ESSENTIAL AMINO ACID BALANCE IN CHANNEL CATFISH

ICTALURUS PUNCTATUS DIETS

By

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Abstract

Channel catfish are among the top cultured aquaculture species in the U.S. Protein is an important factor in catfish feeds to allow for proper health and development and accounts for 50%-60% of the total feed cost. Due to the high cost of protein associated with feeds, cheaper alternatives are being explored both through ingredient replacement and the optimization of proteins. This study sought to examine reduced protein diets in channel catfish using a better-balanced amino acid profile and the evaluation of possible limiting amino acids. Towards this goal, a 12-week experimental growth trial was conducted to investigate the effects that dietary protein reduction and amino acid balance have on the growth performance of fingerling channel catfish. A total of nine diets were formulated to contain varying amounts of protein (30% and 24%) and amino acid supplements. In general fish maintained on diets containing 30% (B30) or 24% (B24) protein showed no significant differences in growth. Lysine was confirmed to be the first limiting amino acid in the B24 diet. To further optimize the 24% protein diets, the indispensable amino acid requirements (100%) were raised to 140% to further reduce the amount of dispensable amino acids relative to indispensable. Additionally, for the diets containing 24% protein at 140% of the requirement select amino acids including arginine, tyrosine, isoleucine, tryptophan and threonine were omitted. There was no response to the removal of most of these amino acids; however, the removal of arginine resulted in a significant improvement in growth and feed conversion ratio. Overall, this study found that protein levels in catfish feeds can be reduced to 24% without inhibiting growth or feed utilization. Raising the amino acid requirements to 140% in the 24% protein diets showed no significant difference in growth or protein conversion efficiency when compared to fish fed the 24% protein diet at 100% of the requirement.

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1. Introduction

Channel catfish (*Ictalurus punctatus)* is the top cultured fish species in the U.S. in terms of total value and quantity. (Zimba and Grimm 2003). In 2018, there were 341 million pounds of catfish produced in the United States (Hanson and Sites 2015; Hanson 2019). Most channel catfish production takes place in the southern states including Alabama, Arkansas, Mississippi, and Louisiana with the highest production taking place in Mississippi. This is a well-established industry which supports thousands of rural jobs (Hanson et al. 2021) and has a rich history of research support, particularly by Land Grant Universities in the Southeastern U.S. As with other agriculture operations, economic success of the aquaculture industry is driven by efficient growth and survival of fish which is tied to feed and water quality management.

Feed is the most expensive variable cost associated with aquaculture production. It accounts for 40%-60% of the total aquaculture production costs (Hertrampf and Piedad-Pascual 2000). Grow-out production diets for catfish typically contain from 28% to 32% protein which is the most expensive dietary component in fish feed. The protein constitutes 50%–60% of ingredient costs for aquaculture and is considered the single most important component of aquatic feeds (Mohanta 2012). Various materials such as soybean meal, meat and bone meal, poultry by-product meal, dried distillers grains with solubles and corn gluten are among the most commonly used protein sources in channel catfish feeds. Proper delivery of protein is not only important from a cost standpoint, but it is critically important for fish growth and development. Amino acids are the structural component of proteins. Fish do not have a true protein requirement; they have requirements for a mixture of both dispensable (nonessential) and indispensable (essential) amino acids (Hardy 2002).

There is considerable interest in reducing the protein content of fish feeds not only to reduce feed production costs but also to reduce environmental nitrogen pollution (Cao *et al.* 2007). In fish diets, the source and balance of protein can influence nitrogenous waste and fish growth. Ingredients with poorer digestibility increase nitrogen excretion by reducing nitrogen retention. Furthermore, those that are imbalanced or have low levels of essential amino acids will also lead to higher nitrogen excretion as the protein is degraded and nitrogen excreted. Because of relatively low prices, good digestibility and fair amino acid profiles, plant-based protein sources are often incorporated into production diets Lech and Reigh (2012). However, as an individual protein source they are often low in one or more indispensable amino acid. Thus, multiple proteins are blended, or synthetic amino acid are supplemented to balance the amino acid profile. Therefore, protein retention and nitrogen excretion of fish is dependent on level of dietary protein as well as amino acid profile (Cheng *et al.* 2003). An increase in nitrogen release into effluents caused by poor protein use reduces water quality or requires additional processing to remove waste products. Consequently, there is considerable interest in improving the utilization of protein (Lazzari and Baldisserotto 2008).

Fish have absolute requirements for 10 essential amino acids, which are considered indispensable (Wilson 2003). An indispensable amino acid (IAA) is one that the animal cannot synthesize or cannot synthesize in quantities sufficient for their needs; thus, it must be supplied in the diet. A dispensable amino acid (DAA) is one that can be synthesized by the animal in quantities sufficient for maximal growth (Robinson *et al.* 2001). These ten indispensable (essential) amino acids: histidine, leucine, threonine, valine, phenylalanine, methionine, arginine, lysine, isoleucine, and tryptophan cannot be synthesized by fish, and therefore must be supplied through dietary sources. Several fish nutrition studies have focused on searching for the appropriate IAA ratios in fish feeds and examined their relationship with different responses in fish nutrition research. The amino acid requirements of channel catfish are relatively well studied and summarized based on National Research Council 2011 recommendations in Table 1.

In commercial feed formulations, lysine and methionine are usually the first limiting amino acids as they are often found in low levels in inexpensive protein sources. These are often referred to as the limiting amino acid, or the deficient essential amino acid, as they limit the synthesis of protein (Webster and Lim 2002).

While protein reduction and the use of alternative protein sources have been extensively studied in terms of evaluating optimal levels for fish growth, the challenge of amino acid balance remains. Both essential and non-essential amino acids, either individually or in combination, can directly and indirectly affect fish health and immune functions (Habte-Michael 2020). Dietary amino acids are required for two purposes including growth, which mainly is comprised of protein deposition, and for a number of processes that are conceptually described as maintenance (Cowey 1994). Different species of fish have various IAA requirements for both immunity and growth. These IAAs are important in regulating vital metabolic pathways related to growth, reproduction, immunity and maintenance, therefore increasing the effectiveness of food utilization, improving protein accumulation, reducing fattiness and improving health (Li *et al.* 2007). Hence, it is critical to meet both the IAA and DAA requirements and provide a well-balanced amino acid profile to maximize protein retention.

In commercial catfish feed formulations it is well established that lysine and methionine are typically the first limiting amino acids as they are often found in low levels in inexpensive protein sources. These are often referred to as the first limiting amino acids, or the

Table 1. NRC 2011 nutrient requirements for channel catfish (*Ictalurus punctatus*) dry matter basis.

Amino Acids	**%**
Arginine	1.2
Histidine	0.6
Isoleucine	0.8
Leucine	1.3
Lysine	1.6
Methionine	0.6
Methionine + cystine	0.9
Phenylalanine	0.7
Phenylalanine + tyrosine	1.6
Threonine	0.7
Tryptophan	0.2
Valine	0.8
Taurine	NR

deficient essential amino acid, as they limit the synthesis of protein. Hence, these amino acids are often at the requirement whereas other IAA are well above the requirement. It is critical that the amino acid requirement is met, but the balance to maximize retention by the fish must also be improved. The aim of this study was to examine reduced protein diets in channel catfish using a better-balanced amino acid profile and to evaluate possible limiting amino acids.

2. Materials and Methods

2.1 Experimental Diet Formulation

To evaluate the concept of reduction of indispensable amino acids (IAA) a series of nine diets were formulated (Table 2). Two diets (B30 and B24 100%) were formulated to be iso-lipidic (6% lipid) and to contain 30% and 24% intact protein (IP) while meeting IDAA requirement of channel catfish, as a minimum expressed as a percentage of the diet. Minimal IAA levels were set as recommended by NRC 2011 for channel catfish, except for methionine, which was maintained at 0.39% diet as recommended by (Wu and Davis 2005). Lysine was supplemented in the B24 100% and to confirm it was the first limiting IAA, it was removed from the diet (B24-lysine). The B24 100% was then supplemented with additional levels of IDAAs to meet 140% of the requirement (B24 140%). This included lysine, methionine, tyrosine, arginine, threonine, isoleucine, and tryptophan which in turn improved the IAA balance and reduced the level of non-essential amino acids relative to essential amino acids. Five additional diets were formulated to evaluate if select supplemented IAAs produced a response when removed, thus confirming limitations. The supplemented IAAs were lysine, methionine, tyrosine, arginine, threonine, isoleucine, and tryptophan.

2.2 Experimental Diet Preparation

The diets were prepared at the Aquatic Animal Nutrition Laboratory, School of Fisheries, Aquaculture, and Aquatic Sciences (SFAAS), Auburn University (Auburn, AL, USA). Ingredients were sourced and proximate and AA analysis conducted to predict formulation values for AA. After formulation, the pre-ground ingredients were weighed, combined, and mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 minutes. After initial mixing, menhaden fish oil was slowly added as the mixture was mixing. Hot water was then added to the mixture until desired

Table 2: Ingredient compositions (g 100 g-1 as is) of nine experimental diets designed to contain 30% or 24% intact protein supplemented with various levels of IAAs fed to juvenile channel catfish *Ictalurus punctatus* over a 12-week growth period.

Diet name	B30	B24 100%-Lys	B24 100%	B24 140%	B24 140%-Tyr	B24 140%-Arg	B24 140%-Thr	B24 140%-Ile	B24 140%-Trp
Poultry by product meal [a]	6.96	5.46	5.46	5.46	5.46	5.46	5.46	5.46	5.46
Soybean meal [b]	38.00	29.79	29.79	29.79	29.79	29.79	29.79	29.79	29.79
CPC[c]	6.96	5.46	5.46	5.46	5.46	5.46	5.46	5.46	5.46
Menhaden fish oil [d]	2.57	2.87	2.87	2.87	2.87	2.87	2.87	2.87	2.87
Corn Starch [e]	10.26	20.91	20.40	18.88	19.17	18.94	18.96	18.98	18.90
Corn [f]	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Mineral premix [g]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix [h]	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Choline chloride [i]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay-C [j]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic [k]	3.55	3.91	3.91	3.91	3.91	3.91	3.91	3.91	3.91
Arginine [l]	0.00	0.00	0.00	0.20	0.20	0.00	0.20	0.20	0.20
Isoleucine [l]	0.00	0.00	0.00	0.10	0.10	0.10	0.10	0.00	0.10
Lysine (76.6%) [l]	0.10	0.00	0.51	1.34	1.34	1.34	1.34	1.34	1.34
Methionine [l]	0.00	0.00	0.00	0.14	0.14	0.14	0.14	0.14	0.14
Threonine [l]	0.00	0.00	0.00	0.08	0.08	0.08	0.00	0.08	0.08
Tryptophan [l]	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.00
Tyrosine [l]	0.00	0.00	0.00	0.29	0.00	0.29	0.29	0.29	0.29

[a] Tyson Foods, Inc., Springdale, AR, USA.

[b] De-hulled Solvent Extracted Soybean Meal, Bunge Limited, Decatur, AL, USA.

[c] Corn Protein Concentrate (CPC) Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.

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

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

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

[g] Trace mineral (g/100 g 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 heptahydrate, 13.193; Alpha-cellulose, 67.964.

[h] Vitamin premix (g/kg premix): Thiamin HCl, 0.438; Riboflavin, 0.632; Pyridoxine HCl, 0.908; Ca-Pantothenate, 1.724; Nicotinic acid, 4.583; Biotin, 0.211; folic acid, 0.549; Cyanocobalamin, 0.001; Inositol, 21.053; Vitamin A acetate, 0.677; Vitamin D3, 0.116; Menadione, 0.889; dL-alpha-tocoperol acetate, 12.632; Alpha-cellulose, 955.589.

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

[j] Stay-C® (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

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

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

pelleting consistency was reached. Diets were extruded through a meat grinder (Hobart Corporation, Troy, OH, USA). using a 3-mm die to form pellets. The pellets were dried in a forced air-drying oven (<50°C) to achieve a moisture content of <10%, ground to an appropriate size, sieved, placed and sealed in plastic bags and stored in the freezer at -20°C until use. Diets were analyzed for proximate composition and amino acid profile at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA) and are presented in Table 3 and 4.

2.3 Experimental System and Growth Trial

A 12-week growth trial was conducted at the E.W. Shell Fisheries Research Center, Auburn, Alabama using channel catfish. Fingerlings were produced in house and placed in holding tanks until they reached adequate stocking size during which time, they were offered a commercial catfish feed. Twenty similar sized fish (mean initial weight 1.5g ± 0.10g) were stocked into each tank with four replicates for diets 1-4 and 9 and 5 replicates for diets 5, 6, 7 and 8. Diets were assigned to aquaria using a random number generator.

Each aquarium was part of an indoor recirculating aquaculture system (RAS) consisting of forty 75-L glass aquaria connected to a common reservoir tank, recirculation through an Aquadyne bead filter, vertical fluidized bed biological filter (625 liter) and a 0.25-hp. centrifugal pump. Each tank was equipped with an air stone connected to an air blower to maintain dissolved oxygen (DO) near saturation. Mix-N-Fine salt (Cargill Inc. Minneapolis, MN) was added to the system sump for salinity maintenance.

Temperature, DO and salinity were monitored twice daily from the system sump (A.M. and P.M.) using a YSI Pro 2030 (YSI, Yellow Springs, OH). Total ammonia-nitrogen (TAN) and

Table 3. Analyzed proximate composition (g 100 g-1 as is) of nine experimental diets designed to contain graded intact protein levels supplemented with IAAs fed to juvenile channel catfish *Ictalurus punctatus* over a 12-week growth period.

	B30	B24-100%-Lys	B24 100%	B24 140%	B24 140%-Tyr	B24 140%-Arg	B24 140%-Thr	B24 140%-Ile	B24 140%-Trp
Crude protein*	27.54	22.98	23.68	24.00	23.72	25.86	24.76	24.57	24.75
Moisture	13.73	14.56	13.53	19.14	14.33	7.66	14.63	11.73	11.31
Crude Fat	4.84	4.36	4.23	3.16	4.52	5.30	3.96	5.52	5.40
Crude Fiber	3.17	4.21	3.01	2.90	3.52	3.29	2.86	2.59	2.66
Ash	6.09	5.86	5.93	5.74	5.88	6.16	5.88	5.93	5.96

[1]Analysis by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Table 4. Mean averages of two amino acid analyses of (g 100 g-1 as is) of nine experimental diets designed to contain 30% or 24% intact protein supplemented with various levels of IAAs fed to juvenile channel catfish *Ictalurus punctatus* over a 12-week growth period.

Diet	B30	B24 100% -lys	B24 100%	B24 140%	B24 140%-Tyr	B24 140%-Arg	B24 140%-Thr	B24 140%-Ile	B24 140%-Trp
Alanine	1.59	1.21	1.28	1.28	1.27	1.41	1.27	1.29	1.24
Arginine	1.74	1.31	1.36	1.46	1.48	1.50	1.48	1.56	1.39
Aspartic Acid	2.63	2.04	2.12	2.12	2.10	2.30	2.11	2.18	1.94
Cysteine	0.44	0.35	0.37	0.37	0.36	0.38	0.36	0.37	0.32
Glutamic Acid	5.11	3.81	4.01	4.07	3.94	4.46	3.98	4.17	3.87
Glycine	1.26	1.00	1.03	1.04	1.03	1.12	1.03	1.07	0.96
Histidine	0.68	0.53	0.54	0.55	0.55	0.60	0.55	0.56	0.52
Hydroxyproline	0.17	0.11	0.09	0.12	0.11	0.13	0.11	0.12	0.12
Isoleucine	1.31	0.98	1.03	1.06	1.26	1.17	1.14	1.06	1.04
Lanthionine	0.03	0.02	0.02	0.02	0.03	0.02	0.03	0.02	0.02
Leucine	2.77	2.02	2.19	2.20	2.15	2.37	2.16	2.17	2.09
Lysine	1.54	1.12	1.47	2.06	2.03	2.36	2.07	2.23	1.99
Methionine	0.51	0.38	0.40	0.43	0.48	0.54	0.46	0.54	0.47
Ornithine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Phenylalanine	1.51	1.12	1.20	1.22	1.18	1.29	1.18	1.20	1.11
Proline	1.72	1.29	1.37	1.39	1.36	1.51	1.37	1.40	1.34
Serine	1.25	0.96	1.03	1.06	0.97	1.12	0.98	0.99	0.91
Taurine	0.23	0.22	0.22	0.23	0.22	0.24	0.22	0.23	0.24

Threonine	1.04	0.81	0.85	0.86	0.85	1.00	0.84	0.92	0.83
Tryptophan	0.31	0.24	0.24	0.27	0.25	0.29	0.23	0.28	0.26
Tyrosine	1.10	0.79	0.84	1.11	0.81	1.19	0.97	1.07	0.96
Valine	1.37	1.03	1.07	1.08	1.08	1.15	1.10	1.12	1.02
∑EAA	10.85	8.75	8.95	9.18	9.41	9.8	9.31	9.31	9.16
∑NEAA	16.62	13.64	14.14	15	14.64	16.1	14.88	15.09	14.69
Total	28.34	21.37	22.76	24.03	23.54	26.18	23.67	24.58	22.67

[1]Analysis by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

nitrite-nitrogen were measured twice a week from water samples taken from the system sump using a YSI 9500 photometer (YSI, Yellow Springs, OH). The pH was also measured twice weekly during the experimental period from the system sump using a Large Display pH Pen (Sper Scientific, Scottsdale, AZ, USA).

The daily feed ration was calculated as percentage of fish weight and divided into two equal feedings (9:00 and 16:00) each day. The ration was calculated on a dry feed basis using bi-weekly weight data as well as visual observations of feed intake and ranged from 4.5% to 7.5% of the fish weight across the growth trial.

2.4 Sample Collection and Body Composition

At the end of the growth trial, fish from each tank were group weighed and individually counted. Five fish from each tank were euthanized and frozen for subsequent analysis. Whole fish were thawed, chopped, and then blended in a food processor to produce a homogenous sample. The entire sample was divided into several samples and frozen. A subsample for whole body composition analysis was analyzed by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA). The following were calculated using fish weight, feed fed and temperature data:

a) Thermal Unit Growth coefficient (TGC) =

$$(\text{final weight}^{1/3} - \text{initial weight}^{1/3})/(\text{mean temperature x days}) \times 100$$

b) Mean weight (g) = Biomass / no. of fish

c) Weight gain (g) = Final mean weight – initial mean weight

d) Weight gain (%) = (Final mean weight-initial mean weight)/ initial mean weight x 100

e) Survival (%) = final fish number/initial fish number x 100

g) Feed conversion ratio (FCR)= feed offered (g)/ weight gain (g)

g) Protein conversion efficiency (PCE, %) = (final weight × final protein content) - (initial weight × initial protein content) × 100 / protein offered

h) Feed offered (g) = sum of average feed offered per fish over 12 weeks

2.5 Statistical Analysis

All data were subjected to a one-way analysis of variance (ANOVA) to determine significant differences ($P<0.05$) among treatments, which was followed by an SNK test to distinguish differences among treatment means. All data were analyzed using SAS (V9.4. SAS Institute, Cary, NC, USA). Regression analysis was performed on lysine levels and weight gain in the 140% diet series.

3. Results

Water quality results for the 12-week experimental trial are presented in Table 5. TAN and nitrite nitrogen values were elevated throughout the experiment despite frequent water exchanges and inoculation of biological filters. TAN ranged from 0.01 – 4.8 mg/L (mean: 1.50 ± 1.40 mg/L). Nitrite levels ranged from 0 – 0.13 mg/L (mean 0.03 ± 0.02 mg/L). While there were elevated levels, TAN and nitrite did not appear to have an impact on the study. Dissolved oxygen averaged 7.27 mg/L and ranged from 5.88 to 9.76 mg/L. Temperature (28.47 ± 0.49 ◦C), pH (7.53± 0.38), and salinity (3.29 ± 0.45 ppt) also remained within levels acceptable for the culture of channel catfish throughout the experimental period.

Performance parameters of the fish fed the test diets are presented in Table 6. No significant differences were observed in initial mean weight of fish, survival (%), and PCE among all treatments ($P > 0.05$). Initial mean weights ranged from 1.43 g to 1.55 g. Survival ranged from 95% (B24 140%-Ile) to 100% (B30, B24 140%-Arg, B24 140%-Thr). PCE ranged from 43.32 (B24 100%-lys) to 48.23 (B24 140%-Tyr).

Significant differences were observed in mean final weight, weight gain, percent weight gain, feed offered per fish, FCR and TGC ($P < 0.05$). Fish offered the B24:140%-Arg (25.10 g) were not significantly larger from fish offered diets B30 (22.60 g) and B24:140-Trp (22.26 g) but were significantly larger than fish offered the other six diets. Percent weight gain which ranged from 936.4 (B24 100%-lys) to 1614.7 (B24 140%-Arg) and TGC ranged from 0.056 (B24 100%-lys) to 0.074 (B24 140%-Arg) mirrored the response for final weight. FCR ranged from 1.17 (B30) to 1.45 (B24 100%-lys) with fish offered the B24:100%-Lys having significantly larger values than fish offered all other diets.

Table 5. Water quality parameters averaged over a 12-week catfish growth trial.

Parameters	Mean ± Std. Dev.	Low	High
DO (mg/L)	7.27±0.72	5.88	9.76
Temp (◦C)	28.47±0.49	27.2	29.7
Salinity ppt	3.29±0.45	2.3	4
pH	7.53±0.38	6.7	7.9
TAN (mg/L)	1.50±1.40	0.01	4.8
Nitrite (mg/L)	0.02±0.03	0	0.13

Ammonia levels were elevated during the majority of experiment.

Table 6. Growth performance of channel catfish fed experimental diets designed to contain graded intact protein levels supplemented with IAAs over a 12-week growth period.

Diet	Initial mean weight (g)	Final mean weight (g)	Weight gain (g)	Weight gain (%)	FCR[1]	Dry feed (g)	PCE[2] (%)	TGC[3]	Survival (%)
B30	1.43	22.60^{b}	21.17^{b}	1480.1^{b}	1.17^{b}	24.80^{b}	43.98	0.070^{b}	100.0
B24 100%-lys	1.55	16.00^{c}	14.45^{d}	936.4^{c}	1.45^{a}	20.91^{d}	43.32	0.056^{d}	98.3
B24 100%	1.47	20.69^{b}	19.23^{bc}	1308.7^{b}	1.26^{b}	23.84^{bc}	45.40	0.066^{bc}	98.3
B24 140%	1.46	19.70^{b}	18.24^{c}	1255.7^{bc}	1.22^{b}	22.23^{cd}	44.34	0.064^{c}	98.3
B24 140%-Tyr	1.46	21.35^{b}	19.89^{bc}	1364.3^{b}	1.22^{b}	24.15^{bc}	48.23	0.067^{bc}	96.7
B24 140%-Arg	1.47	25.10^{a}	23.63^{a}	1614.7^{a}	1.16^{b}	27.46^{a}	47.56	0.074^{a}	100.0
B24 140%-Thr	1.54	21.27^{b}	19.73^{bc}	1280.8^{b}	1.22^{b}	24.07^{bc}	44.58	0.067^{bc}	100.0
B24 140%-Ile	1.47	21.52^{b}	20.05^{bc}	1365.4^{b}	1.20^{b}	24.10^{bc}	47.64	0.068^{bc}	95.0
B24 140%-Trp	1.44	22.26^{b}	20.82^{bc}	1451.6^{b}	1.23^{b}	25.50^{b}	44.01	0.069^{b}	97.5
PSE[4]	0.048	0.718	0.714	65.24	0.033	0.571	0.018	0.001	1.67
P-value	0.554	<0.001	<0.001	<0.001	<0.001	<0.001	0.401	<0.001	0.737

Means in the same column (that do not share a superscript letter) with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by SNK (Student-Newman-Keuls).
[1]FCR: Feed conversion ratio.
[2]PCE: Protein conversion efficiency.
[3]TGC: Thermal growth coefficient.
[4]PSE: Pooled standard error.

Whole body composition

Whole body composition results are presented in Table 7. There were no significant differences among dry matter, crude protein, crude fat, and ash of whole-body samples for fish maintained on the various diets. Dry matter ranged from 26.15% (B30) to 28.62% (B24 140%-Arg). Crude protein ranged from 13.00% (B24 140%) to 14.24% (B24 140%-Arg). Crude fat ranged from 9.21 (B24 140%-Trp) to 10.39% (B24 140%-Arg). Ash content ranged from 2.94 (B24 140%-Ile) to 3.78% (B24 140%-Trp). In general, there were no obvious trends in the data. However, as expected, lipid content linearly increased as moisture content decreased.

Table 7. Proximate analysis (as is) for whole body composition of channel catfish. Each sample represents mean of 5 fish pooled per tank at the conclusion of the 12-week growth period.

Diet #	Dry matter (%)	Crude Protein (%)	Crude Fat	Ash
B30	26.15	14.00	9.30	3.09
B24 100%-Lys	28.18	14.30	9.65	3.58
B24 100%	27.30	13.50	9.66	3.30
B24 140%	27.53	13.00	9.53	3.24
B24 140%-Tyr	27.54	13.86	9.59	3.40
B24 140%-Arg	28.62	14.24	10.39	3.66
B24 140%-Thr	27.88	13.48	9.91	2.99
B24 140%-Ile	26.94	14.02	9.25	2.94
B24 140%-Trp	27.40	13.35	9.21	3.78
PSE	0.622	0.581	0.475	0.325
P-value	0.274	0.807	0.723	0.710

Means in the same column (that do not share a superscript letter) with different superscripts are significantly different at $P < 0.05$ based upon analysis of variance followed by SNK range test.
[1]Fish whole body composition analysis were analyzed by Midwest Laboratories, Inc., Omaha, NE, USA

4. Discussion

Channel catfish is one of the most studied species in aquaculture from a nutritional perspective. Much of this knowledge is a result of channel catfish being the most important aquaculture species by production volume for U.S. aquaculture for several decades. In order to support and grow the U.S. catfish industry, the nutritional requirements of channel catfish have been extensively examined. Rather surprisingly, and despite this large amount of research, a great deal of knowledge gaps still exist in terms of essential amino acid requirements for this species. This study sought to better understand amino acid balance in channel catfish with the goal of improving protein utilization in this species.

Protein retention by channel catfish, which can be related to source quality and quantity, can ultimately influence pond water quality as it affects nitrogen loading. Li and Lovell (1992) evaluated the effects of dietary protein on nitrogenous waste in production ponds. They found that diets with higher protein concentrations increased both the total ammonia nitrogen and nitrite levels in water as compared to lower protein diets. Similarly, (Robinson *et al.* 2004) reported that nitrogenous waste compounds such as total nitrogen (TN), total ammonia nitrogen (TAN), nitrite and nitrate were generally higher in ponds receiving higher protein feeds. Since protein, amino acid balance, and digestibility can influence nitrogen loading these factors can have a significant effect on water quality and production costs which are consequently of commercial importance.

As previously discussed, catfish feeds should be balanced to contain appropriate levels of amino acids, nonspecific nitrogen and nonprotein energy. Amino acids in catfish diets have been studied extensively (summarized in Table 1), but there is no consensus on the level of dietary protein to provide the most cost-efficient growth. Feeds are formulated to meet amino acid requirements. Even though dispensable amino acids can be synthesized by catfish, there are advantages if they are provided in the diet. For instance, if DAA are supplied in the diet, energy is saved as they do not need to be synthesized. Additionally, some dispensable amino acids can partially replace an indispensable amino acid. For example, cystine can replace around 60% of methionine, and tyrosine can replace around 50% of the phenylalanine on a weight-by-weight basis (Council 1993). Cystine and tyrosine are semi-indispensable amino acids because they can only be synthesized from their precursor IAAs methionine and phenylalanine, respectively. The inclusion of these semi-indispensable amino acids in diets saves some of the precursor IAAs (Oliva-Teles 2012).

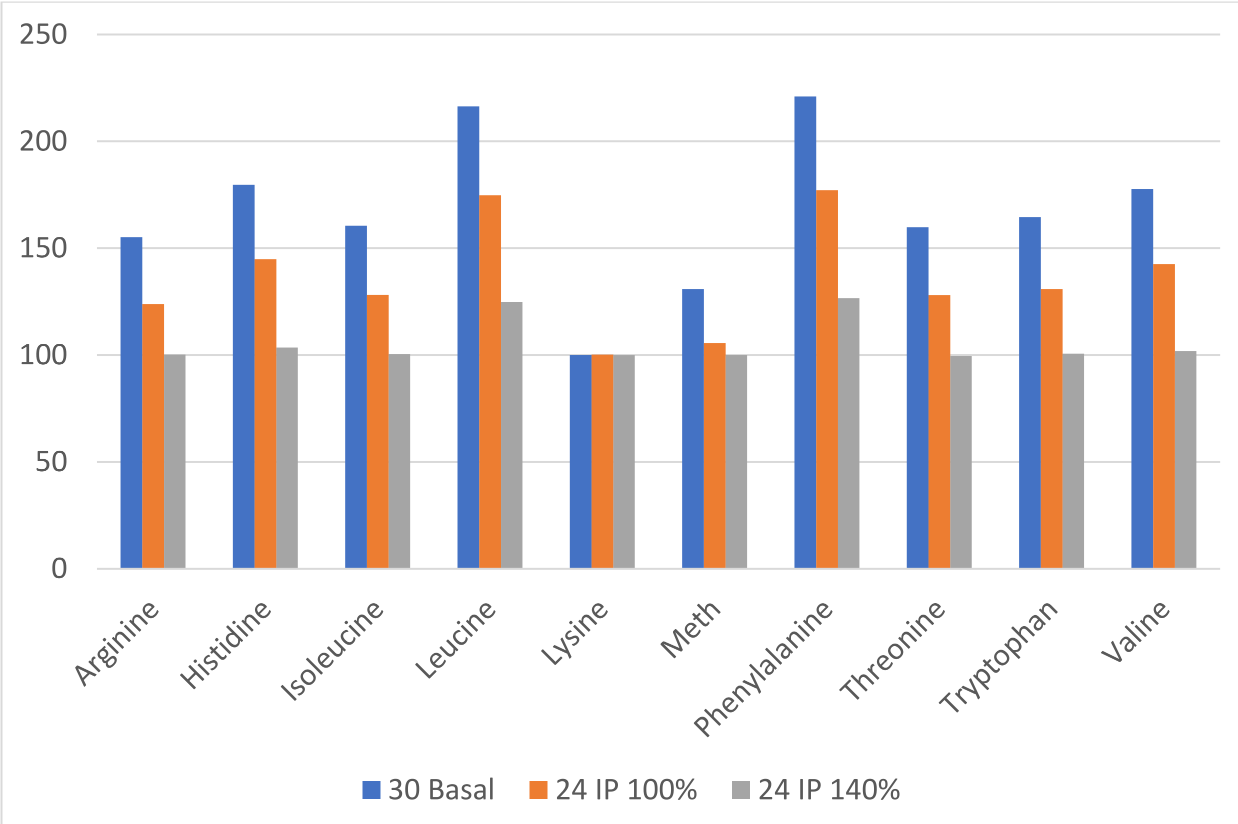
As summarized in Table 1, IAA requirements for channel catfish, are well established. However, there is limited information on the effects of NEAAs and the overall balance or interactions of amino acids. In a previous study (Salem *et al.* 2021b) evaluated how dietary supplementation of EAAs and NEAAs could aid in the reduction of dietary intact protein content in channel catfish. Results showed that final weight increased and FCR decreased as intact protein increased. It also showed that lower protein diets supplemented with DAAs or IAAs did not show a significant improvement in fish growth albeit there was a general increase in weight gain. This indicates further work needs to be conducted on reduced protein diets.

A second study (Salem *et al.* 2021a) looked at the effectiveness of using the crystalline amino acids with dietary IP reduction from 30% to 24% in juvenile channel catfish diets. Growth and FCR of the catfish fed the low intact protein diet (24%) supplemented with crystalline IAAs were not significantly different from fish offered the basal 30% IP diet. They also reported that based on regression analysis of the catfish's final weight, TGC, FCR and apparent net protein retention values for fish fed 24% IP supplemented with crystalline IAAs at 100, 120 and 140% NRC requirements showed linear improvement as IAA supplementation levels increased. The highest values were observed in fish offered diet supplemented at 140% NRC. This response indicates that there may be benefits to increasing the levels of IAA in low protein diets which is essentially reducing the level of DAA intake relative to that of IAA.

In the current study, the previous work of these studies was expanded on with the goal of improving the overall balance of amino acids. As demonstrated in Figure 1, reducing the protein from 30% to 24% improved the amino acid balance by reducing the level of excess IAA. That is to say, the levels of IAAs were reduced making them closer to the requirement albeit still in excess. In this case (24 IP 100%), only lysine was limiting and required supplementation. The balance of IAA was further improved when the formulated levels of EAAs were raised to 140% of the requirement. In the 140% diet series the following amino acids were supplemented: arginine, isoleucine, lysine, methionine, threonine, tryptophan and tyrosine. Most of the IAAs were at or near the requirement with the exception of leucine and phenylalanine which were still well above the requirement (Figure 1). With this approach, excess levels of IAA were reduced while the established dietary requirements were still met. This also resulted in a lower level of DAA relative to IAA.

Looking at the first four diets in this study, reducing the protein to 24% of the diet resulted in no significant differences in performance unless lysine was removed from the diet. This confirms that lysine is the first limiting amino acid in these diets. Reducing the protein to 24% at 100% or 140% of the requirement resulted in a slight reduction in growth although, growth was not significantly different for fish offered the B30 diet. With regard to diet B24, raising the amino acid levels to 140% (B24-140%) it was expected to observe improved growth performance. This would be due to a better-balanced amino acid profile as well as a daily increase in amino acid

Figure 1. Amino acid values of diets B30, B24: 100%, and B24: 140% expressed as a percentage of the NRC 2011 requirement.



intake. However, improvements in performance were not observed indicating other factors were limiting.

(Salem et al. 2021a) ran a similar group of diets where a significant decrease in growth was observed when protein was reduced from 30% to 24%. However, no reduction in fish performance was observed in our study using similar diets. The previous study also demonstrated the 24% IP diets supplemented with 120% and 140% IAAs showed an improvement in growth as supplementation levels increased (Salem *et al.* 2021a). They also found that the addition of DAAs did not improve performance; therefore, in the present work only IAAs were supplemented.

Based on these results production diets could be reduced to 24% without affecting growth if the IAAs were supplemented to meet amino acid requirements. Reducing the protein levels of the feed appears to have worked albeit, further improvements could be made. To better balance the IAAs and reduce levels of DAAs, the supplemental level was increased to 140% of the requirement (Figure 1.). Lysine and methionine are already well established to be first limiting IAAs. Hence, to determine if the IAAs arginine, threonine, isoleucine, and tryptophan were limiting amino acids they were individually deleted from the diets. Additionally, tyrosine a DAA, was supplemented because it is a semi-indispensable amino acid that can replace the IAA phenylalanine. Depression in growth performance would be expected if they were limiting amino acids. There were no significant depressions in fish performance indicating no limitations of these amino acids or that the fish were unable to utilize the increase in IAAs. With the exception of lysine and methionine, which were added at high levels, the other supplemented amino acids were included in relatively low levels ranging from 0.02% of the diet for tryptophan to 0.29% of the diet for tyrosine. This indicates they were close to the requirements and may be another reason no deficiency was observed.

Fish offered the B24:140%-Arg were significantly larger than fish offered B24-140% diet which contained all supplemental amino acids. Percent weight gain and TGC mirrored the response for final weight and FCR was reversed. This indicates that a positive response of the fish was observed when arginine was removed from the diet.

Dietary arginine requirements for channel catfish are relatively well studied and are reported to be 1.2% of the diet or 4.1% of the digestible protein (Table 1.). In the diet series using 140% of the requirement, arginine was formulated to 1.68% of the diet. The analysis of the diets, however, reported a range of 1.39%-1.56% of arginine. Diet B24:140%-Arg had significantly better growth compared to the others in this series of diets. This response could be due to an arginine-lysine antagonism. The excess of arginine in the diets could have inhibited the availability of lysine which was already marginal in lysine relative to the requirement. Therefore, the removal of arginine allowed an increase in the availability of lysine thus possibly increasing growth.

Excess of either lysine or arginine causes a reduction in bioavailability of the other. This interaction is referred to as an arginine-lysine antagonism. The arginine-lysine antagonism has been demonstrated in several animal species. Several fish studies have not established compelling evidence of a lysine-arginine antagonism occurring in fish (National Research Council 2011). (Robinson *et al.* 1981) found no evidence of this antagonism in channel catfish. However, a study by (Zhou *et al.* 2011) found that increasing both lysine and arginine levels by 20% of requirement at the same time, resulted in less growth depression compared to other dietary combinations in blackhead sea bream (*Acanthopagrus schlegelii)* fingerlings. Another study (Cheng *et al.* 2011) found an increase in both feed efficiency and weight gain in juvenile red drum (*Sciaenops ocellatus*) fed diets supplemented with arginine in excess of the established requirement. Hence, it is not clear if an antagonism occurred in the current study.

Another possible explanation is that arginine was not removed from the diet and slightly more lysine was supplemented to the B24: 140%-Arg diet. This could have resulted in an increase in lysine levels that may also be responsible for the increase in growth. The lysine requirement as reported by the NRC is 1.6% of the diet which translates to 2.24% of the diet at 140% of the requirement. The analyzed values of the 140% diet series ranged from 1.99%-2.36%. As lysine is likely the first limiting amino acid, and it was supplemented at or near the requirement, any shifts in lysine levels could have affected growth. A linear regression was performed on the 140% diet series which showed a positive correlation between weight gain and the amount of lysine present in the diet (R^2=0.36). If an increase in lysine levels occurred, it is possible that this could have had a greater impact on fish growth then the omittance of the other amino acids.

5. Conclusion

Research regarding protein reduction and optimization of amino acids is becoming more prevalent due to the potential for better cost efficiency in feed formulation. This is particularly important in channel catfish because it is a top aquaculture species in the United States. This study suggests that protein content can be reduced from 30% to 24% without impacting fish growth performance or nutrient retention. Raising the amino acid levels from 100% to 140% of the requirement to reduce NEAA relative to IAAs did not significantly change growth, FCR, or PCE. Although the diet formulated to exclude arginine resulted in the lowest FCR and highest reported growth, the analyzed amino acid composition did not support the fact that it was omitted and indicated a higher level of lysine which could explain the results. Based on these findings further research is necessary to better understand the balance of amino acids in channel catfish.

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