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CONTENT

AMINO ACID RECOMMENDATIONS FOR TILAPIA: A REVIEW OF AVAILABLE DATA AND PRINCIPLE BEHIND AMINOTILAPIA®

by Karthik Masagounder

KEY INFORMATION

- Available data on amino acid requirements of tilapia show high variability and do not cover the whole production cycle.
- Data generated in the past with conventional Nile tilapia strain (e.g., data by Santiago and Lovell 1988) seem to underestimate the amino acid requirements for genetically improved modern tilapia strains.
- Fundamental principles of factorial model for amino acid recommendations and data behind the AMINOTilapia® tool development are discussed.
- AMINOTilapia® enables users obtain amino acid recommendations for different growth stages of tilapia based on various production features including:
  - feeding rate and feed conversion ratio (FCR)
  - body weight and growth rate of tilapia
  - feeding frequency
  - farming system and presence of natural food
- The tool gives amino acid recommendations on both total and digestible basis expressed as % diet for the chosen dry matter content of feed.

DEAR READER,

Aquaculture is a fast growing industry and Tilapia is one of the most prominent fish produced around the globe. To be successful we need to transfer some basic nutritional concepts learned from terrestrial species. One of the main achievements over the last decades in poultry and swine production has been the switch from the protein requirement feeding concept to one based on amino acid requirements. This shift has greatly helped the poultry and swine industries to become more efficient. AMINOTilapia® is a software tool designed to help you to make a similar change towards improved efficiency of Tilapia diet formulation based on specific amino acid requirements.

In this AMINONews® issue, Dr. Karthik Masagounder has not only introduced AMINOTilapia®, but has also made a comprehensive review on the requirement of the first limiting amino acids: sulfur amino acids, lysine and threonine. Moreover, the calculation method to derive the amino acid requirements of Tilapia based on the factorial approach and management influences is described.

Happy reading!

Vincent Hess

INTRODUCTION

Tilapia is currently the second most produced farmed fish, just after carp. Global production of farmed tilapia exceeded 5 million MT in 2015, and witnessed an average annual growth rate of 10 % during 2010–2015 (Fitzsimmons 2016; FAO 2017). Tilapia production from aquaculture accounted for ~10 % of total finfish produced globally from farming in 2014. Among different tilapia species, Nile tilapia (*Oreochromis niloticus*) is the predominant one, and contributes to 75 % of total farmed tilapia. The rest are contributed by hybrid red tilapia *O. niloticus* x *O. mossambicus* (15 %), Mozambique Tilapia *O. mossambicus* (5 %), blue tilapia *O. aureus* (2–3 %) and other species (2–3 %) (Industry survey by Kevin M. Fitzsimmons, University of Arizona, personal communication, 2017). Important breakthroughs that fueled the rapid growth of tilapia industry include production of mono-sex male population and genetically improved fast growing strain through selective breeding program (e.g. genetically improved farmed tilapia or GIFT strain). In parallel, significant improvements have been made over the years in the feed (nutrition, feed processing) and feeding management of tilapia, which triggered the intensification of tilapia farming. However, the industry is facing significant challenges with rising feed cost which constitutes the major operational cost of tilapia production. Dietary protein has a major share of diet cost and there has been continuous efforts in finding alternative cost-effective protein sources for tilapia. Studies have demonstrated that, with the use of supplemental amino acids, fish meal can be effectively replaced by plant and alternative animal protein sources, while protein utilization can also be improved (e.g., Facts & Figures No. 1610; Furuya *et al.*, 2004; Figueiredo-Silva *et al.*, 2015; Koch *et al.*, 2016). Current fish meal level in tilapia feed is no more than 5 % in main tilapia producing countries. Nevertheless, effective use of alternative protein sources and further improvements in protein utilization require more precise understandings on the amino acid requirements of tilapia. Besides, Nile tilapia has been genetically improved over 15 generations of selection with ~10 % improvement in weight gain per generation (Nguyen 2016). This makes it essential to regularly validate and update the nutrient requirement data of tilapia in order to meet their new genetic demands. Furthermore, past studies generated requirement data mainly for early life stages under ideal experimental condition, indicating lack of data for different growth stages of tilapia under commercial production conditions. This article gives a comprehensive review of available data on the amino acid requirements of tilapia and then, discusses the application of factorial approach in modelling amino acid requirements. The paper overall provides the readers with the fundamental principles behind the development of AMINOTilapia® tool, which is aimed to give amino acid recommendations for different growth stages of tilapia under different production conditions.

AMINO ACID REQUIREMENTS OF TILAPIA – A REVIEW OF AVAILABLE DATA

Tilapia like other fish species cannot synthesize on their own the ten essential amino acids (EAA). These need to be adequately provided through feed, and in case of deficit, protein synthesis and thus, growth will be impaired. Amino acid requirement studies in tilapia have largely used dose-response method, while in the recent years diet-dilution technique and deletion method were also adopted. Requirement values discussed in this section are on total basis (% diet, as fed) unless otherwise mentioned.

METHIONINE AND CYSTEINE

Methionine is typically the first limiting amino acid in soybean based tilapia diet. Methionine requirement of tilapia was first investigated back in 1980s. Study by Jackson and Capper (1982) showed that fingerling-stage Mozambique tilapia (1.7 g, initial weight) require ≤ 0.53 % methionine (0.74 % cysteine) in the diet. Dietary methionine requirement of fry Nile tilapia (62 mg) was later reported to be 0.75 % by Santiago and Lovell (1988), where test diets supplied a constant cysteine level of 0.15 %. Recently, few studies have reported requirements (% diet) for sulfur amino acids for juvenile Nile tilapia: 0.49 % methionine (Nguyen and Davis 2009a) and 0.85 % methionine + cysteine (M+C) (Nguyen and Davis 2009b), 0.91 % methionine (0.10 % cysteine) (DM basis) (He *et al.*, 2017) and 0.99 % M+C (Diogenes *et al.*, 2016). On the contrary, a much higher value was found in other recent studies. Xiang *et al.*, (2014) reported a total sulfur AA requirement of 1.43 – 1.46 % diet (DM basis) for Nile tilapia (68 g, initial weight). Similarly, Evonik-Thaksin University collaborative study found 1.57 % and 1.25 % M+C levels for optimizing body weight and protein gain of hybrid tilapia (*O. nilotics* x *O. mossambicus*) (Figueiredo-Silva *et al.*, 2015). Furthermore, study by He *et al.*, (2017b) (a collaborative study between Evonik and Sun Yat-sen University) showed that juvenile Nile tilapia require 0.99 % methionine with 0.47 % cysteine. While the above discussed studies used dose-response method, Liebert (2009) used diet dilution technique to determine methionine requirement value: the study showed that juvenile tilapia require 0.73 % and 0.94 % dietary methionine for achieving 70 % and 80 %, respectively, of theoretical maximum nitrogen deposition rates. One factor that should be emphasized is that the later studies (Xiang *et al.*, 2014; Figueiredo-Silva *et al.*, 2015; He *et al.*, 2017a; He *et al.*, 2017b) have ensured a dietary lysine level of at least 2 % in the test diets, whereas the other studies provided only 1.50 – 1.67 % dietary lysine. This level (1.50 – 1.67%) is lower than the optimal lysine level estimated for young Nile tilapia in the recent studies (e. g., 2.32 % diet Takishita *et al.* 2009; ≥ 1.8 % diet Bomfim e. g. 2010), and could have suppressed tilapia growth, resulting in underestimation of methionine requirements. Furthermore, study by He *et al.* (2017b) demonstrated that test diets with insufficient energy could also result in underestimation of dietary EAA requirements. The study found that dietary methionine requirement of juvenile Nile tilapia increases from 0.73 % to 0.99 % (with 0.47 % Cys), when the dietary energy level was increased from 10.9 MJ/kg to 12.4 MJ/kg.

Recent studies clearly underscore the importance of balancing the test diets for essential nutrients (excluding the test EAA) and energy. Limitation of dietary nutrients can result in underestimation of requirements for the test EAA which partly explains the variability of requirement data observed among studies. Overall, studies that ensured adequate dietary lysine level (2 %) tend to show M+C requirements of 1.4 – 1.6 % diet for early-stage tilapia.

LYSINE

Lysine was one of the largely investigated EAA for tilapia. First study on the lysine requirement of tilapia was published by Jackson and Capper in 1982. The study showed that fingerling-stage Mozambique tilapia (*Sarotherodon mossambicus*) require 1.62 % lysine in the diet. Santiago and Lovell (1988) later reported even lower dietary lysine requirements (1.43 %) for Nile tilapia fry (41 mg, initial weight). After a long interval, Takishita *et al.*, (2009) investigated lysine requirement (both total and digestible basis) for fingerling tilapia (0.98 g initial weight). The study found an optimal dietary lysine requirement of 2.32 % on total basis and 2.17 % on digestible basis for maximizing weight gain of fingerling Nile tilapia. These values are much higher than those determined for tilapia in earlier studies. In the following year, study by Bomfim *et al.* (2010) showed a linear increase in the weight gain of fingerling Nile tilapia (1.12 g initial weight) in response to increasing lysine levels (1.05 – 1.80 %), suggesting the optimal requirement to be ≥ 1.80 % diet (≥ 1.70 % diet on digestible basis). In a recent study, Furuya *et al.* (2012), using quadratic model, determined the requirement to be only 1.56 % diet on digestible basis for optimizing weight gain of fingerling Nile tilapia (1.44 g initial weight). However, the study by Furuya *et al.* (2012) also recorded a poor growth rate (0.10 g/d) relative to those (0.38 g/d; 0.47 g/d) observed in the other two studies (Takishita *et al.*, 2009; Bomfim *et al.*, 2010), despite all the studies having used a similar size fish (about 1 g). All the above studies have used dose-response method, while there are at least two studies (Liebert 2009; Diogenes *et al.*, 2016) reported lysine requirement values using other methods. Liebert (2009), using diet-dilution technique, showed that lysine requirement of juvenile Nile tilapia (12 g, initial weight) can vary depending on various factors including growth potency of fish, amino acid utilization efficiency and feed intake. The author found that, for the observed utilization efficiency, lysine requirement of Nile tilapia to be 1.63 % and 2.09 % diet for the nitrogen deposition rate being 70 % and 80 % of its theoretical maximum. More recently, Diogenes *et al.* (2016), using deletion method, reported a dietary lysine requirement of only 1.56 % diet for juvenile Nile tilapia (20 g, initial weight). However, the EAA levels used in the experimental diets for meeting dietary requirements of Nile tilapia other than the test amino acid were lower than the requirement levels determined in the same study for several EAA. This indicates that the study likely have underestimated optimal dietary lysine requirement. Lysine requirement studies for adult tilapia is limited. Michelato *et al.*, (2016a) recently determined that adult Nile tilapia (275 g) require a dietary lysine level of 1.46 %.

In summary, despite the discrepancies, recent studies have reported much higher dietary lysine requirement values (1.56–2.32 % diet) than the value (1.43 % diet) reported by Santiago and Lovell (1988) for fry-fingerling stage Nile tilapia. This could be partly attributed to the fact that recent studies use genetically improved strains and all-male sex which likely have higher growth potential compared with those used by Santiago and Lovell (1988).

THREONINE

Next to methionine and lysine, threonine received high attention because not only it is essential for muscle protein synthesis, but also it has other functional roles such as mucin production and immune response. Because of this, threonine requirement in commercial production conditions can be higher if the fish are undergoing immune challenges.

Threonine requirement for tilapia was first reported by Santiago and Lovell (1988). The study found that fry stage Nile tilapia (<1 g) require 1.05 % threonine in the diet. Later, Liebert and Benkendorff (2007) and Liebert (2009) derived, based on diet-dilution technique, that juvenile (50 g) Nile tilapia require 0.83 % and 1.05 % threonine in diets for the 70 % and 80 % of maximum nitrogen deposition rates, respectively. Recent studies, in contrast, showed much higher threonine requirement values: 1.33 % dry feed (Yue *et al.*, 2014) and 1.45 % diet (Diogenes *et al.*, 2016) for juvenile Nile tilapia and 1.62–1.72 % dry feed (Zhou *et al.*, 2014) and 1.15–1.20 % diet for adult Nile tilapia (Michelato *et al.*, 2016b).

Excluding methionine, lysine and threonine, studies for the remaining EAAs are limited. A summary of EAA requirement data published in various studies is presented in Table 1.

TABLE 1 EAA requirements (% diet, as-fed*) of Nile tilapia published in various studies

Amino acid	InWt	Days	FnWt	Model used	Response variable	Requirement	Reference
	g[a]		g[b]			% diet	
Lys	0.04	56	1.35	Broken line	WG[c]	1.43	Santiago & Lovell 1988
	0.98	32	10	Broken line	WG	2.32	Takishita *et al.*, 2009
	1.12	30	15.34	Linear	WG / FCR	1.8 ≤ R[d]	Bomfim *et al.*, 2010
	1.44	45	5.78	Quadratic	WG	1.56[e]	Furuya *et al.*, 2012
	12	56	50	Nonlinear	N deposition (70 & 80 % max)	1.63 & 2.09	Liebert ,2009
	20	57	56	Linear	N deposition	1.56	Diogenes *et al.*, 2016
	87	45	226	Broken line	WG	1.31[e]	Furuya et al., 2013
	275	40	571	Quadratic	Fillet yield	1.46	Michelato *et al.*, 2016a
Met (Met+Cys)	0.06	56	1.24	Broken line	WG	0.75 (0.90)	Santiago & Lovell 1988
	0.86	40	15.93		WG	(0.90)	Bomfim *et al.*, 2008
	1.28	56	8.33	Broken line	WG	(0.85)	Nguyen & Davis 2009b
	1.7	70	71.0	Quadratic	WG	(1.57)[f]	Figueiredo-Silva *et al.*, 2015
	2.3	56	17.92	Quadratic	WG	0.91 (0.99)[g]	He *et al.*, 2017a
	5.62	56	44.12	Broken line	WG	0.49	Nguyen & Davis 2009a
	8.95	56	80–90	Quadratic	WG	0.99 (1.46)	He *et al.*, 2017b
	12	56	50	Nonlinear	N deposition (70 & 80 % max)	0.73 & 0.94	Liebert, 2009
	20	57	35	Linear	N deposition	(0.99)	Diogenes *et al.*, 2016
	68	60	312	Quadratic	WG	1.13 (1.43)[g]	Xiang *et al.*, 2014
Thr	0.05	56	1.3	Broken line	WG	0.75	Santiago & Lovell 1988
	12	56	50	Nonlinear	N deposition (70 & 80 % max)	0.83 & 1.05	Liebert, 2009
	2.97	56	22.4	Quadratic	WG	1.33[g]	Yue *et al.*, 2014
	20	57	35	Linear	N deposition	1.45	Diogenes *et al.*, 2016
	67.08	60	216	Quadratic	WG	1.72[g]	Zhou *et al.*, 2014
	67.08	60	216	Quadratic	FCR & PER[h]	1.62[g]	Zhou *et al.*, 2014
	563	28	829	Quadratic	WG	1.20	Michelato *et al.*, 2016b
Trp	0.06	56	1.35	Broken line	WG	0.28	Santiago & Lovell 1988
	20	57	41	Linear	N deposition	0.37	Diogenes *et al.*, 2016
Arg	0.02	56	1.02	Broken line	WG	1.18	Santiago & Lovell 1988
	2.95	85	63	Quadratic	WG	1.36	Neu *et al.*, 2016
	20	57	52	Linear	N deposition	1.95	Diogenes *et al.*, 2016
	82	60	286	Quadratic	WG	1.51	Wu *et al.*, 2016
	82	60	286	Quadratic	FCR & PER[h]	1.58	Wu *et al.*, 2016
Iso	0.05	56	1.7	Broken line	WG	0.87	Santiago & Lovell 1988
	20	57	58	Linear	N deposition	0.88	Diogenes *et al.*, 2016
Leu	0.05	56	1.46	Broken line	WG	0.95	Santiago & Lovell 1988
	1.94	56	21	Quadratic	WG	1.25[g]	Gan *et al.*, 2016
	20	57	37	Linear	N deposition	1.5	Diogenes *et al.*, 2016
Val	0.09	56	1.4	Broken line	WG	0.78	Santiago & Lovell 1988
	20	57	37	Linear	N deposition	1.18	Diogenes *et al.*, 2016
His	0.05	56	0.92	Broken line	WG	0.48	Santiago & Lovell 1988
	20	57	53	Linear	N deposition	0.54	Diogenes *et al.*, 2016
	4.84	100	50.23	Quadratic	WG	0.82	Michelato *et al.*, 2017
Phe (Phe+Tyr)	0.01	56	0.97	Broken line	WG	1.05 (1.55)	Santiago & Lovell 1988
	20	57	63	Linear	N deposition	(1.57)	Diogenes *et al.*, 2016
	52.7	60	227	Quadratic	WG	1.17 (2.19)	Jiang *et al.*, 2016
	52.7	60	227	Quadratic	FCR & PER	1.21 (2.23)	Jiang *et al.*, 2016

* values are expressed on as-fed basis unless indicated
[a] InWt–Initial body weight
[b] FnWt–Final body weight
[c] WG–weight gain
[d] R–Requirement
[e] on digestible basis
[f] determined for hybrid tilapia (*O. nilotics* x *O. mossambicus*)
[g] on 100 % dry matter basis
[h] PER–protein efficiency ratio

LIMITATIONS OF AVAILABLE DATA

Review of available data on the EAA requirements show several shortcomings: (i) High variability is observed among the data published for similar body weight (or life stage) of tilapia. Variability observed among studies may partly be explained by factors such as those related to fish (e.g., strain, age), experimental design (e.g., method used, housing condition), feed (e.g., digestibility and availability of test amino acid, balance of other nutrients, feed quality), and statistics (e.g. model used for analysis) (ii) Some of the recent studies (e.g., Liebert 2009, Xiang *et al.*, 2014, Figueiredo-Silva *et al.*, 2015 and He *et al.*, 2017b for methionine and cysteine; Takishita *et al.*, 2009 and Bomfim *et al.*, 2010 for lysine) showed higher dietary requirements for the most commonly limiting EAA than the values reported in the past. Studies that used test diets containing EAA levels (except for the test EAA) lower than the levels reported in these studies likely have underestimated the requirements (iii) Only few studies have reported EAA requirements on digestible basis, while most of the EAA requirement data were published on total basis. Formulating diets to meet EAA requirements on total basis can lower the performance of fish, if diets do not meet requirements on digestible basis because of poor digestibility (iv) Most of the requirement studies conducted in tilapia have focused on their early life stages. However, in commercial farming, tilapia are fed several months to obtain a final market weight of 700–1000 g and requirement data are clearly lacking for the whole production cycle (v) Tilapia strain used to date in most of the commercial farming are genetically improved for better growth (e.g., GIFT strain), and likely have higher dietary requirements for EAAs than the values generated in the past with slow growing strain.

Lack of data reinforce the importance of generating amino acid requirement data for the whole life-cycle of Nile tilapia under different production conditions. The remaining article discusses how factorial approach can be used in determining amino acid requirements for tilapia. This approach is similar to those used for carp (AMINOCarp®), salmonids (AMINOSalmonid®) and shrimp (AMINOShrimp®).

FACTORIAL APPROACH FOR DETERMINING AMINO ACID REQUIREMENTS

Factorial approach treats the total requirement for an amino acid as the sum of its amounts needed to meet the physiological demands of animals for maintenance and growth.

Maintenance may be defined as a state in which an animal maintain all its vital functions without any loss or gain in body tissue. Amino acid requirement for maintenance is derived by determining the amount of amino acid needed for zero protein gain. It is basically the amount of amino acid needed for the losses that occur in intestine and integument (skin), oxidative degradation, conversion of non-protein nitrogen containing molecules, and also those used for protein turnover. Maintenance requirement is proportional to the amount of metabolically active tissue in the body rather than total body weight, and therefore the requirement is computed for metabolic body weight.

Amino acid requirement for growth, on the other hand, is the amount of amino acid needed for protein accretion. This requirement value needs to be further adjusted for the rate at which the absorbed amino acid, after detecting for the basal maintenance needs, is utilized for protein synthesis (accounts for inefficiency of utilization). This is because some extra amount of amino acids are needed for maintaining the newly deposited proteinaceous tissue. Together, amino acid requirement, on digestible basis, can be simplified in the following equation:

AA requirement (digestible basis) =
AA maintenance +
(AA deposition / AA utilization)

Adjusting the value for the digestibility of amino acids in the diet gives us AA levels in the diet on a total basis.

AA requirement (total) =
AA requirement (digestible basis) /
AA digestibility coefficient

This means, different diet formulations can have significantly different amino acid levels on total basis, simply because of differences in amino acid digestibility among diets. Diet with poorly digestible ingredients should be avoided in order to minimize amino acid excretion and nutrient pollution in water which can ultimately affect water quality and fish health.

AMINO ACID NEEDS FOR MAINTENANCE

Maintenance requirements for amino acids in fish are determined by feeding diets containing gradient levels of an amino acid from deficient to excessive (called 'dose-response study'), or by increasing the ration levels of a test feed, e.g., 20 %; 40 %, 60 %, 80 % and 100 % satiation (called 'increasing ration level technique' or 'requirement by ration level'). The former method uses non-linear regression model (e.g., exponential) while the later method commonly adopts linear regression method for determining maintenance needs. Several studies have reported maintenance needs for dietary protein and energy in fish (e. g., Lupatsch *et al.*, 1998 and 2001; Fourier *et al.*, 2002; Libert *et al.*, 2006; Glencross and Bermudes 2011; Glencross *et al.*, 2011; Helland *et al.*, 2013). However, information on the maintenance needs of amino acids for fish is limited. Table 2 summarizes the maintenance requirements of amino acids reported for different fish species. Maintenance needs of EAA in fish was first reported by Rodehutscord *et al.* in 1997 (a collaborative study between Evonik and the University of Bonn). The authors determined maintenance requirements of all the EAA except Phe for rainbow trout. This was followed by studies conducted in Atlantic salmon (Helland *et al.*, 2010; Grisdale-Helland *et al.*, 2011 & 2013) and Atlantic cod (Helland *et al.*, 2011). For Nile tilapia, He *et al.* (2013) recently reported maintenance requirements for both juvenile and adult stages (a collaborative study between Evonik and Sun Yat-sen University).

TABLE 2 Maintenance requirements of amino acids in various fish species

Species	IBW*	Unit	Met	Lys	Thr	Trp	Arg	Ile	Leu	Val	His	Phe
Atlantic cod[1]	100	mg/kg$^{0.7}$/d	3.2	9.0	7.4	0.7	0.2	4.4	9.4	7.6	−0.9	4.9
Atlantic salmon[2]	0.80	mg/kg$^{0.75}$/d			7.2							
Atlantic salmon[3]	1.5	mg/kg$^{0.75}$/d		20.0								
Atlantic salmon[4]	39	mg/kg$^{0.7}$/d	9.70	28.6	9.5	0.8	19	15.9	23.1	17.9	10.9	11.6
Atlantic salmon[5]	86	mg/kg$^{0.7}$/d	2.10	12.5	8.3	2.1	7.7	8.4	14.4	6.1	5.4	5.2
Nile tilapia[6]	21	mg/kg$^{0.7}$/d	3.1	16.9	8.3		17.5	19.4	10.7	10.7	10.0	12.8
Nile tilapia[6]	165	mg/kg$^{0.7}$/d	16.5	68.8	37.6		93.5	86.0	47.2	49.6	36.2	59.0
Rainbow trout[7]	0.78	mg/kg$^{0.75}$/d		21								
Rainbow trout[8]	40–51	mg/kg$^{0.75}$/d	9.1	10.9	18.8	5.9	15.4	5.12	46.5	16.4	6.0	
Various[9]		mg/kg0$^{.75}$/d	18.4	15.6	5.4	0.5	7.7	6.4	9.1	9.3	9.8	14.6

* IBW, initial body weight (g)
[1] Grisdale-Helland *et al.*, 2011; [2] Rollin *et al.*, 2006; [3] Abboudi *et al.*, 2006; [4] Grisdale-Helland *et al.*, 2013; [5] Helland *et al.*, 2010; [6] He *et al.*, 2013; [7] Bodin *et al.*, 2009; [8] Rodehutscord *et al.*, 1997; [9] Hua 2013
[3] Protein value was recalculated from N
[8] Values were recalculated from kg body weight to metabolic body weight

Maintenance needs of amino acids is generally low for rapidly growing early life stages. Proportion of maintenance requirement in the total requirement of an amino acid was reported to vary from 4 % (for lysine and isoleucine) to 32 % (leucine) for juvenile rainbow trout (40–50g) (Rodehutscord *et al.*, 1997). Similarly, Hua (2013), based on model estimate of various published data, showed that maintenance requirements of different amino acids range between 2 % and 31 % of total amino acid requirement in fish. Maintenance needs of amino acids are expected to increase with increasing fish age. He *et al.* (2013) found that maintenance requirements markedly increase for adult tilapia (165 g) relative to their juvenile counterparts (21 g). The study showed that adult tilapia require 4–5 times the amount of maintenance requirements needed for their juveniles. Plotting data of protein maintenance requirements and fish weight of various species although showed a positive slope, no strong relationship was detected (R^2 = 0.12 %) (Figure 1).

Species in Figure 1 included Atlantic cod (Grisdale-Helland *et al.*, 2011), Atlantic salmon (Abboudi *et al.*, 2006, Helland *et al.*, 2010 and Grisdale-Helland *et al.*, 2013), barramundi (Glencross and Bermudes 2010), gilthead seabream Lupatsch *et al.*, 1998 and Fourier *et al.*, 2002), European seabass (Lupatsch *et al.*, 2001 and Fourier *et al.*, 2002), hybrid tilapia (Liebert *et al.*, 2006), Nile tilapia (Liebert *et al.*, 2006, Trung *et al.*, 2011 and He *et al.*, 2013), tra catfish (Masagounder *et al.*, 2016), red tilapia (Liebert *et al.*, 2006), rainbow trout (Fourier *et al.*, 2002) and turbot (Fourier *et al.*, 2002). Lack of strong relationship indicates that maintenance needs of protein are likely different for different species.

However, a strikingly strong relationship was found between the maintenance needs of protein and amino acids across data reported for different fish species (Figure 2). Data although show higher maintenance requirement for lysine than that for methionine or threonine, maintenance needs of lysine relative to its total requirement is much lower compared that with other amino acids.

Lysine (maintenance) = 2.25 + 0.062 Protein(maintenance);
R^2 = 0.98 (n = 7)
Methionine (maintenance) = 1.54 + 0.015 Protein(maintenance);
R^2 = 0.90 (n = 6)
Threonine (maintenance) = 2.57 + 0.026 Protein(maintenance);
R^2 = 0.82 (n = 7)

Similar equations can also be derived for other amino acids. Benefits of such equation is that one can easily predict maintenance needs of amino acids from the established protein maintenance requirements, for which more data are available. This data analysis nevertheless included only few species (Atlantic cod, Atlantic salmon, and Nile tilapia for lysine, methionine and threonine; gilthead seabream for threonine) and therefore, needs to be further strengthened by generating data for more and specific fish species and life stages.

Maintenance requirements can be easily influenced by stress and health conditions of fish. Further studies are warranted in this area to understand the influence of fish size and production conditions on maintenance requirements of amino acids.

Figure 1 Maintenance requirement for protein versus body weight, reported for different fish species

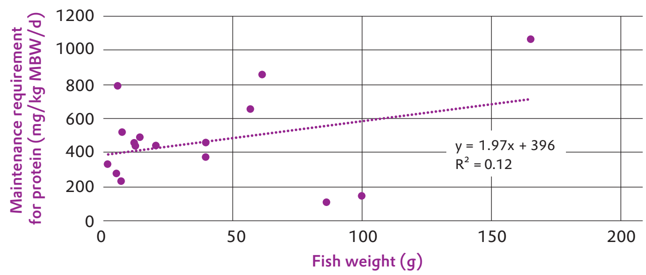
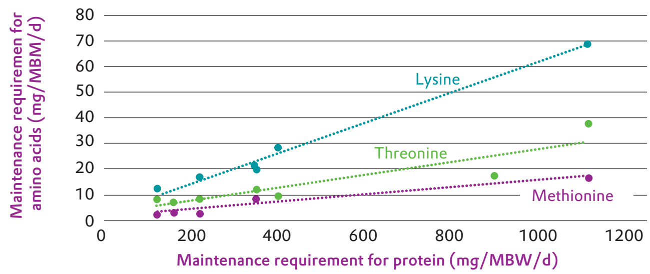


Figure 2 Relationship between maintenance requirement of amino acids and protein across different fish species



AMINO ACID NEEDS FOR WHOLE-BODY PROTEIN DEPOSITION

Amino acids absorbed from the gut, after meeting the obligatory needs for maintenance are diverted for protein accretion, a process that involves both protein synthesis and degradation. In a fast growing fish, major portion of absorbed amino acids are expected to be used for body protein deposition. For genetically improved male Nile tilapia, under ideal growing conditions, weight gain is less than 1 g/d when they are young (<20 g body weight) and is about 7 g/d when they reach 300 g body weight (Santos *et al.*, 2013). Amino acid gain of fish can be determined simply from weight or protein gain, and the amino acid profile of fish. Plotting data from various studies of our own and other published data (n = 85) show that body protein content of Nile tilapia vary from 11 % to 17 %, with values sharply increasing at the early stages and then, plateauing as they approach adult stage (Figure 3). Trung *et al.* (2011) showed that protein level vary from 14 to 17 % for 19 to 963 g size Nile tilapia, which matches quite well with the model fit for the data in Figure 3.

Whole-body protein and amino acid content of Nile tilapia (n = 274) has been analyzed since the year 2010 in Evonik's lab using wet chemistry (AMINOLab®). Interestingly, amino acid profile, expressed as % body protein, showed less variation for all the EAA (around 4 % CV, except for

Figure 3 Relationship between whole-body protein content and body weight of Nile tilapia (n = 85)

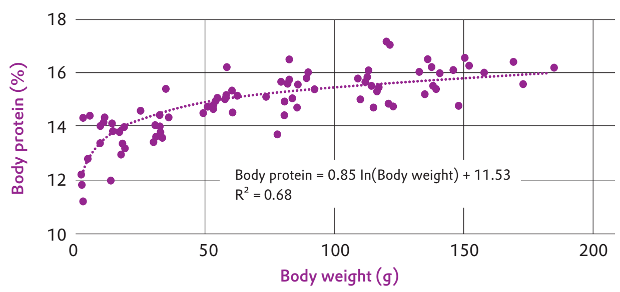


TABLE 3 Whole body amino acid profile expressed as ratio to lysine for Nile tilapia (n = 274 samples) and others species

Species	Met	Met+Cys	Lys	Thr	Trp	Arg	Ile	Leu	Val	His	Phe
Nile tilapia	34	46	100	57	13	88	53	95	62	32	53
Common carp[1]	34	46	100	53		76	52	92	59	38	51
Atlantic salmon[2]		51	100	57	14	75	53	92	63	36	52
Rainbow trout[2]		50	100	58	13	81	54	97	64	37	53

[1] Lemme 2012;
[2] Figueiredo-Silva, 2014

His (7.4 % CV)). Ratio of EAA to lysine showed to vary from 13 % (Trp) to 95 % (Leu) with none exceeding lysine as has been observed in other species (carp, salmonids) (Table 3).

AMINO ACID UTILIZATION FOR PROTEIN DEPOSITION

As the protein deposition increase, cost associated with the maintenance of those newly accreted protein also increase. Efficiency for marginal utilization of an amino acid is computed by feeding fish with test diets containing increasing levels of the test amino acid or by feeding fish with a single diet at increasing ration levels. In both the methods, excess supply of amino acid or feed should be avoided in order to avoid underutilization of amino acid. Utilization of amino acid for its retention is then determined from the slope value after fitting the data with linear regression.

Several reports are available on overall protein utilization efficiency for growth beyond maintenance in fish. Reported protein utilization values are 0.69 for Atlantic cod (Grisdale Helland *et al.*, 2011), 0.64-0.88 for different stages of Atlantic salmon (Helland *et al.*, 2010; Grisdale-Halland *et al.*, 2011 & 2013), 0.55 for barramundi (Glencross and Bermudes 2010), 0.40 and 0.52 for European seabass (Fourier *et al.*, 2002; Lupatsch *et al.*, 2011), 0.28 and 0.38 for gilthead seabream (Lupatsch *et al.*, 1997 & Fourier *et al.*, 2002), 0.52 and 0.34 for Nile tilapia (Trung *et al.*, 2011; He *et al.*, 2013) 0.32 for pangasius (Glencross *et al.*, 2011), and 0.44 for rainbow trout (Fourier *et al.*, 2002). However, studies on the amino acid utilization efficiency for protein accretion beyond the maintenance needs is limited. He *et al.* (2013) (study by Evonik-Sun Yat-sen University) recently reported amino acid utilization efficiency for both juvenile and adult stage tilapia. The study found lowest utilization efficiency for isoleucine (0.29 for juveniles and 0.21 for adult) and highest value for leucine (0.87 for juvenile and 0.63 for adult). In general, utilization efficiency of amino acids (e. g., methionine, threonine, tryptophan) that have several functional roles beyond muscle protein synthesis are expected to be low. Study reports on amino acid retention efficiency can also be used to understand the amino acid utilization efficiency. If the maintenance amino acid requirement is known, utilization efficiency can be calculated from the reported amino acid retention efficiency.

Amino acid utilization efficiency can also be influenced by dietary factors. Utilization of an amino acid for protein growth depends on the availability of other amino acids needed for the protein synthesis. Deficiency of an amino acid can limit the protein synthesis while excess of an amino acid cannot be stored by cells, both of which can lead to underutilization of amino acids. In hybrid tilapia (*O. nilotics* x *O. mossambicus*), Figueiredo-Silva *et al.*, 2015 (FF 1610, a collaborative study between Evonik and Thaksin University) showed that as the methionine level increased in the soybean based diets, retention efficiency of lysine (from 39 to 53 %) and protein (from 26 to 46 %) have significantly increased. The study found that methionine was inadequate in the control diet, and increase of methionine supply improved the utilization of other amino acids, and thus, the utilization of dietary protein. However, the study observed decrease in methionine utilization from 55 % to 33 % for the increasing dietary methionine levels. This is because as the fish approaches its maximum capacity of protein synthesis, efficiency of amino acid utilized for protein synthesis drops, in line with the law of diminishing return. Similar observation was also made in another study by Yangtze River Fisheries Research Institute (FF 1613) in hybrid tilapia (*O. nilotics* x *O. aureus*). These studies bolster the importance of providing fish with ideal balance of amino acids. It should be further noted that limitations in the availability of other nutrients can also affect amino acid utilization for protein synthesis. A recent study by He *et al.* (2016) showed that amino acid retention efficiency of juvenile Nile tilapia significantly reduces when the dietary energy was dropped from 12.4 to 10.9 MJ/kg.

OPTIMIZING FEEDING FREQUENCY TO MAXIMIZE PROTEIN UTILIZATION

Tilapia is an omnivorous species with relatively small stomach and long intestine. They have a tendency to eat small meals all day long and benefit from feeding several times a day as they lack big stomach to store food. Several studies show evidence for increase in feeding frequency improving the performance of Tilapia. Pouomogne and Ombredane (2001) fed 31 g size Nile tilapia at 2, 3 and 6 meals a day for 109 days in earthen ponds. The study found that feeding tilapia 6 times a day elicits a daily growth of 1.3 g as opposed to only 0.9 g daily growth for lower feeding frequency (2 or 3 times a day). In addition, significant improvements were observed with FCR (from 1.6 to 1.3) and protein efficiency ratio (from 2.3 to 2.8), as the feeding frequency increased from 2 to 6 meals per day. Villarroel *et al.* (2011) observed a tendency of improvements in the performance of Nile tilapia (24 g, initial weight) reared in tanks, when the daily feeding frequency was increased from 2 to 4. A 28-d study conducted in Bangladesh (Ferdous *et al.*, 2014) evaluated three feeding frequencies (3, 4 and 5 times per day) for Nile tilapia fry (0.018 g initial weight) in hapa system. Results showed significant improvements in final body weight (by 71 %), FCR (by 13 points) and survival (by 14 %) as the daily feeding frequency increased from 3 to 5. Benefits of continuously feeding Nile tilapia using automatic feeding system was evaluated by Sousa *et al.* (2012) for juvenile Nile tilapia (16 g, initial weight). This study, lasted for 126 days, show that feeding tilapia during day once per hour (12 times per day) versus once in every two hours (6 times per day) produce only numerical improvements in performance. Additionally, the study recorded no significant improvements in the performance of tilapia when extending the feeding from only day to both day and night feeding. It is important to note that in all the above studies, fixed amount of feed computed according to body weight or feeding table was split equally across the number of daily feedings and fed to fish. Satiation feeding at every meal with increased daily feeding frequency can cause gastric overload, and thus, increased gut evacuation rate (Riche *et al.*, 2004). Therefore, while increasing daily feeding frequency, it is equally important to optimize amount of feed to be fed to tilapia for different life stages.

Overall, available data show evidence of performance improvements in tilapia with a daily feeding frequency of up to at least 6 times. This should be coordinated with optimal amount of daily feeding to ensure better nutrient utilization and thus, maximize profitability. These factors are considered in the development of factorial-approach based amino acid recommendations for Nile tilapia.

NATURAL FOOD IN DIFFERENT FARMING SYSTEMS

Nutrition and feeding management are extremely important for the successful farming of fish. Tilapia farming is practiced largely in earthen ponds and cages, but also in pens, tanks and raceways. Fish grown in outdoor systems can have access to natural food, contribution of which varies depending on the farming system, management practice, intensity of production, and life stage of fish.

In the earthern ponds, farming is practiced at different levels of intensity. In the extensive farming system, fish rely predominantly on natural food which are produced by feeding ponds with organic and inorganic fertilizers. Supplemental feed is not normally provided in the extensive farming. Fish yield in this type of farming is low, and does not exceed 2 tonnes per ha. On the other hand, in the semi-intensive farming, along with natural food, produced by pond fertilization, fish are also fed with commercial feed. The fish yield is moderate and can range from 6 to 10 tonnes per ha. In the intensive farming, ponds are stocked with fish at very high density. Availability of natural food is very limited or nil, because of high density of fish and continuous renewal of water. Fish production is completely dependent on the commercial feed. High fish harvest is expected, and the yield can vary from 15 to 20 tonnes or even more per ha. Cage farming of tilapia is practiced largely in lakes, reservoirs and river where the contribution of natural food is low or negligible. However, cages and net pens installed in fertilized ponds can have access to natural food. Semi-intensive and intensive farming systems are practiced in cage farming, with production completely depending on commercial feeds and yields varying from 30 to 150 kg/m^3. Intensive farming system is practiced in concrete ponds, tanks, and raceways where contribution of natural food is nil.

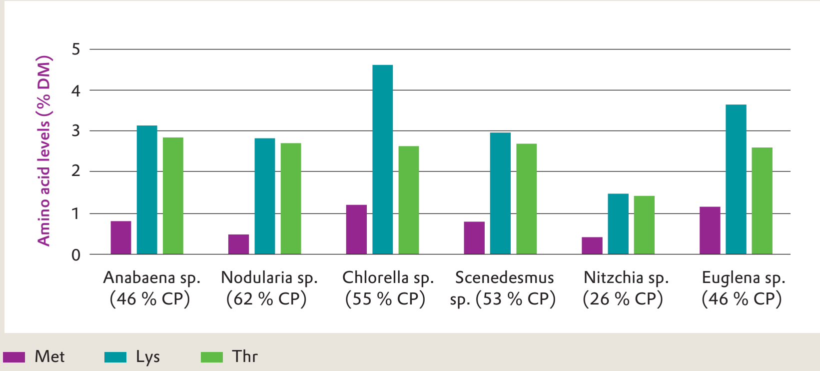
DIETARY CONTRIBUTION OF NATURAL FOOD IN FERTILIZED EARTHERN PONDS

Among the different farming types, feed management of semi-intensive earthern pond system needs special attention, as fish generally have access to both natural food and the commercial feed. Fertilization of ponds results in development of natural food dominated by phyto and zooplankton communities. Understandings of the amount of natural food contributed to the total feed intake of tilapia is important to adjust the feeding and nutrition of fish in semi-intensive farming. Diana *et al.* (1994) investigated growth performance of tilapia in ponds by comparing treatments, (i) pond fertilization without supplemental feeding, (ii) feeding only without pond fertilization, and (iii) both pond fertilization and supplemental feeding. The trial lasted for 162 days showed that relative to the group fed commercial feed alone, growth rate was about 7 % lower for the group had access to both pond fertilization and supplemental feed and 44 % lower for the group had access only to pond fertilization. Further analysis of the data revealed that feed input was 36 % lower in the group had both pond fertilization and supplemental feed, indicating the compensation from the natural food. The author further showed that the feed input can be restricted (up to 50 %) in ponds where natural food is available to fish. Similarly, Focken *et al.* (1999) based on stable isotope analysis found that 30 % of the carbon in fat-free matter and of the nitrogen originate from natural food in tilapia. Furthermore, study by Focken *et al.* (2000) in hybrid tilapia suggested that although proportion of natural food can be very low in the gut at a given time, gut evacuation rate is much faster for the natural food versus the supplemental feed and therefore, overall contribution by natural food is still significant in semi-intensive farming system. This differential gut evacuation is likely attributed to the fact that natural food is nutritionally less dense with very high water content (up to 90 %) relative to the supplemental feed.

In this type of farming system the challenge for the nutritionist is then to adjust the nutrient profile of feed given to fish based on the composition and nutrient profile of plankton in order to maximize overall amino acid utilization. Tilapia are visual feeders when they are young, feeding actively on selective zooplankton, and shift to filter feeding mode when they reach 6–7 cm standard length (about 10 g) (Beveridge and Baird 2000). Similarly, other studies showed that Nile tilapia consume zooplankton when they are less than 5 cm total length (about 2 g) and then gradually switch to phytoplankton (Moriarty and Moriarty 1973; Yada 1982). Abdel-Tawwab (2003), by analyzing the stomach content of Nile tilapia (15–20 g, initial weight) grown in ponds over a period of 125 days, documented that main microalgae species consumed by tilapia belonged to the groups, Cyanobacteria, Clorophyta, Bacillariophyta and Euglenophyta. His study further revealed that zooplankton did not exceed 1.5 % of total stomach components. Overall, these studies indicate that phytoplankton plays a major role in the nutritional contribution of Nile tilapia during grow-out phase.

Several studies have been published on the amino acid profile of phytoplankton or microalgae that are used as food source by tilapia (Boyd 1973; Brown 1991; Pushparaj *et al.*, 1995; Becker 2007). Protein content (% dry matter) of these algae varied from 26 % (Nitschia closterium) to 62 % (Nodularia sp.) (Figure 4.). Amino acid profile expressed as % CP show similar pattern across different species of micro algae (Brown 1997). However, actual content of amino acids varied among species because of differences in protein levels (Figure 4). In addition, methionine to lysine ratio found to be low (17–32 %) for different microalgal species reported. While presence of natural food spares the amount of commercial feed, amino acid profile of feed to be fed in this type of system is to be defined.

Figure 4 Crude protein and amino acid (Met, Lys, Thr) content (% DM) of phytoplankton typically consumed by tilapia



Our EAA recommendations are adjusted for this type of farming, considering the amino acid profile of plankton typically consumed by tilapia.

In summary, data on the EAA requirements of tilapia published in the literature is largely limited to juvenile stage and given as a fixed value. AMINOTilapia® tool enables users optimize amino acid recommendations for different growth stages, by simulating different production scenarios, and thus, to maximize tilapia production and profitability.

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Facts & Figures No. 1610: Responses of red hybrid tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*) to incremental supplementation of DL-Methionine to low fish meal diets.

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