FEED SUSTAINABILITY (/ADVOCATE/CATEGORY/FEED-SUSTAINABILITY)

Evaluating excess supplementation in tilapia diets

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Isoleucine, leucine support rapid growth of juvenile Nile tilapia

Tilapias are widely cultured in the tropical and subtropical regions of the world. They are the most diversified species geographically, with increasing production expected to be around 6.4 million metric tons in 2017. Markets are growing steadily with the rapid expansion of tilapia production capacity in Asia and Latin America. Given its importance for global aquaculture, nutritional research geared towards the optimization of feeds for Nile tilapia (*Oreochromis niloticus*) is vital for the tilapia aquaculture industry.

Amino acids are biologically important compounds for animals. Fish cannot synthesize all amino acids, and the 10 essential amino acids (EAAs) must be supplied in feeds at adequate levels. EAAs play important roles in fish growth, nutritional physiology, immunity, behavior, larval metamorphosis, reproduction and resistance to environmental stressors.

The branched-chain proteinogenic amino acids (BCAAs) Ile, Leu and valine (Val), play very important roles in certain biochemical reactions and growth. Ile and Leu deficiency results in biochemical malfunction, including growth retardation in fish. On the other hand, excess dietary Ile and Leu have been found to depress growth of other farmed fish species, and the three BCAAs can display mutual antagonism, with the Ile *vs.* Leu or Leu *vs,* Val antagonism being the most potent.

Earlier research by others defined the dietary requirements of Ile, Leu, and Val of Nile tilapia defined to be 0.87, 0.95 and 0.78 percent, respectively, resulting in a relative ratio of 1:1.1:0.9. This proportional relationship differs relative to ratios found in a range of practical feed ingredients, which display a relative surplus of Leu, but resemble ratios found in whole-body profile (Table 1).

Therefore, the purpose of our study was to evaluate whether excess supplementation of Ile and Leu (at a level of 2.5 percent) in a practical diet would affect the production performance and health indicators of juvenile Nile tilapia. Our research was supported by NSF-HBCU-UP Award #1436426 Targeted Infusion Project: Promoting Minority Students Participation in STEM-Undergraduate Degree Programs at Kentucky State University; and by USDA NIFA-CBG Grant #2013-38821120 Strengthening Environmental Science Program for Preparing Minority Young Scientists for the 21st Century.

Nutritional research to optimize tilapia aquafeeds is vital for the global tilapia aquaculture sector.

Table 1. Isoleucine, leucine, and valine requirement, concentrations, and relative ratios as in requirement, fish whole-body, experimental diets, and selected practical ingredients.

BCAA	Nile tilapia		Experimental diets			Practical ingredients								
	Requirement*	Whole-body	Control	Ile+	Leu+	FM	PBM	SM	SBM	SPC	WG	CPC	BSF	DDGS
						percent as is								
Isoleucine	0.87	0.57	1.69	4.19	1.69	2.50	2.33	3.19	2.31	3.00	2.74	3.15	2.17	1.16
Leucine	0.95	0.98	2.87	2.87	5.37	4.31	4.15	5.27	3.75	4.96	5.24	12.54	3.47	3.38
Valine	0.78	0.66	1.93	1.93	1.93	2.97	3.22	3.35	2.37	3.17	3.07	3.47	3.45	1.49
						Ratios								
Isoleucine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Leucine	1.09	1.72	1.70	0.68	3.18	1.72	1.78	1.65	1.62	1.65	1.91	3.98	1.60	2.91
Valine	0.90	1.16	1.14	0.46	1.14	1.19	1.38	1.05	1.03	1.06	1.12	1.10	1.59	1.28

* percent of the diet (Santiago and Lovell 1988). FM = menhaden fish meal; PBM = poultry by-product meal; SM = squid meal; SBM = soybean meal; SPC = soy protein concentrate; WG = wheat gluten; CPC = corn protein concentrate; BSF = black soldier fly (defatted); DDGS = distillers dried grains w/ solubles.

Table 1. Isoleucine, leucine, and valine requirement, concentrations, and relative ratios as in requirement, fish whole-body, experimental diets, and selected practical ingredients.

Study setup

Three isonitrogenous (40 percent crude protein, CP) and isolipidic (10 percent lipid) experimental diets were formulated to contain different levels of the BCAAs Ile and Leu. A control diet (Control) was formulated using practical ingredients and contained 1.69 and 2.87 percent Ile and Leu, respectively. Two additional diets were formulated identically to Control, but were supplemented with Ile (Ile+) or Leu (Leu+) each at an excess of 2.5 percent substituting for L-aspartic acid (Table 2).

Nile tilapia (~1.5 grams) were obtained from Louisiana Specialty Aquafarms LLC in Harvey, La., and transported to the Aquaculture Nutrition Laboratory located at the Aquaculture Research Center of Kentucky State University. Following acclimation, fish were stocked into a 2,000-liter fiberglass tank operating as a recirculating aquaculture system (RAS) and were fed with a commercial feed (40 percent CP, 12 percent lipid) until they attained the adequate size for the feeding trial.

First author feeding the fish during the trial.

Upon commencement of the feeding trial, groups of 20 juvenile Nile tilapia (4.0 grams, initial weight) were sorted by hand and stocked into nine 110-liter glass aquaria operating as a RAS, and were randomly grouped into three treatments. After a seven-day conditioning period, fish in each of three aquaria were fed three times daily to apparent satiation for eight weeks. Water quality parameters were maintained within acceptable ranges for Nile tilapia. At the end of the feeding trial, after a 24-hour fasting, fish from each aquarium were group-weighed, counted and a representative number of fish (seven total) were sampled for data collection.

Teets, Table 2

Diet	Control	Ile+	Leu+
Supplemental Ile (%)	0	2.5	0
Supplemental Leu (%)	0	0	2.5
	Percent, dry matter basis		
Menhaden fishmeal	10.0	10.0	10.0
Poultry byproduct meal	10.0	10.0	10.0
Soybean meal	35.0	35.0	35.0
Wheat gluten	3.53	3.53	3.53
Wheat flour	27.02	27.02	27.02
Carboxymethyl cellulose	1.5	1.5	1.5
Menhaden oil	2.0	2.0	2.0
Soybean oil	4.7	4.7	4.7
Vitamin premix	0.6	0.6	0.6
Stay C (35 percent vitamin C)	0.3	0.3	0.3
Choline chloride	0.2	0.2	0.2
Mineral premix	0.4	0.4	0.4
Calcium phosphate dibasic	2.0	2.0	2.0
DL-Methionine	0.25	0.25	0.25
L-Aspartate	2.5	0.0	0.0
L-Isoleucine	0.0	2.5	0.0
L-Leucine	0.0	0.0	2.5
Crude Protein (%)	40	40	40
Lipid (%)	10	10	10

Table 2. Design and analyzed composition of the experimental diets fed to juvenile Nile tilapia (4.0 grams, initial weight) for eight weeks.

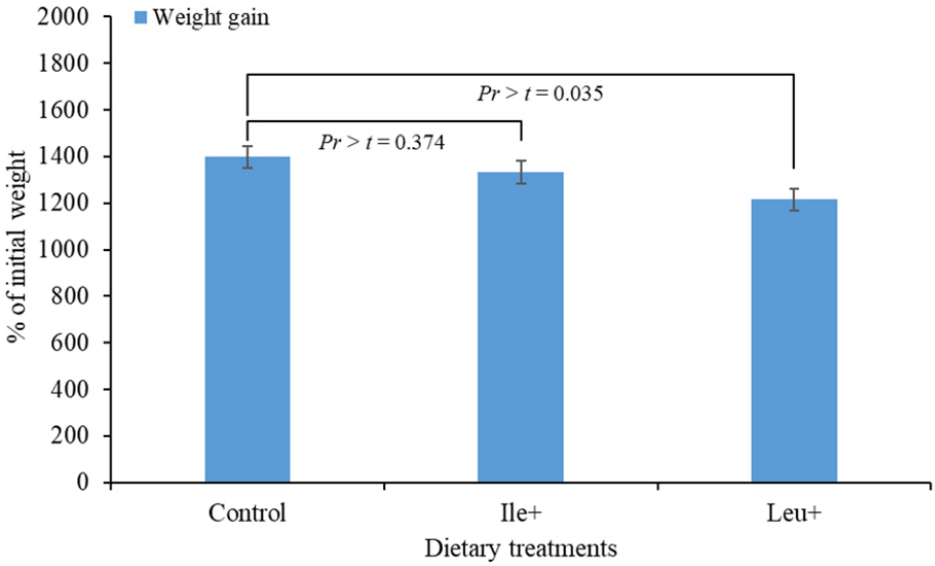

Fig. 1: Weight gain of juvenile Nile tilapia (4 grams, initial weight) after feeding the experimental diets for eight weeks. Connecting lines denote orthogonal contrasts between dietary treatments. Error bars represent SE.

Results and discussion

Teets, Table 3

Diet	Initial weight (g)	Final weight (g)	FI (% BW/day)	Survival (%)
Control	4.0 ± 0.01	59.9 ± 2.0	6.5 ± 0.1	100 ± 0
Ile+	3.9 ± 0.11	56.3 ± 2.4	6.7 ± 0.4	98 ± 2
Leu+	4.0 ± 0.03	52.6 ± 1.6	6.5 ± 0.5	95 ± 0
Contrasts (Pr>t)				
Control vs Ile+	0.493	0.256	0.738	0.267
Control vs Leu+	0.972	0.042	0.946	0.010

Table 3. Production performance of juvenile Nile tilapia (4.0 g initial weight) after 8 weeks of feeding the experimental diets. Mean ± SE.
SE = standard error; FI = feed intake; BW = body weight (g).

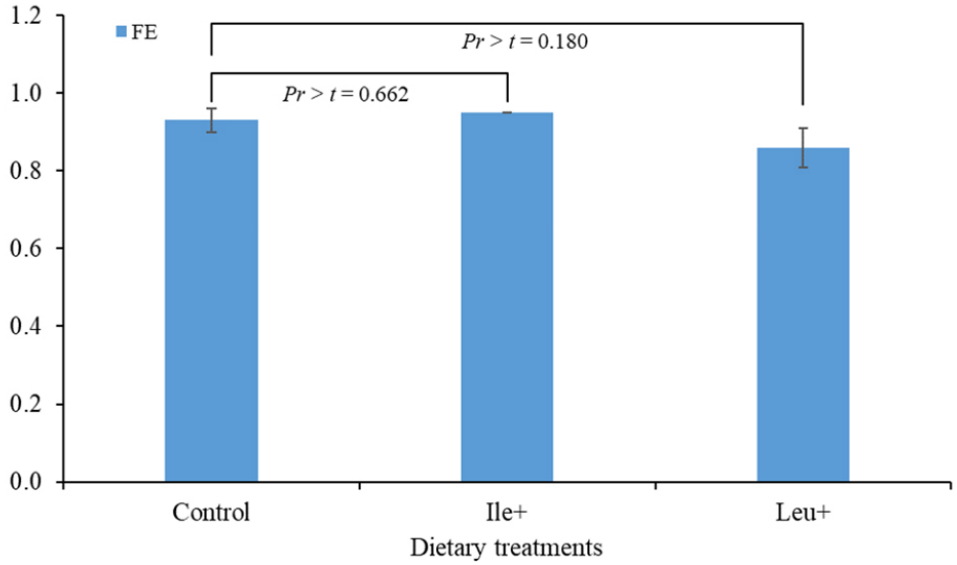

Fig. 2: Feed efficiency (FE) of juvenile Nile tilapia (4 grams, initial weight) after feeding the experimental diets for eight weeks. Connecting lines denote orthogonal contrasts between dietary treatments. Error bars represent SE.

Teets, Table 4

Diet	Albumin g/dL	Globulin g/dL	TBIL mg/dL	ALT U/L	ALP U/L	Amylase U/L	Glucose mg/dL	Phosphorous mg/dL	Sodium mmol/L
Control	1.80 ± 0.35	1.87 ± 0.12	0.50 ± 0.06	23.3 ± 3.3	28.7 ± 1.2	11.3 ± 2.9	55.0 ± 5.5	9.5 ± 1.4	154 ± 3.8
Ile+	1.63 ± 0.26	1.90 ± 0.10	0.43 ± 0.07	25.0 ± 1.5	40.3 ± 4.7	13.7 ± 4.2	50.0 ± 1.0	8.7 ± 0.4	150 ± 0.3
Leu+	2.17 ± 0.37	2.20 ± 0.36	0.40 ± 0.06	27.3 ± 2.4	32.0 ± 3.5	20.5 ± 2.5	52.0 ± 5.5	9.3 ± 0.6	154 ± 1.5
Pr>t									
Control vs. Ile+	0.734	0.921	0.468	0.655	0.054	0.641	0.465	0.583	0.283
Control vs. Leu+	0.463	0.339	0.289	0.303	0.519	0.142	0.656	0.901	0.925

Table 4. Blood parameters of juvenile Nile tilapia (4.0 grams, initial weight) after eight weeks of feeding the experimental diet. Mean ± SE.
SE = standard error; TBIL = total bilirubin; ALT = alanine aminotransferase; ALP = alkaline phosphatase.

Perspectives

The calculated ratios of the BCAAs in the Control diet used in this study indicated a surplus of Leu if compared to the ratios calculated from quantitative requirement values, but were very similar to those calculated based on the profile of whole-tilapia juveniles (~30 grams). Overall, the dietary treatments evaluated in this study supported rapid growth of juvenile Nile tilapia.

Supplementation of BCAA is not a common practice in commercial feed formulations as practical ingredients commonly used would supply adequate amounts of these essential amino acids. Based on our evaluation, only diets formulated using corn protein concentrate as the sole protein source would exceed the total Leu level (5.37 percent) evaluated in this study, and such costly formulation would be economically impractical for Nile tilapia. Nevertheless, from a scientific point of view, identifying the mechanisms underlying potential antagonistic interactions among, or toxicity of, BCAA continues to be an interesting research topic in aquatic animal nutrition.

References available from corresponding author.

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