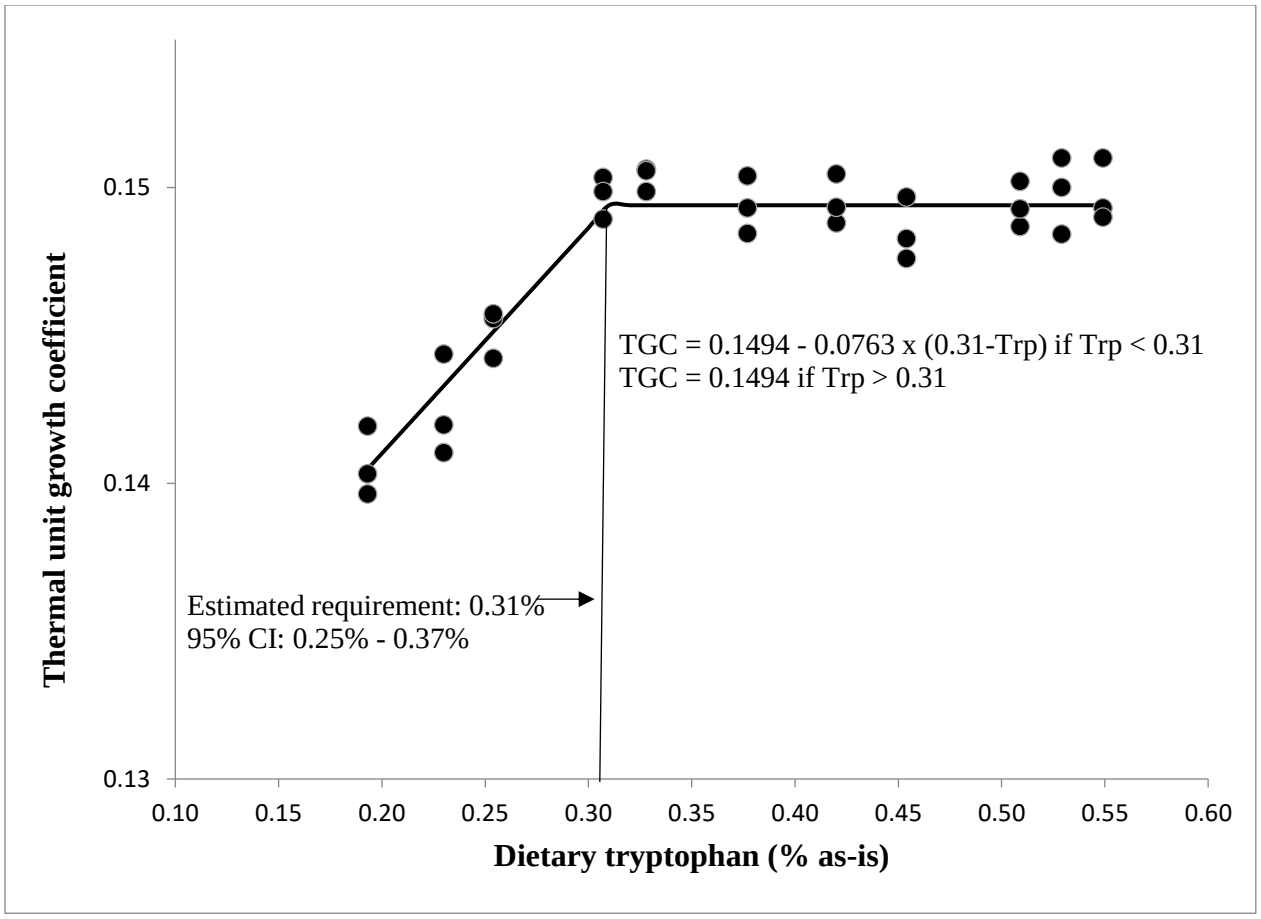


Fig. 1. Regression of thermal-unit growth coefficient against dietary Trp level of Nile tilapia (7.4 ± 0.1) after 8 weeks. Each point represents one replicate of a treatment. The solid line represents the best fit one-slope straight broken line analysis. CI: confidence interval.

Mean FE, FCR and ANPR of fish were significantly affected by various inclusion rates of Trp. Fish fed higher Trp levels exhibited higher FE, ANPR and lower FCR. Dietary Trp requirement was estimated at 0.25% (0.24 - 0.25%) and 0.27% (0.25 - 0.31%) for optimum feed efficiency and apparent net protein deposition (95% of maximum value) obtained from saturation kinetic model. These numbers are close to the value previously found by Zamihan et al., (2017) who reported Trp requirement at 0.31% for the same species of fish. In the present study, the fitted values of dietary Trp against FE and ANPR resulted in lower Trp requirement compared to Trp requirement for maximizing weight gain (0.31%) of fish. Contrary, Bureau and Encarnacao (2006) indicated that the requirement for maximizing protein gain is higher than that required to maximized weight gain.

In the present study, mean whole-body moisture, crude protein, lipid and ash content were not affected by different inclusion levels of dietary Trp. Similarly, Zaminhan et al. (2017) observed no significant differences in proximate compositions of Nile tilapia fed diets with different inclusion levels of dietary Trp. The protein (14.0 – 15.1%), lipid (10.9 – 11.9%) and ash (4.0-4.7%) of whole body fish samples in that study were relatively close to our study with values obtained at 13.13 to 15.30%, 8.86 to 11.09%, and 2.90 to 4.23%, respectively. Similarly, Pewitt et al. (2017) found that whole-body composition of *S. ocellatus* was not significantly affected by dietary Trp levels. Ahmed (2012), however, observed significant differences in whole-body composition of Indian catfish, *Heteropneustes fossilis*, fed different dietary Trp levels. In his study, body fat increased significantly ($P < 0.05$) in response to the increase in dietary Trp concentrations while low body protein content was noted in fish fed diets containing lower levels of Trp. The same pattern was observed in his previous study in Indian major carp, *Cirrhinus mrigal*a (Hamilton) (Ahmed and Khan, 2005). The impacts of different inclusion levels of Trp were not

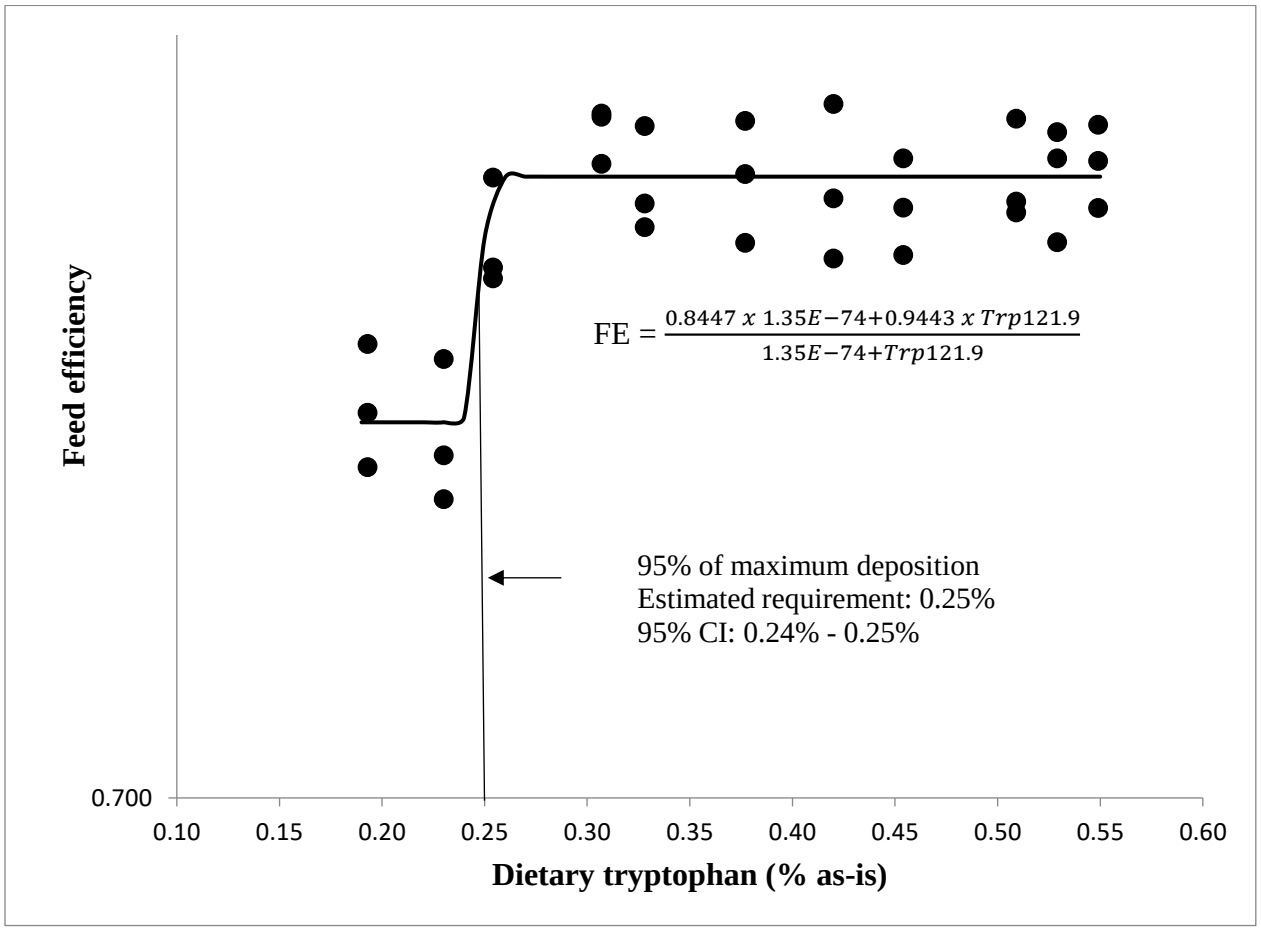


Fig. 2. Regression of feed efficiency against dietary Trp level of Nile tilapia (7.4 ± 0.1) after 8 weeks. Each point represents one replicate of a treatment. The solid curve represents the best fit saturation kinetic model. CI: confidence interval.

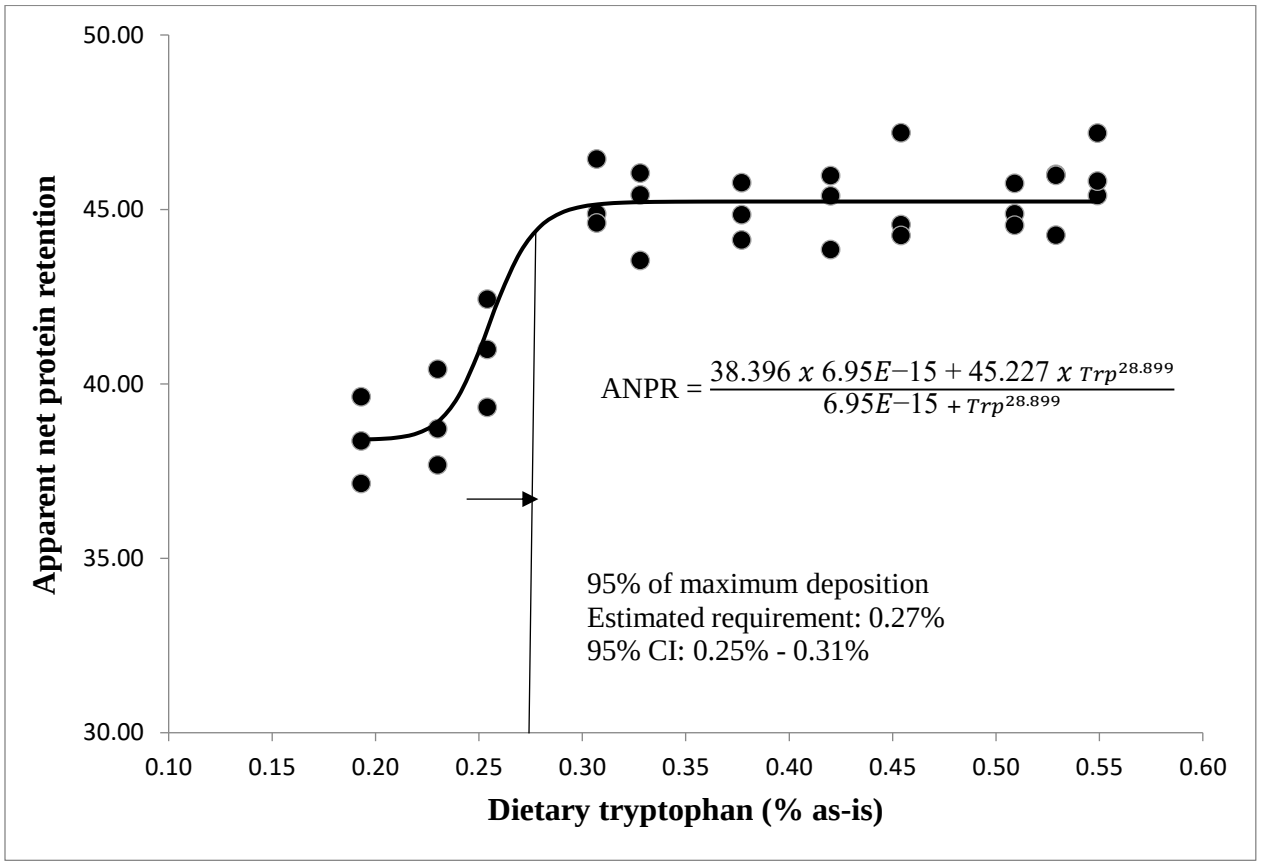


Fig. 3. Regression of apparent net protein retention against dietary Trp level of Nile tilapia (7.4 ± 0.1) after 8 weeks. Each point represents one replicate of a treatment. The solid curve represents the best fit saturation kinetic model. CI: confidence interval.

observed on condition factor (2.14 – 2.43%), IPF (1.94 - 2.74%) and HSI (1.99 - 2.39%) of Nile tilapia fed various rates of Trp. However, in the study conducted by Pewitt et al. (2017) on red drum *S. ocellatus*, HSI and condition factor were highly correlated to weight gain, being low in fish fed the basal diet and gradually reaching a plateau at the requirement level. Gaylor et al. (2005) also found that HSI and IPF ratio of hybrid striped bass *Morone chrysops x M. saxatilis* were correlated to dietary Trp levels in which IPF was inversely related to dietary Trp concentration. In other studies conducted by Ahmed (2012) and Coloso et al. (2004) on Asian sea bass and Indian catfish, respectively, the HSI was highest in fish fed the Trp-deficient diets. The possible reasons for discrepancies among these published data might result from the utilization of different protein sources, energy content and physiology of fish.

The deposition of body Trp can also be used as an indicator of dietary IAA profile required by the animal. In this study, Trp concentration of fish was relatively similar (adjusted mean at 0.14%) among fish fed different inclusion levels of Trp. There was, however, a consistent increasing trend in Trp deposition of Nile tilapia fed increasing levels of Trp from 0.19 - 0.33%; the increasing of Trp above that did not help the fish to attain better Trp deposition. The fitted values of BLM detected that Trp inclusion level of 0.33% (0.26 - 0.39%) was sufficient to meet the optimal Trp deposition of Nile tilapia. This Trp level is a bit high compared to the estimated values of Trp for optimal TGC (0.31%), FE (0.25%) and ANPR (0.27%). On the other hand, a decreasing trend of Trp deposition efficiency occurred as the inclusion levels of Trp increased. Nile tilapia fed diets containing high inclusion levels of Trp had significantly reduced Trp deposition efficiency. The distance between fish exhibiting highest and lowest Trp deposition efficiency is about 26.50% (22.76 and 49.26%). Zaminhan et al. (2017), however, observed huge

distance of Trp deposition efficiency between 4.8 and 45.8% as fish fed very low and high levels of Trp.

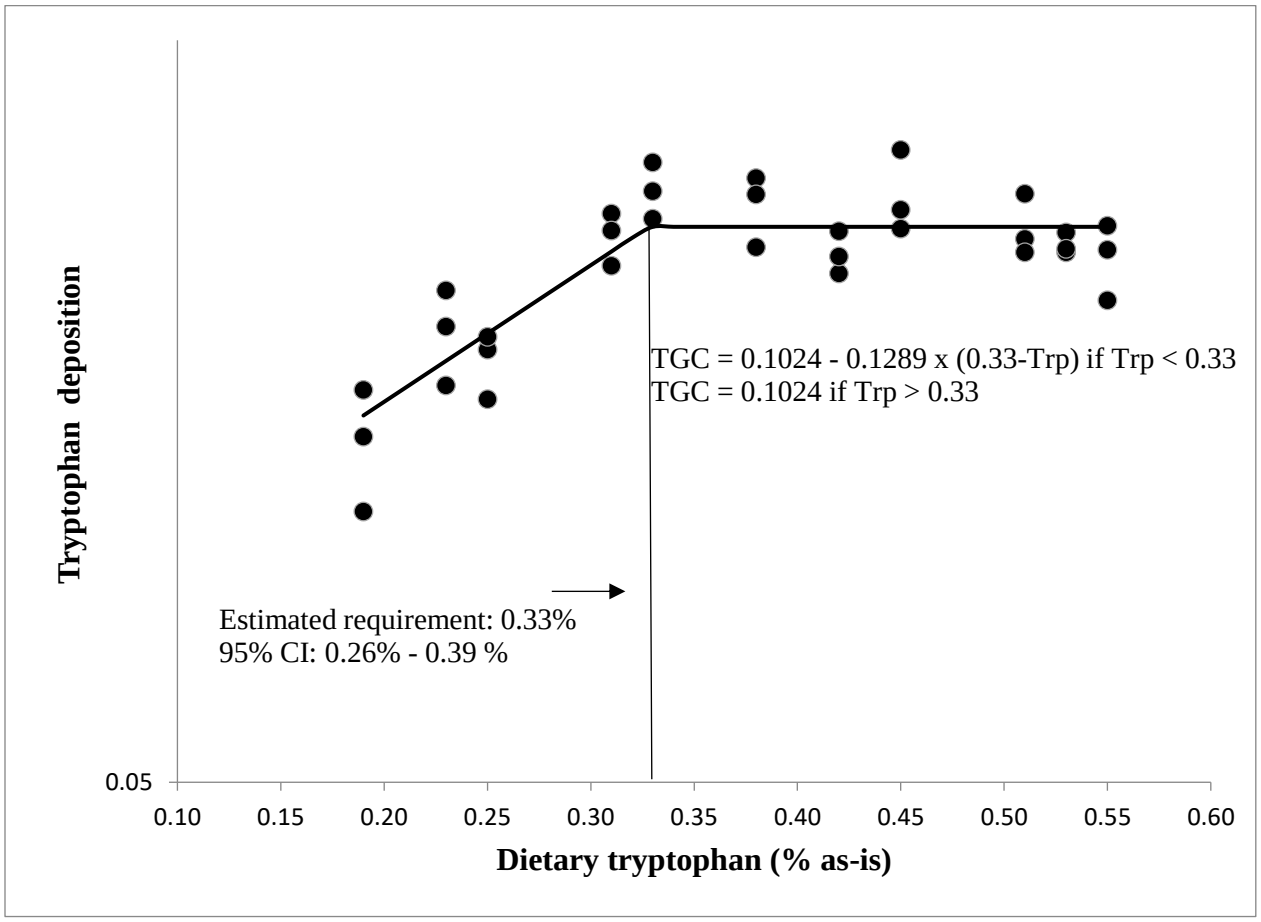


Fig. 4. Regression of tryptophan deposition against dietary Trp level of Nile tilapia (7.4 ± 0.1) after 8 weeks. Each point represents one replicate of a treatment. The solid line represents the best fit one-slope straight broken line analysis. CI: confidence interval.

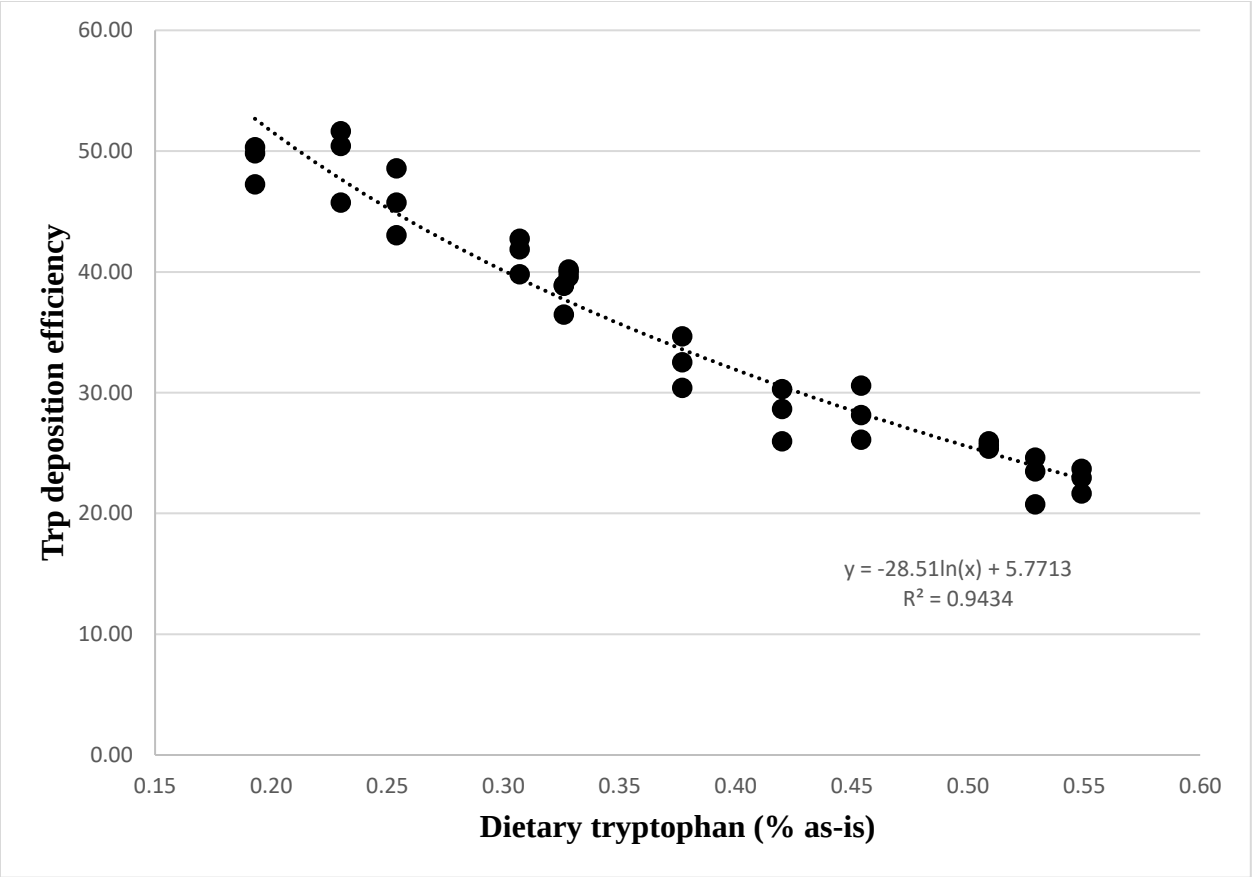


Fig. 5. Trp deposition efficiency of juvenile Nile tilapia (7.4 ± 0.1 g) after 8 weeks being fed practical diets containing graded levels of dietary taurine. CI: confidence interval.

## 5. Conclusion

The tryptophan requirement of juvenile Nile tilapia was confirmed at 0.31% (0.25 - 0.37%), 0.33% (0.26 - 0.39%), 0.25% (0.24 - 0.25%), 0.27% (0.25 - 0.31%) of the diet for optimum growth, tryptophan deposition, feed efficiency and apparent net protein deposition, respectively.

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# Chapter V

# THE BALANCE OF INDISPENSABLE AND DISPENSABLE AMINO ACID SUPPLEMENTATION IN DIETS OFFERED TO NILE TILAPIA *Oreochromis niloticus*


## Abstract

Nutritional methodologies applied to formulate cost-effective feeds using low protein diets or alternative protein ingredients from cheaper sources as fish meal replacement can result in an imbalance of AA profiles of feed, causing impaired growth and reduced feed efficiency. The use of balanced AA profile is important in formulating diets to allow reduced costs while still maintaining an adequate complement of protein. The objectives of this study were to further optimize AA balance of the diets offered to Nile tilapia by investigating the role of dispensable AA (DAA) across the range of IAA intakes. The possible limitation of indispensable AA (IAA) intake in diets with high inclusion of crystalline AA (CAA) supplemented in the diets was also confirmed. The basal diet was formulated to contain 22.2% intact protein in which IAA was adjusted to reach 100%, 110% and 120% of NRC requirements with or without DAA supplementation. Glycine and glutamic acid were used as DAA sources at 1:1 and 2:2 ratio to assign 2 levels of DAA at 2% and 4% of the diet. A total of 660 fish were randomly stocked into 44 aquaria with 15 fish per/tank. Fish were fed to near satiation four times a day for a 10-week period. The effects of feeding regimes (two times versus four times) on the efficacy of CAA utilization was also evaluated in which the feed offered to fish fed 100% IAA without DAA supplements and 110% IAA with 4% DAA supplements were paired for two and four feedings per

day. Results indicated that DAA plays an important role in meeting the nitrogen requirement of fish in which fish fed diets with DAA supplementation had significantly higher growth rates (thermal-unit growth coefficient, TGC) and lower FCR compared to fish fed diets without DAA supplementation. Growth performance of fish fed diets without DAA supplementation was not comparable to fish fed diets supplemented with DAA in spite of their IAA supplements up to 120% NRC requirement. Fish fed diets with DAA supplements at 4% exhibited superior growth compared to fish fed diets with 0 and 2% DAA supplements. The supplementation of IAA at 110% NRC requirement helped the fish get better growth performance compared to fish fed 100% NRC. However, no further benefit was observed on growth performance of fish fed diets with IAA supplements up to 120%. Fish fed diets with IAA supplements at 110% NRC and 4% DAA supplements exhibited the best response. The results of this study indicated that although DAA provided benefits in balancing AA profiles of feed, limitation of IAA intake at 100% NRC might cause impaired growth of Nile tilapia. In addition to this, no influence of feeding frequency was obtained on growth performance of fish feed, suggesting that fish can be fed successfully two times per day regardless of high inclusion levels of CAA.

## 1. Introduction

Fish require a continuous supply of protein as a source of AA for maintenance, growth, and other physiological functions. If protein intake is inadequate, fish might withdrawal protein from their less vital tissues to maintain the function of more vital ones. This process will result in retardation or cessation of growth, or loss of weight (Lim and Webster, 2002). As fish cannot synthesize all AAs in sufficient quantities to meet its needs, the provision of dietary protein or mixtures of AA is necessary.

Protein is considered the most important component of fish feed as it has a high cost per unit. Providing cost-effective feed meeting all of the IAAs required for growth of fish is a challenge for feed producers. Generally, the use of low protein diets or alternative protein ingredients from cheaper sources as fish meal replacements has been investigated to reduce feed cost (Botaro et al., 2007; El-Saidy and Gaber, 2002, 2003; Furuya and Furuya, 2010; Furuya et al., 2004; Gaylord and Barrows, 2009; Gonzales et al., 2007; Koch et al., 2016; Nguyen et al., 2009; Righetti et al., 2011; Thompson et al., 2012). However, these shifts in fish feed formulation can result in an imbalanced AA profile of feed that can affect growth performance of cultured species. Rigorous studies are needed to improve diet formulations associated with AA balance to allow reduced costs while still maintaining optimal growth of fish.

When considering the balance of dietary AA, more focus has been given to indispensable AA (IAA) as a proportion of protein. Numerous studies have been dedicated to investigation of specified requirements of IAAs to optimize growth and health of fish (Espe and Lied, 1994; Espe et al., 2006; Murai et al., 1987; Pérez-Jiménez, 2014; Rollin, 1999; Rollin et al, 2003; Williams et al., 2001; Yu and Zhang, 2012; Zarate et al., 1999). However, proper care should also be taken to

explore the role of dispensable AAs (DAAs) which are parts of the overall nitrogen requirement and participate in protein synthesis. The importance of IAA:DAA balance has been documented in several fish species (Cowey, 1995; Gaye-Siessegger et al., 2007; Green et al., 2002; Peres and Oliva-Teles, 2006; Silva et al., 2009; Vilhelmsson et al., 2004). Abboudi et al. (2009) indicated the critical role of DAA as protein sparing in maintenance of Atlantic salmon in which fish fed the DAA – protein free diet likely gained in metabolic efficiency through re-balancing the pattern of the body AA pool while fish fed diet without DAA supplements lost their nitrogen gain. Furthermore, DAA such as glutamine, is required in the functions of several cell populations of fish and helps them to attain improved growth performance and immune system (Cheng et al., 2011; Pereira et al., 2017; Pohlenz et al., 2012a, 2012b, 2012c). Firmer fillets of Atlantic salmon were also observed on fish fed diets supplemented with glutamate (Larsson et al., 2014).

The supplementation of crystalline AA (CAA) to meet a respective requirement of Nile tilapia in term of IAA to diets containing high levels of plant protein ingredients have positive effects on growth performance of these species (Figueiredo-Silva et al., 2015; Gan et al., 2015; He et al., 2015; Nguyen and Davis, 2016; Yue et al., 2013). Like other fish species, research on the balance of IAA:DAA for promoting maximum growth and protein deposition on Nile tilapia has been rather limited (Mambrini and Kaushik, 1994). Given the debate over the importance of meeting AA requirements and the efficient use of CAA, this research sought to further optimize AA balance of the diets offered to Nile tilapia *Oreochromis niloticus* by investigating the role of dispensable AA (DAA) across the range of IAA intakes.

## 2. Materials and Methods

### *2.1. Experimental design and diets*

The basal diet was formulated to contain 22.2% intact protein originating primarily from 3.60% fishmeal, 25.55% soybean meal, 5.83% corn protein concentrate and 25.00% whole wheat as protein sources. Crystalline AA (CAA) were then provided at 1.78% to this diet to assign the IAA profile meeting the respective requirement of NRC (2011), except methionine and histidine which optimal values were obtained from suggested numbers of Santiago and Lovell (1988) and Nguyen and Davis (2009), respectively. IAA profile was adjusted to reach 110% and 120% of NRC requirement with or without DAA supplementation. Glycine and glutamic acid were used as DAA sources at 1:1 and 2:2 ratio to assign 2 levels of DAA at 2% and 4% of the diet (Table 1).

The test diets were prepared in the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn, AL, USA). Pre-ground feed ingredients and oil were placed into a food mixer (Hobart Corporation, Troy, Ohio, USA) for 15 minutes. Hot water was then added to the mixture in order to attain an appropriate consistency for pelleting. Diets were then extruded through a 4-mm diameter die in a meat grinder, air dried at < 50° C to a moisture less than 11%, and stored in the freezer at -20° C until used. A sample of each feed was collected and analyzed for proximate composition by Midwest Laboratories (Omaha, NE, USA) and AA compositions by Ajinomoto Heartland Inc, (Chicago, IL, USA) (Table 2).

### *2.2. Culture methods*

The trial was conducted at the E.W. Shell Fisheries Center, Auburn, Alabama. Sex-reversed Nile tilapia *Oreochromis niloticus* were obtained from a commercial fingerling producer (Spring Genetic, Miami, Florida, USA), which were stocked in the nursery tank two weeks before

**Table 1** Ingredient compositions (g 100 $g^{-1}$ as-is) of nine experimental diets[a] offered to juvenile Nile tilapia (3.19 ± 0.04 g) over a ten week period.

| Diet name | 100I | 100I+2D | 100I+4D | 110I | 110I+2D | 110I+4D | 120I | 120I+2D | 120I+4D |
|---|---|---|---|---|---|---|---|---|---|
| Menhaden fishmeal[b] | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 | 4.00 |
| Soybean meal[c] | 27.30 | 27.30 | 27.30 | 27.30 | 27.30 | 27.30 | 27.30 | 27.30 | 27.30 |
| CPC[d] | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| Menhaden fish oil[e] | 6.32 | 6.32 | 6.32 | 6.32 | 6.32 | 6.32 | 6.32 | 6.32 | 6.32 |
| Lecithin[f] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Corn Starch[g] | 25.02 | 23.00 | 20.98 | 24.33 | 22.31 | 20.29 | 23.33 | 21.31 | 19.29 |
| Whole wheat[h] | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| Mineral premix[i] | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin premix[j] | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 | 0.80 |
| Choline chloride[k] | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Stay C[l] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| CaP-dibasic[m] | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 | 2.20 |
| Arginine[g] | | | | | | | 0.07 | 0.07 | 0.07 |
| Isoleucine[g] | | | | 0.10 | 0.10 | 0.10 | 0.20 | 0.20 | 0.20 |
| Leucine[g] | 0.00 | | | | | | 0.11 | 0.11 | 0.11 |
| Lysine 78.8%[n] | 0.69 | 0.69 | 0.69 | 0.89 | 0.89 | 0.89 | 1.10 | 1.10 | 1.10 |
| Methionine[n] | 0.08 | 0.08 | 0.08 | 0.13 | 0.13 | 0.13 | 0.18 | 0.18 | 0.18 |
| Phenylalanine[g] | | | | 0.01 | 0.01 | 0.01 | 0.12 | 0.12 | 0.12 |
| Threonine[g] | 0.28 | 0.28 | 0.28 | 0.39 | 0.39 | 0.39 | 0.50 | 0.50 | 0.50 |
| Tryptophan[n] | 0.02 | 0.02 | 0.02 | 0.05 | 0.05 | 0.05 | 0.08 | 0.08 | 0.08 |
| Valine[n] | 0.38 | 0.38 | 0.38 | 0.53 | 0.53 | 0.53 | 0.69 | 0.69 | 0.69 |
| Cysteine[g] | 0.11 | 0.11 | 0.11 | 0.15 | 0.15 | 0.15 | 0.20 | 0.20 | 0.20 |
| Glutamic Acid[g] | | 1.01 | 2.02 | | 1.01 | 2.02 | | 1.01 | 2.02 |
| Glycine[g] | | 1.01 | 2.02 | | 1.01 | 2.02 | | 1.01 | 2.02 |

[a] Diet designations: 100I: diet was formulated to meet the respective requirement of indispensable amino acid (IAA) based on NRC recommendations for Nile tilapia; 100I+2D: diet 100I plus 2% dispensable amino acid (DAA); 100I+4D: diet 100I plus 4% DAA; 100I: diet was formulated to meet 110% the respective requirement of IAA based on NRC recommendations for Nile

tilapia; 100I+2D: diet 110I plus 2% DAA; 110I+4D: diet 110I plus 4% DAA; diet was formulated to meet 120% the respective requirement of IAA based on NRC recommendations for Nile tilapia; 120I+2D: diet 120I plus 2% DAA; 120I+4D: diet 120I plus 4% DAA.

[b] Omega Protein Inc., Houston, Texas, USA

[c] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA

[d] Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA

[e] Omega Protein Inc., Reedville, VA, USA.

[f] The Solae Company, St. Louis, MO, USA

[g] MP Biochemicals Inc., Solon, OH, USA

[h] Bob's Red Mill Natural Foods, Milwaukie, OR, USA

[i] Trace mineral (g/100g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.250; Furrous sulfate, 4.000; Magnesium sulfate anhydrous, 13.862; Manganous sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate hepahydrate, 13.193; Alpha-cellulose, 67.964

[j] Vitamin (g/kg premix): Thiamin HCl, 0.44; Riboflavin, 0.63; Pyridoxine HCl, 0.91; DL pantothenic acid, 1.72; Nicotinic acid, 4.58; Biotin, 0.21; Folic acid, 0.55; Inositol, 21.05; Menadione sodium bisulfite, 0.89; Vitamin A acetate, 0.68; Vitamin $D_3$, 0.12; dL-alpha-tocoperol acetate, 12.63; Alpha-cellulose, 955.59

[k] Amresco Inc., Solon, Ohio, USA

[l] Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[m] Alfa Aesar, Ward Hill, MA, USA

[n] Ajinomoto Heartland Inc, Chicago, IL, USA

the beginning of the trial. After acclimating, juvenile Nile tilapia (3.19 ± 0.04 g) were randomly stocked into thirty-six rectangular aquaria of a 3,800-L indoor recirculation system at 15 fish per aquarium. Each of the nine treatments was assigned to four randomly chosen tanks. Samples of fish from the initial stocking were retained for later protein retention analysis.

The aquaria were provided with continuous water flow rate of 0.45 L/min and aeration by diffused air. The forty-four 55-L aquaria shared a single 3.65-$m^3$ recirculating system, with a sump, a suspended bead biofilter and a bead mechanical filter. Water temperature was maintained at 27 to 29$^o$C using a submerged 3600-W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA). Dissolved oxygen (DO) and water temperature were measured twice per day using YSI 650 multi-parameter instrument (YSI, Yellow Springs, Ohio) while pH, total ammonia nitrogen (TAN) and nitrite-nitrogen were measured twice per week. Photoperiod was set at 14 h light and 10 h dark. During the experimental period, DO, temperature, salinity, pH, TAN, and nitrite were within acceptable ranges for tilapia at 5.27 ± 1.07 mg/L, 28.09 ± 0.56°C, 1.15 ± 0.12 ppt, 7.21 ± 0.63, 0.33 ± 0.27 mg/L and 0.13 ± 0.06 mg/L, respectively.

Diets were offered to fish at 6-5% body weight daily four times per day at 08:00, 11:00 13:00, and 16:00 h according to fish size. Feed of fish fed 100% IAA without DAA supplements and 110% IAA with 4% DAA were paired for two and four feeding times. Fish were bulk-weighed and enumerated every week in the first two weeks and every other week thereafter. Daily feed rations were calculated and adjusted weekly based on percent body weight after each weighing and for intermittent weeks a 30 % increase in weight gain was assumed across all treatments. Upon termination of the experiment, fish were counted and group weighed. Four fish per aquarium were randomly sampled after being euthanized with tricaine methanesulphonate to measure

conditionindex including individual weight and length, hepatosomatic index (HSI), intraperitoneal fat (IPF) ratio. These whole-body fish samples were frozen at -20$^{o}$C and then ground homogenously before being freeze dried for crude protein analysis using the Kjeldahl method (Williams 1984). Subsequently, weight gain, thermal-unit growth coefficient (TGC), apparent net protein retention (ANPR), feed conversion ratio (FCR), condition factor (K), hepatosomatic index (HSI) and intraperitoneal fat (IPF) were determined using the following calculations:

a) Thermal-unit growth coefficient (TGC) = (final weigh$^{1/3}$ - initial weight$^{1/3}$) / (temperature × day) × 100.
b) Apparent net protein retention (ANPR, %) = (final weight × final protein content) - (initial weight × initial protein content) × 100 / protein intake.
c) Feed conversion ratio (FCR) = dry feed intake / weight gain.
d) Condition factor (K, 100 × g/cm$^3$) = 100 × individual body weight / (individual body length$^3$).
e) Hepatosomatic index (HSI, %) = [(liver weight/fish weight) × 100].
f) Intraperitoneal fat (IPF ratio, %) = [(IPF weight/fish weight) × 100].

*2.3. Statistical Analysis*

All data were subjected to one-way and two-ways analysis of variance to determine significant differences ($P < 0.05$) among the treatments, which was followed by Tukey's multiple comparison test to distinguish significant differences among treatment means. The analysis of covariance ANCOVA was used to compare the two regression lines of Nile tilapia responses to different inclusion levels of indispensable and dispensable AA. All the data were analyzed using SAS (V9.4. SAS Institute, Cary, North Carolina, USA).

**Table 2** Analyzed proximate composition[a] and amino acid profile[b] of the experimental diets (g 100 $g^{-1}$ as-is) fed to tilapia (3.19 ± 0.04 g) over a ten week period.

| Composition | 100I | 100I+2D | 100I+4D | 110I | 110I+2D | 110I+4D | 120I | 120I+2D | 120I+4D |
|---|---|---|---|---|---|---|---|---|---|
| Crude | 26.00 | 27.40 | 29.80 | 27.40 | 28.70 | 30.10 | 27.10 | 30.00 | 30.50 |
| Moisture | 8.37 | 8.41 | 8.00 | 7.61 | 7.79 | 7.04 | 8.07 | 7.16 | 9.74 |
| Crude Fat | 8.47 | 8.47 | 8.86 | 10.20 | 8.84 | 9.02 | 8.77 | 8.79 | 8.57 |
| Crude Fiber | 3.08 | 5.39 | 4.89 | 4.44 | 4.37 | 3.75 | 3.54 | 3.93 | 3.58 |
| Ash | 4.98 | 5.42 | 4.83 | 5.22 | 5.66 | 4.69 | 4.64 | 4.76 | 4.73 |
| **IAA** | | | | | | | | | |
| Arginine | 1.376 | 1.354 | 1.357 | 1.379 | 1.349 | 1.344 | 1.37 | 1.398 | 1.366 |
| Histidine | 0.559 | 0.559 | 0.56 | 0.569 | 0.55 | 0.56 | 0.554 | 0.556 | 0.54 |
| Isoleucine | 0.931 | 0.996 | 0.979 | 1.102 | 1.073 | 1.076 | 1.209 | 1.22 | 1.186 |
| Leucine | 2.144 | 2.194 | 2.163 | 2.241 | 2.191 | 2.188 | 2.303 | 2.311 | 2.259 |
| Lysine | 1.652 | 1.632 | 1.643 | 1.809 | 1.776 | 1.795 | 1.911 | 1.941 | 1.884 |
| Methionine | 0.492 | 0.488 | 0.497 | 0.543 | 0.533 | 0.538 | 0.566 | 0.57 | 0.558 |
| Phenylalanin | 1.265 | 1.25 | 1.252 | 1.29 | 1.252 | 1.257 | 1.319 | 1.341 | 1.322 |
| Taurine | 1.117 | 1.073 | 1.106 | 1.245 | 1.183 | 1.204 | 1.271 | 1.284 | 1.269 |
| Threonine | 0.261 | 0.254 | 0.258 | 0.278 | 0.305 | 0.264 | 0.318 | 0.318 | 0.308 |
| Tryptophan | 1.363 | 1.406 | 1.401 | 1.583 | 1.544 | 1.553 | 1.696 | 1.699 | 1.66 |
| Valine | 1.376 | 1.354 | 1.357 | 1.379 | 1.349 | 1.344 | 1.37 | 1.398 | 1.366 |
| **DAA** | | | | | | | | | |
| Alanine | 1.257 | 1.281 | 1.272 | 1.296 | 1.279 | 1.278 | 1.268 | 1.277 | 1.252 |
| Aspartic | 2.142 | 2.111 | 2.122 | 2.156 | 2.109 | 2.114 | 2.066 | 2.097 | 2.048 |
| Cysteine | 0.455 | 0.455 | 0.458 | 0.498 | 0.494 | 0.484 | 0.523 | 0.528 | 0.515 |
| Glutamic | 4.932 | 5.713 | 6.869 | 4.992 | 5.713 | 6.619 | 4.78 | 5.726 | 6.497 |
| Glycine | 1.03 | 1.994 | 2.995 | 1.057 | 1.995 | 2.979 | 1.017 | 1.988 | 2.895 |
| Proline | 1.55 | 1.551 | 1.576 | 1.619 | 1.611 | 1.643 | 1.565 | 1.58 | 1.515 |
| Serine | 1.173 | 1.158 | 1.166 | 1.191 | 1.17 | 1.165 | 1.14 | 1.153 | 1.127 |
| Tyrosine | 0.68 | 0.693 | 0.669 | 0.724 | 0.7 | 0.68 | 0.693 | 0.684 | 0.669 |

[a] Diets were analyzed at Midwest Laboratories (Omaha, NE, USA).

[b] Diets were analyzed at Ajinomoto Heartland Inc, (Chicago, IL, USA).

## 3. Results

Performances of Nile tilapia, *Oreochromis. niloticus* offered diets containing different AA profiles are presented in Table 3. The experimental groups did not differ significantly from each other with respect to survival, which ranged from 95 – 100%. Significant differences ($P < 0.05$) existed among mean final weight (FW), growth rate (TGC), feed intake (FI), feed conversion ratio (FCR), and apparent net protein retention (ANPR) of fish receiving various dietary treatments. Two-way analysis of variance indicated no significant differences in the interaction effect of IAA and DAA on growth performance and feed utilization efficiency ($P > 0.05$). The individual effect of different DAA and IAA supplements was, however, observed on FW, TGC, ANPR of fish.

According to analysis of covariance, FW, TGC and ANPR of fish were positively correlated with levels of DAA supplements in which fish fed diets without DAA supplements had lowest adjusted mean FW (85.28), TGC (0.248) in all levels of IAA (100, 110, 120% NRC recommended levels), followed by fish fed diets with 2% DAA supplements with FW (93.47) and TGC (0.259). Fish fed diets with 4% DAA supplements had highest adjusted mean FW (100.24), and TGC (0.268) (Table 4). ANPR of fish was, however, negatively affected by the supplements of DAA in which fish fed 0% DAA supplements had highest adjusted mean ANPR (47.67%), followed by fish fed 2% DAA supplements (45.18%) and fish fed 4% DAA supplements exhibited lowest adjusted mean ANPR (43.50%). The increasing levels of DAA from 0 – 4%, however, had no impact on FCR of fish with lowest FCR was observed in fish fed diet 110% IAA plus 4% DAA.

Significant differences ($P < 0.05$) were observed in mean FW, TGC of fish fed 100I, 110I and 120I diet in which fish fed 110I and 120I diets had significantly higher mean FW, TGC compared to those of fish fed 100I diet in both sets of diets irrespective of DAA levels (Table 5).

No significant differences were observed on mean ANPR and FCR of fish with adjusted means ranging from 45.31 to 45.69% and 0.94 to 0.95, respectively. Fish fed diet 110I+4D exhibited the highest mean FW, TGC and lowest FCR. No significant differences were observed on mean HSI (2.09 – 2.62%), IPF (1.05 – 1.62%) and condition factor (1.86 – 2.30%) of fish fed diets with different inclusion levels of IAA and DAA. In addition to this, no influence of feeding frequency was obtained on growth performance of fish even though fish with four times feeding exhibited higher mean TGC, ANPR and lower FCR compared to those of fish fed two times.

**Table 3** Mean response of Nile tilapia (3.19 ± 0.04 g) fed diets containing different amino acid profiles for ten weeks.

| Diet | Final weight (g) | Thermal-unit growth coefficient | Feed conversion ratio | Apparent net protein retention (%) | Survival (%) | Hepatosomatic index (%) | Intraperitoneal fat (%) | Condition factor (100 × g/cm$^3$) |
|---|---|---|---|---|---|---|---|---|
| 100I | 79.64$^{de}$ | 0.239$^{d}$ | 0.99$^{ab}$ | 48.63$^{a}$ | 96.7 | 2.22 | 1.23 | 2.01 |
| 100I+2D | 86.70$^{cd}$ | 0.250$^{c}$ | 0.93$^{cd}$ | 44.83$^{bcd}$ | 96.7 | 2.62 | 1.14 | 2.07 |
| 100I+4D | 91.90$^{cd}$ | 0.257$^{c}$ | 0.95$^{bcd}$ | 42.60$^{de}$ | 96.7 | 2.15 | 1.53 | 1.91 |
| 110I | 89.16$^{c}$ | 0.254$^{c}$ | 0.96$^{bcd}$ | 47.67$^{ab}$ | 96.7 | 2.10 | 1.35 | 1.94 |
| 110I+2D | 93.58$^{bc}$ | 0.259$^{bc}$ | 0.94$^{bcd}$ | 44.21$^{bcd}$ | 100.0 | 2.43 | 1.53 | 2.03 |
| 110I+4D | 105.41$^{a}$ | 0.275$^{a}$ | 0.91$^{d}$ | 44.05$^{bcd}$ | 96.7 | 2.20 | 1.46 | 1.90 |
| 120I | 87.04$^{cd}$ | 0.251$^{c}$ | 0.97$^{abc}$ | 46.70$^{abc}$ | 98.3 | 2.42 | 1.53 | 1.89 |
| 120I+2D | 100.14$^{ab}$ | 0.268$^{ab}$ | 0.92$^{cd}$ | 46.52$^{abc}$ | 98.3 | 2.09 | 1.44 | 1.87 |
| 120I+4D | 103.41$^{a}$ | 0.273$^{a}$ | 0.92$^{cd}$ | 43.86$^{cd}$ | 95.0 | 2.23 | 1.62 | 2.30 |
| 100I-2 | 77.49$^{e}$ | 0.236$^{d}$ | 1.02$^{a}$ | 45.76$^{abcd}$ | 95.0 | 2.17 | 1.05 | 1.86 |
| 100I+2D-2 | 100.09$^{ab}$ | 0.268$^{ab}$ | 0.96$^{bcd}$ | 39.67$^{e}$ | 96.7 | 2.42 | 1.55 | 1.86 |
| *P*-value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.5096 | 0.8096 | 0.6096 | 0.0753 |
| PSE[a] | 1.5648 | 0.0020 | 0.0112 | 1.0093 | 0.4256 | 0.4256 | 0.5256 | 0.8165 |
| **Two-way ANOVA** | | | | | | | | |
| DAA | <.0001 | <.0001 | <.0001 | <.0001 | 0.1236 | 0.2132 | 0.1123 | 0.1142 |
| IAA | <.0001 | <.0001 | 0.1242 | 0.8195 | 0.7401 | 0.1531 | 0.1121 | 0.1361 |
| DAA*IAA | 0.0519 | 0.0662 | 0.4053 | 0.1012 | 0.4471 | 0.6256 | 0.5161 | 0.4401 |

Means in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by Tukey's multiple range test.

[a] PSE: Pooled standard error.

**Table 4** Analysis of covariance output of final weight, thermal-unit growth coefficient, feed conversion ratio, apparent net protein retention of Nile tilapia (3.19 ± 0.04 g) fed diets with increasing levels of DAA over a ten-week growth period

| | Adjusted mean least-squares means of fillet mineral contents | | | |
|---|---|---|---|---|
| DAA | Final weight (g) | Thermal-unit growth coefficient | Feed conversion ratio | Apparent net protein retention (%) |
| 0% | 85.28[c] | 0.248[c] | 0.97 | 47.67[c] |
| 2% | 93.47[b] | 0.259[b] | 0.93 | 45.18[b] |
| 4% | 100.24[a] | 0.268[a] | 0.92 | 43.50[a] |
| Slope *P* value | 0.3693 | 0.5376 | 0.8925 | 0.0658 |
| Intercept *P* value | <0.0001 | <0.0001 | 0.113 | <0.0001 |

Means (n = 4) in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of covariance

**Table 5** Analysis of covariance output of final weight, thermal-unit growth coefficient, feed conversion ratio, apparent net protein retention of Nile tilapia (3.19 ± 0.04 g) fed diets with increasing levels of IAA over a ten-week growth period.

| Adjusted mean least-squares means of fillet mineral contents | | | | |
|---|---|---|---|---|
| IAA | Final weight (g) | Thermal-unit growth coefficient | Feed conversion ratio | Apparent net protein retention (%) |
| 100% | 86.08[b] | 0.249[b] | 0.954 | 45.35 |
| 110% | 96.05[a] | 0.263[a] | 0.936 | 45.31 |
| 120% | 96.87[a] | 0.264[a] | 0.939 | 45.69 |
| Slope *P* value | 0.4555 | 0.6579 | 0.9102 | 0.1569 |
| Intercept *P* value | <0.0001 | <0.0001 | 0.1524 | 0.838 |

Means in the same column with different superscripts are significantly different at $P < 0.05$ based upon analysis of covariance

## 4. Discussion

Results of this study indicated that DAA plays an important role in meeting the nitrogen requirement of fish in which fish fed diets with DAA supplementation exhibited significantly higher mean growth rates (TGC), ANPR, and lower FCR compared to those of fish fed diets without DAA supplements. Growth performance of fish fed diets without DAA supplements was not comparable to fish fed diets supplemented with DAA in spite of their supplements in IAA up to 120% NRC requirement. Growth performance of fish fed diet with 4% DAA was superior to fish fed diet with 0 and 2% DAA supplements. This result confirmed our previous finding that DAA needs to be supplied into low protein diets with balanced IAA profiles to overcome nitrogen limitation. Gaye-Siessegger et al. (2007) also indicated the important role of DAA for growth of Nile tilapia. In their study, the utilization of free DAA resembled those of fish meal by helping the fish attain higher body mass compared to fish fed precursor AAs (phenylalanine, serine, aspartate, glutamate). Similarly, Schuhmacher et al. (1995) found that rainbow trout fed diets without DAA supplementation (IAA:DAA at 100:0) had lower growth and feed efficiency ratios compared to those of fish fed diets with DAA supplements at IAA:DAA ratios of 40:60 and 60:40. Abboudi et al. (2009) also showed the significant role of DAAs under near maintenance conditions in which Atlantic salmon fed DAA protein free diets had better performance compared to fish diets without DAA supplements. According to Heger (2003), if DAA of the diet is limited, IAA from the body pool must supply substrates for their own synthesis, thereby, reducing their availability. Dabrowski and Guderley (2002) also demonstrated that supplementation of DAA at specific levels is critical to satisfy the nitrogen requirement of animals as IAA might not be used as efficiently as DAA for DAA synthesis. The utilization of free DAA, however, could not help the fish to get comparable

weight gain to that of fish fed intact protein diet as observed previously by Mambrini and Kaushik (1994). Retardation of growth in their study was explained as a deficiency in IAAs in conjunction with the reduction of intact protein. In our study, the IAA profile of feed was, however, formulated to meet the respective requirement of Nile tilapia based on NRC recommendations.

The shift in the ratio of IAA:DAA in this study as DAA supplements increased can also affect the growth performance of fish. As DAA of feed was supplemented at fixed levels at 0, 2 and 4% while the IAA was kept constant within experiments, the increase of DAA supplements will result in a reduction in IAA:DAA ratio. Peres et al. (2006) indicated the importance of IAA:DAA ratio which can affect the growth performance of fish. According to their results, IAA:DAA ratio of 50:50 and 60:40 promoted maximum growth performance and feed utilization efficiency, respectively. Higher IAA:DAA of 57:43 was needed to provide highest growth performance (TGC) of rainbow trout (Green et al., 2002). Although, optimum IAA:DAA of tilapia has not been determined, Mambrini and Kaushik (1994) showed the significant effects of having a balance of IAA:DAA ratio in which DAA supplements exceeded 60% of total AA supply induced poor growth of fish. In our study, the calculated IAA:DAA ratios of experimental diets ranged from 40:60 to 50:50, which were all above the recommended levels of Mambrini and Kaushik (1994).

The utilization of higher IAA at 110% NRC stimulated superior growth performance of fish compared to those of fish fed 100% NRC regardless of DAA supplements. The supplementation of IAA up to 120% did not have any further benefit on growth performance of fish as no significant differences in weight gain was observed on fish fed this diet and fish fed 110% NRC diet. One of the possible reasons for impaired growth of fish fed 100% NRC might be

because limitation of daily IAA feed intake. In this study, fish were fed close to apparent satiation based on their body weight. As a similar daily feed intake was offered, fish fed diets with higher IAA levels would have higher IAA intake compared to fish fed diets low in IAA. Another possible reason for reduced growth of fish fed 100% NRC is the higher utilization of CAA might induce feed utilization efficiency. Gaye-Siessegger (2007) indicated that Nile tilapia poorly used free AA and the reason might be because of rapid uptakes of AA into the plasma. Several studies conducted on sea bass and salmon also pointed out the limitation of CAA compared to intact protein diets related to palatability and stability (Dabrowski et al., 2010; El Haroun and Bureau, 2006; Hauler et al., 2007; Liu et al., 2002; Sveier et al., 2001; Yamada et al., 1981).

Excessive IAAs in 120% NRC diet (AA catabolized for energy) cannot be used by Nile tilapia to spare all of the deficient DAA in low protein diet. The increase of IAA up to 120% NRC did not have further improvement on performance of fish. Similarly, several studies conducted on Nile tilapia also indicated no added benefit of fish fed in excess of individual or mixture IAAs on growth performance of fish. Nguyen and Davis (2009) found that supplementing feed with excessive levels of methionine (above the optimum requirement) did not have any greater benefit on weight gain of Nile tilapia (2.32 ± 0.06 g). Similarly, Pereira et al. (2017) and Neu et al. (2016) found that feeding i an excess of arginine had no added benefit on growth performance of Nile tilapia. Neu et al. (2017) also observed the same trend on Nile tilapia fed excessive levels of isoleucine (0.7 – 1.17%). Moreover, Santiago and Lovell (1998) even observed deleterious growth of Nile tilapia fed excess of arginine, histidine, valine, isoleucine, leucine, and phenylalanine. Similarly, Furuya et al. (2012), Gan et al. (2016), He et al., (2017), Michelato et al. (2017) and Zaminhan et al. (2017) observed reduced growth of fish fed excessive levels of lysine, histidine,

tryptophan, leucine, methionine, threonine diet above the requirement (0.29%).

In our study, while no added benefit was observed on adjusted mean protein retention of fish fed diets with increasing levels of IAA, significant negative correlation ($P < 0.05$) between ANPR of fish and DAA supplements was observed. Nitrogen retention correlated to IAA:DAA ratio of feed was also observed in rainbow trout in which maximum mean N retention of 46% was achieved by fish fed the diet containing an IAA:DAA ratio of 57:43 (Green et al., 2002). Similarly, Gomez-Requeni et al. (2003) found that gilthead seabream fed reduced IAA:DAA ratio exhibited poor feed conversion ratio and protein retention. However, in their study the increase of DAA supplements resulted in reduction of IAA contents. Feed containing the lowest IAA:DAA ratio at 0.83 had lower IAA profiles, which were all below the IAA profile of muscle and whole body of fish. This IAA profile might be inadequate for protein synthesis of fish. Whereas the reduced ANPR of fish fed increased, DAA supplements in our study might result from the higher total nitrogen used by fish. In the present study, no significant differences were observed on mean HSI (2.09% – 2.62%), IPF (1.05% – 1.62%) and condition factor (0.86% – 2.30%) of fish fed diets with different inclusion levels of IAA and DAA. Similarly, no significant differences were observed on HSI of fish fed different AA profiles (different IAA:DAA ratio) (Figueiredo-Silva et al., 2010).

No significant influence of feeding frequency was obtained on growth performance of fish even although fish with four feeding times exhibited higher mean TGC, ANPR and lower FCR compared to fish fed two times. Similarly, increasing feeding frequency did not affect either growth performance or body composition of Nile tilapia (Lanna et al., 2016). According to their

study, fish fed diets supplemented with commercial AA (lysine, methionine, threonine) can be fed efficiently 2 times/day without deleterious effect of growth observed.

## 5. Conclusion

Results indicated that DAA plays an important role in meeting the nitrogen requirement of Nile tilapia. Growth performance of fish fed diets without DAA supplementation was not comparable to fish fed diets supplemented with sufficient DAA in spite of their IAAs supplements up to 120% NRC requirement. The results of this study also indicated the inferior growth of fish fed diet with IAA supplements at 100% NRC requirements, which might result from limitation of daily IAA intake. No influence of feeding frequency was obtained on growth performance of fish, indicating that fish can be fed successfully two times per day regardless of high inclusion levels of CAA.

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# Chapter VI

## SUMMARY AND CONCLUSIONS

Protein is considered the most important dietary component as it comprises a significant proportion of whole body dry matter of fish. As it has high cost per unit, provision of feed meeting an exact requirement for protein is a prerequisite for efficient and economical fish production. To do this, a diet has to be formulated properly with regard to AA balance. Concurrently, nutritional methodologies applied to formulating cost-effective feeds are often geared towards using low protein diets or inexpensive protein sources. However, this can result in an imbalance of AA profiles of feed, causing impaired growth, and reduced feed efficiency. Therefore, it is necessary to optimize AA balance in diets for fish. A dietary requirement for protein is essentially a requirement of IAA to meet the need for protein synthesis and growth and sufficient DAA or nitrogen to enable fish to synthesize IAA. Like other fish species, research on the balance of IAA and DAA for promoting maximum growth and protein deposition on Nile tilapia has been limited and inconsistent. Considering the importance of meeting AA requirements, this research is to further optimize AA balance of the diets offered to Nile tilapia *Oreochromis niloticus* by applying and validating the use of the ideal protein concept.

Generally, the ideal protein concept is useful as a guideline to establish an IAA profile in formulating diets of fish, especially those with low and moderate protein levels for which the AA proportions need to be precisely adjusted to avoid deficiencies. The results of this study indicate that the utilization of balanced IAA profiles of feed (under the application of the ideal protein concept) can help the fish to attain better growth performance and feed utilization efficiency. With

the use of balanced IAA diets, the percentage of intact protein inclusion of feed can be reduced from 32% to 27.2% without causing impaired growth performance and feed utilization efficiency of fish. Further reduction of intact protein levels to 24.7% and 22.2% of diet, however, induced growth depression which could result from the deficiency of nonspecific nitrogen as a source of energy or limitation of daily IAA intake.

In our ingredient matrix, in addition to lysine, methionine and threonine, it was hypothesized that tryptophan, isoleucine, arginine, histidine and valine could also be limiting. Hence, a study was conducted to confirm a potentially limiting IAA in our matrix of ingredients. These IAAs were individually deleted from the IAA profile of the diet with enhanced IAA and DAA supplements. The results illustrated that with the exception of valine, the deletion of the other crystalline IAA supplements (tryptophan, arginine, threonine and isoleucine) did not cause any deleterious effects on growth performance and protein utilization efficiency of fish. Therefore, in addition to lysine, methionine and threonine, valine is limiting in our ingredient matrix and the supplementation of this IAA is necessary to meet the requirement of Nile tilapia, while tryptophan, histidine, arginine and isoleucine are likely adequate for growth of fish. Even though tryptophan is not limiting in our ingredient matrix, the reported requirement of this IAA is inconsistent so we confirmed the requirement. Results of this study confirmed the tryptophan requirement of juvenile Nile tilapia at 0.31% (0.25 - 0.37%), 0.33% (0.26 - 0.39%), 0.25% (0.24 - 0.25%), 0.27% (0.25 - 0.31%) of the diet for optimum growth, tryptophan deposition, feed efficiency and apparent net protein deposition (95% of maximum value), respectively.

The supplementation of DAA to spare the use of relatively expensive IAA was also taken into consideration by revising the “ideal protein concept”. Based on the growth data obtained from

this study, it can be concluded that DAA plays an important role in meeting the nonspecific nitrogen requirement of fish. In a low protein diet (22.2%), enhancing IAAs above the requirement (120% NRC or to the IAA profile of the reference diet which supported highest performance of fish) did not help the fish to reach comparable weight gain to fish fed the diet with DAA supplements (4%). The results of this study also indicated the inferior growth of fish fed diet with IAA supplements at 100% NRC and 4% DAA supplements, which might have resulted from limitation of daily IAA intake. The effects of feeding regimes (two times versus four times) on the efficacy of crystalline amino acid utilization was also investigated. Results indicated that Nile tilapia can be fed successfully two times per day regardless of high inclusion levels of CAA.

Based on the data obtained from this study, it can be concluded that the ideal protein concept can be applied in formulating the diets for Nile tilapia juveniles to optimize the amino acid profile of the diets. Well-balanced IAA profile can be used to reduce the intact protein levels of feed without causing impaired growth of fish. However, if low intact protein levels are to be formulated, the supplementation of DAA might be required to satisfy the nitrogen requirement of fish. In our ingredient matrix, in addition to lysine, methionine and threonine, valine is limiting and the supplementation of this IAA is necessary to satisfy the requirement of Nile tilapia.

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