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Methionine Requirement in Practical Diets of Juvenile Nile Tilapia, *Oreochromis niloticus*

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Abstract

Two feeding experiments were conducted to confirm methionine requirement in practical diets of juvenile Nile tilapia, *Oreochromis niloticus*. Test diets used in both experiments contained 414 kcal gross energy, 28 g protein, and 5 g lipid per 100 g diet. In the first experiment, seven diets were made using cottonseed meal (CSM), dehulled solvent-extracted soybean meal (DSESM), and gelatin as intact protein sources. Methionine was added to five of these diets at 0.03 or 0.06% increments to produce methionine levels ranging from 0.33 to 0.57% of the diet. Each diet was fed to four replicate groups of male juvenile Nile tilapia (5.62 ± 0.13 g) in a recirculation system for 8 wk. Broken-line regression analysis of weight gain indicated that methionine requirement of juvenile Nile tilapia was 0.49% of the diet or 1.75% of dietary protein at cystine level of 0.45% of the diet. The second experiment was designed based on methionine requirement determined in the first experiment and also contained seven test diets. The first six diets contained CSM and DSESM as protein sources. Methionine was added to five of these diets at an increasing rate of 0.06% to produce methionine levels ranging from 0.49 to 0.79% of the diet. In the last diet (Diet 7), a portion of DSESM was replaced by gelatin to reduce methionine level to 0.33% of the diet in order to test whether methionine is limited. Each diet was also fed to four replicate groups of male juvenile Nile tilapia (2.32 ± 0.06 g) in a recirculation system for 9 wk. At the termination of the second experiment, there were no significant differences in terms of weight gain, survival, and feed efficiency ratio (FER) among the fish fed the first six diets. However, weight gain and FER of the fish fed these diets were significantly better than those fed Diet 7, confirming the methionine requirement value as has been determined in the first experiment.

The main constraint in formulating nutritionally balanced diets for tilapia cultured in intensive and semi-intensive systems is the paucity of nutritional knowledge (Jackson and Capper 1982). Traditionally, fish meal has been used as the main protein source in aquatic feed because of its high protein content and balanced essential amino acid profile. However, the production of fish meal based on captured fisheries is at or beyond sustainable limits. The limited supply coupled with an increasing demand from the animal feed industry results in a high price of this ingredient. Given the escalating cost of fish meal, it is critical that all animal production systems reduce their reliance on fish meal. This is particularly true in case of feeds for aquatic animal species as they often contain high levels of fish meal (El-Sayed 1999). Therefore, many studies have been conducted to totally or partially replace fish meal with cheap and locally available protein sources. The inclusions of non-fish meal protein sources into tilapia feeds have allowed the production of cost-effective feeds. This makes tilapia culture more competitive in the global market.

To formulate cost-effective feeds, nutrient requirements of tilapia must be known, especially those that are limiting in low-cost feed ingredients. Quite often, methionine is one of the most limiting essential amino acids in non-fish meal protein sources commonly used in tilapia

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feeds. Thus, to maximize the utilization of cheap plant and animal protein sources, especially those that have low methionine levels, the determination of methionine requirement in semipurified diets and its application to practical diets are important for the development of cost-effective feed for tilapia. It is also critical to investigate the effects of free methionine supplementation to practical diets that are deficient in methionine. Generally, feeds produced by supplementation of free methionine to methionine-deficient protein sources are often cheaper than those formulated from ingredients with high levels of methionine such as fish meal. If Nile tilapia could use free methionine efficiently, there would be a great potential to produce cost-effective feeds using non-fish meal protein sources.

Nguyen (2007) determined that the total sulfur amino acid (TSAA) requirement of juvenile Nile tilapia in semipurified diets was 0.85% of the diet or 3.04% of dietary protein and cystine could replace up to 49% methionine requirement based on an equimolar sulfur basis. This means that methionine requirement of juvenile Nile tilapia fed semipurified diets was 0.43% of the diet or 1.54% of dietary protein in the presence of cystine at 0.42% of the diet or 1.50% of dietary protein.

Because semipurified test diets used purified protein sources (gelatin and casein) in which the nutrients are highly digestible, the TSAA requirement value determined using these diets is often lower than that determined using practical diets. Therefore, it is also critical to determine the TSAA requirement in practical diets before the application of this value to formulate commercial tilapia feeds. The objectives of this study were to determine and to confirm methionine requirement in practical diets for juvenile Nile tilapia based on the value obtained from semipurified diets.

Materials and Methods

Experimental Fish and Diet Preparation

Two feeding experiments were conducted at the E. W. Shell Fisheries Center in Auburn, Alabama. Both experiments used Nile tilapia fry (Ivory Coast strain) spawned at this center, collected as swim-up fry from earthen ponds. Fry were stocked into 45-L aquaria of an indoor recirculation system and fed with methyltestosterone-treated feed (Rangen Inc., Buhl, ID, USA) for 4 wk and then with a commercial fry feed (AquaMax, St. Louis, MO, USA) until the beginning of each experiment. Experimental diets were made and stored at the Fish Nutrition Laboratory, E. W. Shell Fisheries Center. Feed ingredients were ground, weighed, and then homogenized in a Hobart mixer (Hobart Inc., Troy, OH, USA) for 20 min. After mixing, hot water was added to produce a mash appropriate for extruding. All diets were then extruded using a 3-mm die, dried at 35 C in a forced-air oven for 24 h, and stored at −20 C until fed. Diet 1 (first experiment) and Diet 7 (second experiment) were sent to New Jersey Feed Laboratory, Inc., Trenton, NJ, USA, for amino acid profile (Table 1) and methionine and cystine analyses, respectively.

Experiment 1

The first experiment was designed to determine the methionine requirement in practical diets of juvenile Nile tilapia. Seven test diets were formulated using cottonseed meal (CSM), dehulled solvent-extracted soybean meal (DSESM), and gelatin as intact protein sources. Methionine was added to Diets 3–7 at 0.03 or 0.06% increments to provide methionine levels ranging from 0.33

TABLE 1. *Amino acid profile (analyzed values) of the basal diet in the first experiment.*

Amino acid	Percent of sample
Methionine	0.33
Cystine	0.45
Lysine	1.31
Phenylalanine	0.99
Leucine	1.52
Isoleucine	0.81
Threonine	0.82
Valine	0.86
Histidine	0.47
Arginine	2.22
Glycine	3.49
Aspartic acid	2.90
Serine	1.30
Glutamic acid	4.99
Proline	1.64
Hydroxyproline	0.96
Alanine	1.70
Tyrosine	0.49

to 0.57% of the diet. For other dietary essential amino acids, which have lower levels than those reported by Santiago and Lovell (1988), crystalline forms were added (except Diet 1) to reach 110% requirement to ensure they are not limited (Table 2). Each diet was fed to four replicate groups of juvenile Nile tilapia (5.62 ± 0.13 g) in a recirculation system for 8 wk.

Experiment 2

The second experiment was designed to confirm methionine requirement in practical diets for juvenile Nile tilapia. This experiment also consisted of seven test diets. The first six diets used CSM and DSESM as protein sources. Methionine was added to five of these diets at 0.06% increment to produce methionine levels ranging from 0.49 to 0.79% of the diet. In the last diet (Diet 7), a portion of DSESM was replaced by gelatin to reduce methionine level to 0.33% of the diet in order to test whether methionine is limited (Table 3). Each diet was also fed to four replicate groups of juvenile Nile tilapia (2.32 ± 0.06 g) in a recirculation system for 9 wk.

Experimental Procedures

Juvenile Nile tilapia were randomly stocked into 45-L aquaria of a 2500-L indoor recirculation system of 15 fish (first experiment) and 12 fish (second experiment) per aquarium. There

TABLE 2. *Composition of seven practical diets fed to tilapia in the first experiment.*

	Diet						
Ingredient (g/100 g)	1	2	3	4	5	6	7
Cottonseed meal[1]	15.00	15.00	15.00	15.00	15.00	15.00	15.00
DSESM[2]	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Gelatin[3]	10.00	9.20	9.20	9.20	9.20	9.20	9.20
Menhaden fish oil[4]	3.25	3.25	3.25	3.25	3.25	3.25	3.25
Wheat starch[3]	44.25	44.23	44.20	44.17	44.11	44.05	43.99
Trace mineral premix[5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix (choline and vitamin C free)[6]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Stay C (25% vitamin C activity)[7]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calcium phosphate, dibasic[3]	2.90	2.90	2.90	2.90	2.90	2.90	2.90
L-Histidine[3]	0.000	0.032	0.032	0.03	0.03	0.03	0.03
L-Isoleucine[3]	0.000	0.117	0.117	0.11	0.11	0.11	0.11
L-Lysine[3]	0.000	0.185	0.185	0.18	0.18	0.18	0.18
L-Phenylalanine[3]	0.000	0.058	0.058	0.05	0.05	0.05	0.05
L-Threonine[3]	0.000	0.360	0.360	0.36	0.36	0.36	0.36
L-Tryptophan[3]	0.000	0.070	0.070	0.07	0.07	0.07	0.07
L-Methionine[3]	0.00	0.00	0.03	0.06	0.12	0.18	0.24
Methionine (% of the diet)	0.33	0.33	0.36	0.39	0.45	0.51	0.57
Cystine (% of the diet)	0.45	0.45	0.45	0.45	0.45	0.45	0.45

DSESM = dehulled solvent-extracted soybean meal.

[1] Faithway Feed Co. Inc., Guntersville, AL, USA.

[2] Southern Sates Cooperative Inc., Richmond, VA, USA.

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

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

[5] Contained (as g/kg premix): cobalt chloride, 0.04; cupric sulfate pentahydrate, 2.50; ferrous sulfate, 40.00; magnesium sulfate anhydrous, 138.62; manganous sulfate monohydrate, 6.50; potassium iodide, 0.67; sodium selenite, 0.10; zinc sulfate heptahydrate, 131.93; and cellulose, 679.64.

[6] Contained (as g/kg premix): thiamine-HCl, 0.438; riboflavin, 0.632; pyridoxine-HCl, 0.908; D-pantothenic acid hemicalcium salt, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B_{12}, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; vitamin A acetate (500,000 IU/g), 0.677; vitamin D_3 (1,000,000 IU/g), 0.116; DL-alpha-tocopheryl acetate (250 IU/g), 12.632; and alpha-cellulose, 955.589.

[7] Hoffman-La Roche Vitamins Inc., Parsippany, NJ, USA.

TABLE 3. *Composition of seven practical diets fed to tilapia in the second experiment.*

	Diet						
Ingredient (g/100 g)	1	2	3	4	5	6	7
Cottonseed meal[1]	15.00	15.0	15.0	15.0	15.0	15.0	15.0
DSESM[2]	37.00	37.0	37.0	37.0	37.0	37.0	21.0
Gelatin[3]	0.00	0.00	0.00	0.00	0.00	0.00	10.0
Menhaden fish oil[4]	3.25	3.25	3.25	3.25	3.25	3.25	3.25
Wheat starch[3]	31.35	31.2	31.2	31.1	31.1	31.0	37.3
Whole wheat[3]	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Trace mineral premix[5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix (choline and vitamin C free)[6]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Stay C (25% vitamin C activity)[7]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calcium phosphate, dibasic[3]	2.80	2.80	2.80	2.80	2.80	2.80	2.80
L-Methionine[3]	0.00	0.06	0.12	0.18	0.24	0.30	0.00
Methionine (% of the diet)	0.49	0.55	0.61	0.67	0.73	0.79	0.33
Cystine (% of the diet)	0.51	0.51	0.51	0.51	0.51	0.51	0.45

DSESM = dehulled solvent-extracted soybean meal.

[1] Faithway Feed Co. Inc., Guntersville, AL, USA.

[2] Southern Sates Cooperative Inc., Richmond, VA, USA.

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

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

[5] Contained (as g/kg premix): cobalt chloride, 0.04; cupric sulfate pentahydrate, 2.50; ferrous sulfate, 40.00; magnesium sulfate anhydrous, 138.62; manganous sulfate monohydrate, 6.50; potassium iodide, 0.67; sodium selenite, 0.10; zinc sulfate heptahydrate, 131.93; and cellulose, 679.64.

[6] Contained (as g/kg premix): thiamine-HCl, 0.438; riboflavin, 0.632; pyridoxine-HCl, 0.908; D-pantothenic acid hemicalcium salt, 1.724; nicotinic acid, 4.583; biotin, 0.211; folic acid, 0.549; vitamin B_{12}, 0.001; inositol, 21.053; menadione sodium bisulfite, 0.889; vitamin A acetate (500,000 IU/g), 0.677; vitamin D_3 (1,000,000 IU/g), 0.116; DL-alpha-tocopheryl acetate (250 IU/g), 12.632; and alpha-cellulose, 955.589.

[7] Hoffman-La Roche Vitamins Inc., Parsippany, NJ, USA.

were four replicates per treatment. In this system, water temperature was maintained at around 28 C using a submerged 3600-W heater (Aquatic Eco-Systems Inc., Apopka, FL, USA). Dissolved oxygen was maintained near saturation using air stones in each aquarium and the sump tank connected to a regenerative air blower. Dissolved oxygen and water temperature were measured once a day using a YSI-55 digital oxygen/temperature meter (YSI Corporation, Yellow Springs, OH, USA), while pH, total ammonia-N (TAN), and nitrite-N were measured twice per week. pH was measured by an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, OH, USA). TAN and nitrite-N were measured using the methods described by Solorzano (1969) and Parsons et al. (1985), respectively. Photoperiod was set at 14-h light and 10-h dark. Diets were offered to fish at 4.5–6.0% body weight (BW) daily according to fish size and divided into two equal feedings at 0800–0900 h and 1600–1700 h. Fish were weighed weekly, and feed rations adjusted accordingly. At the end of each experiment, fish were counted and group weighed to determine weight gain, survival, and feed efficiency ratio (FER).

Statistical Analyses

Statistical analyses were performed using SAS (version 9.1; SAS Institute, Cary, NC, USA). Data from both experiments were analyzed using one-way ANOVA to determine if there were significant differences ($P \leq 0.05$) in weight gain, survival, and FER. Student–Newman–Keuls multiple comparison test (Steel and Torrie 1980) was used to determine differences among treatment means. Regression analysis of weight gain using broken-line model (Robbins 1986) was used to determine methionine requirement in practical diets for juvenile Nile tilapia.

Results

Experiment 1

The means (±SD) of water quality variables during the first experiment were as follows: dissolved oxygen, 6.56 ± 0.27 mg/L; water temperature, 28.3 ± 0.4 C; TAN, 0.070 ± 0.062 mg/L; nitrite-N, 0.041 ± 0.028 mg/L; and pH, 8.0 ± 0.2. These values were within optimum ranges for normal growth and health of juvenile Nile tilapia (El Gamal 1988; Wangead et al. 1988; Watanabe et al. 1993; El-Shafai et al. 2004).

There were no significant differences in survival and FER among treatments (Table 4). Juvenile tilapia offered diets with different methionine levels had the same survival and FER. The addition of crystalline essential amino acids (except methionine) to Diet 2 had no positive effects on weight gain, survival, and FER compared to nonsupplemented diet (Diet 1), indicating that they were not limited in test diets using CSM, DSESM, and gelatin as intact protein sources. However, there were significant differences in terms of weight gain among treatments as dietary methionine levels increased from 0.33 to 0.51% of the diet (Diets 1–6). Increasing dietary methionine level to 0.57% of the diet did not further increase weight gain (Diet 7). Broken-line regression analysis of weight gain indicated that methionine requirement of juvenile Nile tilapia was 0.49% of the diet or 1.75% of dietary protein at cystine level of 0.45% of the diet or 1.61% of dietary protein.

TABLE 4. *Weight gain, survival, and FER of juvenile Nile tilapia fed different test diets in the first experiment.*[1]

Diet	Weight gain (%)[2]	Survival (%)	FER
1	474^{d}	98.3^{a}	0.64^{a}
2	488^{cd}	93.3^{a}	0.60^{a}
3	516^{bcd}	100.0^{a}	0.68^{a}
4	593^{abc}	85.0^{a}	0.63^{a}
5	626^{ab}	91.7^{a}	0.71^{a}
6	685^{a}	91.7^{a}	0.73^{a}
7	697^{a}	93.3^{a}	0.73^{a}
PSE	25.2	5.7	4.6
P value	<0.0001	0.62	0.34

FER = feed efficiency ratio; PSE = pooled standard error.

[1] Values are means of four replicates. Means with the same superscript in the same column are not significantly different ($P > 0.05$).

[2] Weight gain (%) = ([final weight − initial weight]/initial weight) × 100.

Experiment 2

The means (±SD) of water quality variables during the second experiment were as follows: dissolved oxygen, 6.56 ± 0.38 mg/L; water temperature, 28.5 ± 0.9 C; TAN, 0.061 ± 0.055 mg/L; nitrite-N, 0.014 ± 0.009 mg/L; and pH, 8.1 ± 0.1. These values were within optimum ranges for normal growth and health of juvenile Nile tilapia (El Gamal 1988; Wangead et al. 1988; Watanabe et al. 1993; El-Shafai et al. 2004).

There were no significant differences in survival rate among treatments. The survival in all treatments ranged from 89.6 to 95.9%. However, there were significant differences among weight gain and FER between the first six treatments and the Treatment 7 (Table 5). Fish offered Diets 1–6 grew at about the same rate but faster and used feeds more efficiently than those offered Diet 7. Weight gain and FER of juvenile Nile tilapia of Treatments 1–6 varied from 1126 to 1264% and from 0.79 to 0.85, respectively, compared to 804% and 0.64, respectively, for the group fed Diet 7. Weight gain and FER of tilapia in the first six treatments were not significantly different. The results of the second experiment indicated that methionine level of Diet 7 was deficient, but those of Diets 1–6 were sufficient to meet the requirement as determined in the first experiment. When methionine requirement has been met, supplementation of crystalline methionine to Diets 2–6 had no positive effect on weight gain, survival, and FER.

Discussion

The utilization of crystalline amino acids in practical diets of fish depends on the species ability to absorb and use such amino acids for protein synthesis as well as for other physiological

TABLE 5. *Weight gain, survival, and FER of juvenile Nile tilapia fed different test diets in the second experiment.*[1]

Diet	Weight gain (%)[2]	Survival (%)	FER
1	1126^{a}	95.9^{a}	0.79^{a}
2	1130^{a}	95.9^{a}	0.81^{a}
3	1170^{a}	89.6^{a}	0.80^{a}
4	1208^{a}	95.9^{a}	0.83^{a}
5	1160^{a}	95.9^{a}	0.82^{a}
6	1264^{a}	95.9^{a}	0.85^{a}
7	804^{b}	91.7^{a}	0.64^{b}
PSE	50.5	3.4	1.6
P value	<0.0001	0.73	<0.0001

FER = feed efficiency ratio; PSE = pooled standard error.

[1] Values are means of four replicates. Means with the same superscript in the same column are not significantly different ($P > 0.05$).

[2] Weight gain (%) = ([final weight − initial weight]/initial weight) × 100.

functions. In tilapia, varying results have been obtained with crystalline amino acid supplementation. Weight gain of *Oreochromis niloticus* was improved when a 35% protein diet formulated from casein was supplemented with either arginine and lysine or tryptophan and methionine (Teshima et al. 1986). Growth and feed efficiency of hybrid tilapia, *O. niloticus* × *Oreochromis aureus*, were not improved when either methionine and lysine or threonine were added to a 24% protein diet containing soybean meal as the main protein source (Liou et al. 1986). The supplementation of lysine to practical diets containing white fish meal, heat-treated solvent-extracted soybean meal, and expeller groundnut cake as intact protein sources improved growth of Mozambique tilapia, *Sarotherodon mossambicus* (Jackson and Capper 1982). Tacon et al. (1983) reported that growth of Nile tilapia was improved to a level comparable to that obtained from a fish meal-based diet when 0.8% D,L-methionine was supplemented to a diet in which 75% of brown fish meal was replaced by soybean meal. In the first experiment, weight gain of juvenile Nile tilapia increased significantly as methionine levels increased by supplementation of crystalline methionine, indicating that juvenile Nile tilapia could use crystalline methionine efficiently when methionine levels in practical diets were deficient. The study that showed no positive effect of crystalline amino acid supplementation to practical diets for tilapia (Liou et al. 1986) may be because the amino acid in question was not deficient. Results of the second experiment supported this interpretation. Because methionine level in Diet 1 of the second experiment (0.49% of the diet or 1.75% of dietary protein) has met the requirement as determined in the first experiment, supplementation of methionine to Diets 2–6 had no positive effect on weight gain and FER. Nguyen (2007) also demonstrated that supplementation of methionine to practical diets containing DSESM and expeller-pressed soybean meal as protein sources, or a combination of DSESM, CSM, and meat and bone meal, did not improve growth, survival and, feed conversion ratio of juvenile tilapia, *Oreochromis* spp., because methionine levels in non-supplemented diets have met the requirement. The test diets used in either experiment contained cystine at levels similar to those of methionine. Because cystine could replace up to 49% of methionine requirement for juvenile Nile tilapia as determined using semipurified diets (Nguyen 2007), the conversion of methionine to cystine was assumed not to occur.

When methionine requirement has been met, increasing levels of free methionine supplementation to diets containing groundnut, soya, fish meal, and crystalline amino acids as protein sources resulted in a significant reduction in specific growth rate of Mozambique tilapia (Jackson and Capper 1982). However, the results of the second experiment showed no adverse effects of excessive levels of methionine on weight gain, survival and, FER of juvenile Nile tilapia. The difference in the response of tilapia to excessive levels of methionine may be because of tilapia species used in feeding experiments. The results of the present experiments demonstrated that juvenile Nile tilapia could efficiently use crystalline methionine, and no negative effects in terms of weight gain, survival, or FER were found in diets that had methionine at levels above the requirement.

Nguyen (2007) determined that the TSAA requirement of juvenile Nile tilapia in semipurified diets was 0.85% of the diet or 3.04% of dietary protein and cystine could replace up to 49% methionine requirement based on an equimolar sulfur basis. This means that methionine requirement of juvenile Nile tilapia fed semipurified diets was 0.43% of the diet or 1.54% of dietary protein. Because these diets used purified protein sources (gelatin and casein) in which the nutrients are highly digestible, the methionine requirement value determined is often lower than that of practical diets. The methionine requirement of 0.49% of the diet or 1.75% of dietary protein determined in this study was slightly higher than that obtained from semipurified diets. These results confirm that for a typical practical diet formulation with 0.45% cystine (or 1.61% of dietary protein), methionine at a level of 0.49% of the diet or 1.75% of dietary protein is adequate for meeting the requirement of juvenile Nile tilapia.

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