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Using of commercial amino acids in the channel catfish *Ictalurus punctatus* diets and their effects on growth and health

Shimaa M. Salem[*1, 2], Lay Nguyen[1], Tarek I. Mohamed[2], Ola A. Orma[2], and D. Allen Davis [1]

ABSTRACT

A nine weeks growth study was carried out to investigate, how the dietary supplementation of essential amino acids (EAA) could help in the reduction of intact protein (IP) level in diets designed for juvenile (10.6g) channel catfish *Ictalurus punctatus*. The effect on fish growth, feed utilization, proximate composition, and serum indicators was measured. Five iso-lipid containing (8%) lipid experimental diets were formulated with graded levels of intact protein (32, 30, 28, 26 and 24 %IP) supplemented with EAA (lysine and histidine)to maintain the in EAA level as recommended in NRC 2011. The results of fish growth; final mean weight (g), weight gain, weight gain percent, feed conversion ratio (FCR), feed intake (g) of channel catfish were not significantly different as IP was decreased from 32 to 26 %, with EAA supplementation. Fish growth was significantly reduced, while FCR significantly increased as IP was lowered to 24%. The reduction of IP in-channel catfish diet with EAA supplementation did not affect whole-body crude protein, moisture, ash, crude fiber, and amino acid contents. While whole-body fat significantly increased with lowering of the IP content in the diet. Serum total protein, albumin, total immunoglobulin, and glucose level were not significantly affected by the reduction of the dietary IP level for 32 to 24%. While, serum ALT, AST, ALP and triglycerides levels showed a significant increase, while serum lysozyme and total hemoglobin decreased as protein was decreased to 24%. In conclusion, commercial amino acids could be used as a dietary supplement to reduce intact protein content from 32 to 26% in channel catfish diets with maintaining a comparable fish growth and health. But, fish growth, body composition, and serum metabolites were adversely affected by further lowering of protein to 24%.

Keywords: Channel catfish, Essential amino acids, intact protein, commercial amino acids.

INTRODUCTION

Channel catfish (*Ictalurus punctatus)* aquaculture constitutes a significant portion of aquaculture production in the USA. The rapid expansion in intensive catfish farming has increased the need for using commercial feed (Gaylord and Barrows, 2009). Catfish commercial feed contains about 32–35% protein to support optimal fish growth and health (Robinson and Li, 2007). So, the cost of the commercial aquafeed represents about 50% of the total production cost under intensive culture system. The strategies of reducing the cost of feed production and lowering the environmental pollution with nitrogen could be happened via reducing the dietary protein level of fish diets.

Catfish need a continuous dietary supply of protein as a source of amino acids and nonspecific nitrogen to maintain optimal fish growth. In reality, they do not require protein, so the actual percentage of dietary protein is not as critical as the quantity and proportions of amino acids provided in the protein. Hence, satisfying amino acid needs with a proper balance is a precise consideration that should be taken in mind, during the formulating of fish diets (Wilson et al., 1981). Amino acids can be provided by feeding mixtures of complementary protein sources in proper amounts and ratios. Additionally, supplementing deficient proteins with appropriate crystalline amino acids; to achieve the proper balance of amino acids and raise protein quality (Ambardekar and Reigh, 2007). Li and Robinson (2015) have reported that if the feed mixture contains an amino acid level below the requirement (deficient), growth will be reduced. That amino acid is called the "limiting amino acid". The deficiency of an amino acid can be eliminated by supplementing the diet with the deficient amino acid or by using a feedstuff high in

* Corresponding author: dr_sh84_sh87@yahoo.com
[1]School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, AL 36849 USA [2]Department of Nutrition & Nutritional Deficiency Diseases, Faculty of Veterinary MedicineMansoura University, 35516 Egypt.

that particular amino acid (Robinson and Li, 2007).

Fishmeal is considered the standard source for providing essential amino acids required for fish growth. The limited availability and increased prices of fishmeal led to the increase of using the alternative protein sources and/or the reduction of the feed protein level. Plant protein sources; for example, soybean meal is deficient in sulfur-containing amino acid (methionine). Using of SBM as a protein source, will cause imbalanced amino acids profile, and subsequently reduce protein utilization efficiency and fish growth. Thus, using supplemental purified amino acids has been increased; to avoid amino acid deficiencies, as well as, stimulate feed consumption. For better utilization of supplemented amino acid; the exact requirements of fish species, the amino acid composition of feedstuffs, their digestibility, and the proper balance of protein and energy should be well known (Righetti et al., 2011).

The ability to reduce the dietary protein level with maintaining optimal fish growth and feed efficiency by using crystalline amino acids as a supplement has had a big interest in fish nutrition research. Viola et al. (1992) demonstrated that total dietary protein could be reduced when lysine was supplemented to carp diets. Cheng et al. (2003) found that dietary crude protein could be reduced from42 to 37% in fish meal-based diets for rainbow trout when lysine, methionine, threonine, and tryptophan were supplemented. However, other studies have shown that the ability of lysine supplementation of lysine and methionine to reduce the dietary crude protein in channel catfish was ineffective. This is because, another amino acid was limiting instead of lysine or methionine (Gaylord et al., 2002; Li and Robinson, 1998).

Consequently, this study focused on how the dietary supplementation of EAA(lysine and histidine) could help in the reduction of dietary intact protein content in channel catfish *Ictalurus punctatus* diets and their effect on fish growth performance, the whole body proximate composition, and serum health indicators.

MATERIALS AND METHODS

Experimental design and diet preparation

The basal and test diets were designed to be iso-lipidic (8% lipid), isoenergetic and containing graded levels of dietary intact protein. EAA levels were set as recommended by NRC (2011) for channel catfish, *Ictalurus punctatus,* except methionine which was maintained at 0.39% of the diet, as recommended by Wu and Davis (2005). The test diets were designed to contain graded levels of intact protein (32, 30, 28, 26 and 24 IP) as presented in Table 1.

The experimental diets were prepared in the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn, AL, USA). Dry feed ingredients were finely ground, weighed, and mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 minutes. As the mash was mixing, oil was slowly added followed by hot water (25-30% of diet) which was blended in to attain an appropriate consistency for pelleting. Diets were then extruded through a 3mm diameter die in a meat grinder, dried at < 45°C in air ventilation oven to moisture less than 10%, crumbled to an appropriate size, packed in sealed plastic bags and stored in the freezer at -20° C until used. Samples from experimental diets were collected and analyzed for proximate and amino acid compositions ($g100^{-1}$), (Table2) following AOAC (1990) procedures by Midwestlaboratory (Omaha, Nebraska), and amino acids laboratory Ajinomoto Heartland, Inc (Chicago, Illinois).

Culture environment

A nine-week growth trial was conducted at the E.W. Shell Fisheries Research Center, Auburn, Alabama using juvenile (10.56g ± 0.1g), channel catfish *Ictalurus punctatus*.

Table 1: Ingredient compositions (g 100 g-1 as is) of nine experimental diets designed to containgraded intact protein levels supplemented with EAA fed to juvenile channel catfish (10.56 ± 0.1g) *Ictalurus punctatus* over 9 weeks growth period.

IP	**32**	**30**	**28**	**26**	**24**
EAA		**+**	**+**	**++**	**++**
Soybean meal[1]	40.00	37.28	34.40	31.52	28.64
Meat & bone meal [2]	8.00	7.46	6.88	6.30	5.73
CPC-Empareal 75[3]	8.00	7.46	6.88	6.30	5.73
Corn[4]	30.00	30.00	30.00	30.00	30.00
Corn starch[5]	5.10	8.58	12.16	15.77	19.31
Menhaden fish oil[6]	4.30	4.43	4.58	4.72	4.87
Trace mineral premix[7]	0.50	0.50	0.50	0.50	0.50
Vitamin premix[8]	0.80	0.80	0.80	0.80	0.80
Choline chloride[9]	0.20	0.20	0.20	0.20	0.20
Stay C[10]	0.10	0.10	0.10	0.10	0.10
CaP dibasic[11]	2.00	2.14	2.28	2.42	2.56
Lecithin[12]	1.00	1.00	1.00	1.00`	1.00
L-Lysine 78% [13]	0.00	0.06	0.22	0.35	0.50
L-Histidine 98.5%[13]	0.00	0.00	0.00	0.01	0.06

[1]Dehulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA.
[2] Griffin industries, Cold Spring, KY,USA.
[3] Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.
[4]Yellow corn, Auburn University feed mill, Auburn, AL,USA.
[5] MP Biomedicals Inc., Solon, OH, USA.
[6] Omega Protein Inc., Houston TX, USA.
[7] Trace mineral premix: Cobalt chloride 0.04g/kg , Cupric sulfate pentahydrate 2.5g/kg , Furrous sulfate 40g/kg, Magnesium sulfate anhydrous 138.62g/kg, Manganous sulfate monohydrate 6.50g/kg, Potassium iodide 0.68g/kg, Sodium selenite0.1g/kg, Zinc sulfate hepahydrate 131.92 g/kg, cellulose(filler) 679.64g/kg
[8] Vitamin premix: Thiamin HCl (B1) 0.44g/kg , Riboflavin (B2) 0.63g/kg, Pyridoxine HCl (B6) 0.91g/kg , DL pantothenic acid 1.72g/kg, Nicotinic acid (Niacin) 4.58g/k, Biotin 0.21g/kg , Folic acid 0.55g/kg, Vitamin B_{12}, Inositol 21.05g/kg, Vitamin A acetate 0.68 IU, Vitamin D_3 0.12 IU, DL-alpha-tocoferol Vitamin E 12.63IU,Menadione sodium bisulfite 0.89 IU, Alpha- cellulose 955.59g/kg
[9] Choline chloride: Amresco Inc., Solon, Ohio, USA, need to include separately.
[10] Stay-C® (L-ascorbyl-2-polyphosphate 25% Active C): DSM Nutritional Products., Parsippany, NJ, USA. Need to include separately
[11] Alfa Aesar, Ward Hill, MA, USA.
[12] The Solae Company, St. Louis, MO, USA.
[13] Ajinomoto Heartland Inc., Chicago, IL, USA.
[+] L-Lysine 78% was supplemented to maintain minimum level of lysine as recommended by NRC 2011.
[++] L-Lysine 78% and Histidine were supplemented to maintain minimum level of lysine as recommended by NRC 2011.

Fifteen fish were group weighed and randomly stocked into 20 aquaria allowing four replicates per dietary treatment. These aquaria are components of a 3,800-L indoor recirculation system. Samples of fish from the initial stocking were retained for proximate and amino acids analysis. Dissolved oxygen (DO), salinity and water temperature were measured twice a day using YSI 650 multi-parameter instrument (YSI, Yellow Springs, OH). Water pH was measured twice weekly using a waterproof pHTestr30 (Oakton instrument, Vernon Hills, IL, USA). Water samples were taken to measure total ammonia nitrogen (TAN) and nitrite twice a week. TAN and nitrite were determined using the methods described by (Solorzano, 1969) and (Spotte, 1970), respectively. During the experimental period, DO, temperature, salinity, pH, TAN, and nitrite were maintained within acceptable ranges for channel catfish at 5.99±0.76 mg/L, 27.37±1.51°C, 2.00±1.17 ppt, 7.10±0.25, 0.20±0.17 mg/L, and 0.11±0.10 mg/L, respectively.

Daily feed offered on a dry weight basis was calculated according to the wet weight of fish, which were weighed biweekly, as a percentage of the mean body weight (3.5-6%) based on growth and observation of the feeding activity. The daily feed was offered in two equal meals each day (at 08h00AM and 16h00PM). At the end of the feeding period, fish were counted, and the group weighed. Four fish per aquarium were

Table 2: Analyzed proximate [a] composition and amino acids[b] profile (g 100 g-1 as-is) of the nine experimental diets designed to contain graded intact protein levels supplemented with EAA fed to juvenile channel catfish (10.56 ± 0.1g) *Ictalurus punctatus* over 9 weeks growth period.

IP	**32**	**30**	**28**	**26**	**24**
EAA		**+**	**+**	**++**	**++**
Composition %					
Protein(crude)	31.89	30.20	28.04	25.90	24.63
Dry matter	91.54	91.57	92.27	90.12	90.85
Moisture	8.46	8.42	7.72	9.88	9.14
Fat(crude)	7.19	7.31	7.51	7.26	7.40
Fiber(ADF)	5.18	4.81	4.23	3.94	3.79
Ash	6.08	6.12	5.65	5.71	5.48
GE(kcal/kg)	4367.9	4373.1	4328.0	4335.8	4233.8
Arginine	1.91	1.79	1.67	1.53	1.39
Histidine	0.76	0.71	0.67	0.63	0.62
Isoleucine	1.33	1.24	1.18	1.10	1.01
Leucine	3.11	2.93	2.76	2.53	2.34
Lysine	1.56	1.52	1.53	1.53	1.51
Methionine	0.55	0.51	0.49	045	0.42
Phenylalanine	1.61	1.53	1.43	1.31	1.21
Threonine	1.17	1.09	1.03	0.94	0.86
Tryptophan	0.35	0.33	0.31	0.28	0.27
Valine	1.46	1.37	1.30	1.21	1.11
Meth+Cys	1.02	0.97	0.92	0.83	0.78
Tyrosine	0.99	0.93	0.86	0.81	0.73

[a]Experimental diets proximate analysis were analyzed byMidwest laboratories, Omaha,Nebraska, USA.
[b]Experimental diets amino acids profile were analyzed by Amino acids analysis Laboratory.Ajinomoto Heartland Inc.,Chicago,IL,USA.
[+] L-Lysine 78% was supplemented to maintain minimum level of lysine as recommended by NRC 2011.
[++]L-Lysine 78% and L-Histidine 98.5% were supplemented to maintain minimum level of lysine as recommended by NRC 2011.

Table 3: Mean response of juvenile channel catfish (10.56±0.1g)*Ictalurus punctatus* fed diets with graded intact protein levels supplemented with EAA over a nine week growth period.

IP-EAA	**Final weight g**	**Weight gain g**	**Weight gain %**	**FCR***	**Feed intake g**	**PER****	**Survival percent**
32	60.66^{ab}	50.09^{ab}	473.59^{a}	1.52^{b}	76.36^{a}	2.056^{de}	100
30^{+}	61.48^{a}	50.89^{a}	480.73^{a}	1.51^{b}	76.98^{a}	2.187^{bcd}	100
28^{+}	58.69^{abc}	48.14^{abc}	456.46^{ab}	1.53^{ab}	73.67^{ab}	2.328^{abc}	100
26^{++}	54.76^{abc}	44.22^{abc}	420.98^{ab}	1.62^{ab}	72.06^{ab}	2.36^{ab}	100
24^{++}	52.00^{c}	41.42^{c}	391.94^{b}	1.68^{a}	69.54^{b}	2.42^{a}	100
P-value	0.003	0.003	0.003	0.011	0.006	0.000	
PSE[a]	0.6883	0.6874	6.473	01338	0.5661	0.0297	

Means (n = 4) in the same column with different superscripts are significantly different at P < 0.05 based upon analysis of variance followed by Tukey's multiple range test.
[a]PSE: Pooled standard error.
[*]Feed conversion ratio.
[**]Protein efficiency ratio
[+] L-Lysine 78% was supplemented to maintain minimum level of lysine as recommended by NRC 2011.
[++]L-Lysine 78% and L-Histidine 98.5% were supplemented to maintain minimum level of lysine as recommended by NRC 2011.

randomly retained and frozen at -20Cº for proximate and amino acid analysis. The fish samples were homogenized and freeze-dried in the freeze dryer at -40F or less and subdivided for analysis. Subsamples were sent to the Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri, for analyzing moisture, crude protein, crude lipid, crude fiber, and ash. Other subsamples were sent to Ajinomoto Heartland Inc., (city, Chicago), for amino acid analysis (Table 4). Further, final mean weight, weight gain, weight gain %, FCR, and survival were calculated using the following calculations (Table 3).

a) Mean weight (g):
= Total weight g of fish in aquarium / no. of fish in the same aquarium
b) Weight gain (g):
= W2 - W1
W1 = Initial mean weight & W2 = Final mean weight
c) Percent weight gain (WG %)
= W2-W1/W1×100
d) Survival percentage = final fish number/ initial fish number × 100.
e) Feed conversion ratio (FCR) = dry feed intake g / weight gain g.
f) Feed intake g /fish = feed (DM) offered in a known period/no. of fish.

Table 4: Analyzed*whole body proximate and amino acids composition (g 100 g-1 as-is)** of juvenile channel catfish (10.56±0.1g)*Ictalurus punctatus* fed diets with graded intact protein levels supplemented with EAA over a nine week growth period.

IP	32	30	28	26	24	*P*-value	PSE
EAA		+	+	++	++		
Composition %							
Crude protein	46.25	45.46	45.39	45.02	44.16	0.605	0.239
Moisture	68.57	68.18	66.48	66.35	66.78	0.253	0.3122
Crude Fat	32.66^{ab}	30.24^{b}	32.41^{ab}	34.49^{ab}	34.65^{a}	0.044	0.341
Crude Fiber	0.23	0.22	0.24	0.31	0.27	0.939	0.017
Ash	10.95	10.96	10.58	9.58	10.23	0.311	0.161
Arginine	2.85	2.79	2.77	2.71	2.73	0.603	0.017
Histidine	1.027^{a}	0.98^{ab}	0.98^{ab}	0.96^{ab}	0.95^{ab}	0.033	0.007
Isoleucine	2.13^{a}	1.99^{ab}	2.04^{ab}	1.96^{ab}	1.96^{ab}	0.068	0.017
Leucine	3.46^{a}	3.24^{ab}	3.30^{ab}	3.21^{ab}	3.17^{ab}	0.055	0.028
Lysine	3.98^{a}	3.74^{ab}	3.80^{ab}	3.69^{ab}	3.65^{ab}	0.047	0.032
Methionine	1.23^{a}	1.16^{ab}	1.17^{ab}	1.16^{ab}	1.14^{ab}	0.038	0.008
Phenylalanine	1.92^{a}	1.81^{ab}	1.84^{ab}	1.80^{ab}	1.78^{ab}	0.082	0.014
Taurine	0.65^{a}	0.59^{ab}	0.57^{abc}	0.56^{abc}	0.53^{bc}	0.000	0.010
Threonine	1.99	1.90	1.92	1.89	1.86	0.129	0.014
Tryptophan	0.46	0.45	0.48	0.47	0.43	0.548	0.004
Valine	2.33^{a}	2.21^{ab}	2.25^{ab}	2.22^{ab}	2.16^{ab}	0.037	0.017
Cysteine	0.46^{a}	0.430^{ab}	0.43^{ab}	0.43^{ab}	0.42^{ab}	0.076	0.004
Tyrosine	1.52	1.43	1.47	1.44	1.43	0.102	0.012

*Whole fish body proximate and amino acids (dry lyophilized samples) analyzed by the Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri, USA.
Means (n = 4) in the same row with different superscripts are significantly different at P < 0.05 based upon analysis of variance followed by Tukey's multiple range test.
** Values analyzed W/W% (g 100 g-1 as-is).
+ L-Lysine 78% was supplemented to maintain minimum level of lysine as recommended by NRC 2011.
++ L-Lysine 78% and L-Histidine 98.5% were supplemented to maintain minimum level of lysine as recommended by NRC 2011.

Blood collection and analysis

Four fish per tank were randomly chosen, euthanized by buffered Tricaine Methane Sulfonate 50 mg/L (MS.-222). Blood samples were collected from the caudal vein, then part of the blood was placed into heparinized Eppendorf using heparin sodium salt 100IU/ml, for determination of blood hemoglobin g/dl and hematocrit percent. Another part of the blood was kept into non-heparinized Eppendorf, then serum was separated by centrifugation at 5000rpm, 10 min and 40c. Separated serum was analyzed for; Total protein (TP) g/L, Albumin (ALB) g/L, Total immunoglobulins g/L, Alkaline phosphatase (ALP) U/L, Alanine transferase (ALT) U/L, Aspartate transferase (AST) U/L, Ammonia mg/L, Triglycerides mg/L, Cholesterol mg/L, Glucose mg/L, and lysozyme concentration unit/ml in clinical pathology lab, veterinary medicine school, Auburn

Table5: Analyzed*serum parameters of juvenile channel catfish (10.56±0.1g) *Ictalurus punctatus* fed diets with graded intact protein levels supplemented with EAA over a nine week growth period.

IP-EAA	Total protein g/dl	Albumin g/dl	TIg** g/dl	Triglyceride mg/dl	Glucose mg/dl	ALP U/L	ALT U/L	AST U/L	Ammonia mg/dl
32	3.342	0.958	2.460	124.14^{ab}	102.75	56.55^{ab}	9.50^{ab}	63.75^{ab}	827.50^{ab}
30^{+}	3.320	0.940	2.350	120.00^{b}	94.20	56.00^{ab}	8.80^{ab}	60.40^{ab}	712.75^{ab}
28^{+}	3.208	0.931	2.383	119.50^{b}	94.00	46.46^{b}	7.200^{ab}	56.20^{b}	609.40^{b}
26^{++}	3.230	0.938	2.328	127.375^{ab}	93.60	61.025^{a}	8.166^{ab}	58.50^{ab}	690.33^{b}
24^{++}	3.102	0.930	2.200	148.433^{a}	106.40	62.65^{a}	10.00^{a}	67.60^{a}	837.25^{a}
P-	0.362	0.733	0.358	0.009	0.011	0.000	0.016	0.003	0.027
PSE[a]	0.0252	0.0079	0.0238	23.21	2.140	1.068	0.0297	0.974	0.3205

Means (n = 4) in the same column with different superscripts are significantly different at P < 0.05 based upon analysis of variance followed by Tukey's multiple range test.
[a]PSE: Pooled standard error.
*Serum samples analyzed at clinical pathology lab, school of veterinary medicine, Auburn University by using a Roche Cobas c311 chemistry analyzer instrument.
** Total immunoglobulins
+ L-Lysine 78% was supplemented to maintain minimum level of lysine as recommended by NRC 2011.
++ L-Lysine 78% and L-Histidine 98.5% were supplemented to maintain minimum level of lysine as recommended by NRC 2011.

Table6: Analyzed*serum and hematologic parameters of juvenile channel catfish (10.56±0.1g) *Ictalurus punctatus* fed diets with graded intact protein levels supplemented with EAA over a nine week growth period.

IP-EAA	Lysozyme activity units/ml	Hemoglobin g/dl	Hematocrit (PCV)%
32	414.0^{a}	8.027^{a}	32.416^{ab}
30^{+}	402.0^{ab}	7.758^{ab}	32.333^{ab}
28^{+}	398.^{ab}	6.988^{ab}	32.166^{ab}
26^{++}	391.5^{ab}	7.240^{ab}	31.833^{ab}
24^{++}	361.25^{b}	5.946^{b}	29.1667^{b}
P-value	0.003	0.040	0.007
PSE[a]	3.7060	0.14198	0.2824

Means (n = 4) in the same column with different superscripts are significantly different at P < 0.05 based upon analysis of variance followed by Tukey's multiple range test.
[a]PSE: Pooled standard error.
*Serum samples analyzed at clinical pathology lab, school of veterinary medicine, Auburn University by using a Roche Cobas c311 chemistry analyzer instrument.
+ L-Lysine 78% was supplemented to maintain minimum level of lysine as recommended by NRC 2011.
++ L-Lysine 78% and L-Histidine 98.5% were supplemented to maintain minimum level of lysine as recommended by NRC 2011.

university by using a Roche Cobas c311 chemistry analyzer instrument (Table 5).

Hemoglobin concentration (g/dl)

Total hemoglobin concentration was determined by using (Pointe scientific, INC. hemoglobin reagent set) (Table 6). It depends on the oxidation of hemoglobin and its derivatives to methemoglobin by Potassium ferricyanide in alkaline medium, then reaction with Potassium cyanide producing more stable cyanmethemoglobin which has a maximum absorbance at 540nm, color intensity is proportional to total hemoglobin concentration (Wolf et al., 1973).Total hemoglobin concentration (g /dl) which estimated from the following equation :

= Abs. of unknown/Abs. of Standard × Concentration of standard (g/dl).

Serum lysozyme activity (units/ml)

Serum samples were analyzed by using sigma Aldrich lysozyme Detection Kit; for detection the presence of lysozyme activity (Table 6). This simple assay to detect lysozyme activity uses *Micrococcus lysodeikticus* cells as the substrate. Lysozyme activity results in the lyse of the *Micrococcus lysodeikticus* cells. During incubation of the lysozyme sample and substrate at 25cº, the reaction is followed by monitoring the decrease in absorbance at 450 nm (Wang et al., 2016; Shugar, 1952).

Units/mL enzyme = (ΔA450/min Test – ΔA450/min Blank) (df)/ (0.001) (0.03)
df = dilution factor
0.001 = A450 as per the Unit Definition
0.03 = Volume (in milliliters) of enzyme solution

Statistical Analysis

All data were subjected to a one-way analysis of variance to determine the significant differences ($P < 0.05$) among the treatments, which was followed by Tukey's multiple comparison test to distinguish significant differences among treatment means. The data were analyzed using SAS (V9.4. SAS Institute, Cary, North Carolina, USA)

RESULTS

Fish growth response

Fish final weight, weight gain, weight gain percent, FCR, and fish are presented in Table 3. Based on the results of ANOVA, fish growth, FCR and survival were not significantly affected by the reduction of the dietary IP from 32 to 26% with EAA supplementation as protein was decreased. While the reduction of protein to 24% resulted in a significant reduction in the performance of the fish as compared to those fed on 32 and 30 % IP diets. Whereas, FCR significantly increased and feed intake decreased as the IP level was reduced to 24%.

Whole fish body proximate composition

Whole fish body crude protein, moisture, ash, and crude fiber (Table 4) were not significantly affected by reducing the dietary intact protein from 32 to 24%. While whole fish body fat content significantly increased at 24% IP level. Except for taurine, whole fish body AA content (Table 4) was not significantly affected by lowering the intact protein from 32 to 24%.

Serum and whole blood analysis

In the present growth study, reducing the dietary intact protein content from 32 to 24% did not significantly change the level of some serum health indicators; total protein, albumin, total immunoglobulins, and glucose. The level of other serum parameters; ALT, AST, ALP, triglycerides, and ammonia showed a significant increase, but serum lysozyme, total blood hemoglobin, and hematocrit percent demonstrated a substantial decrease as the dietary intact protein was decreased to 24%.

DISCUSSION

Growth and feed utilization parameters

In conjunction with the high prices of protein sources used in catfish feed formulation, the lowering of the intact protein sources in fish feed has become necessary economically and environmentally. Hence, the use of crystalline amino acids as a supplement for optimizing, satisfying the amino acids need especially the limiting amino acids has had a critical interest. Further, for improving protein utilization and body protein growth (NRC, 2011; Wu, 2013).

In this study the dietary intact protein was reduced from 32 to 24%, and then crystalline L-lysine87.5% and L-histidine 98.5%were supplemented up to the level as recommended by the NRC (2011). Fish growth parameters and FCR were not significantly affected as intact proteins were reduced to 26% (Table 3). These results suggested that optimizing the amino acid balance is the key to the reduction of protein with maintaining growth and feed utilization efficiency.

As demonstrated by, Cowey and Walton (1988) and Furuya et al. (2004) using crystalline amino acids as a supplement in the reduced protein diets; could help in restoring the amino acid balance, stimulate of feed consumption and fish growth. Also, multiple amino acid supplementations are required to improve protein utilization by maintaining the continuous absorption rate of AAs to avoid the risk of AA's deficiencies and antagonism. As illustrated by other studies with achieving the exact amino acids balance free fishmeal diets could be used with maintaining considerable fish growth (Gatlin III et al., 2007).Also, as found by, Viola and Lahav (1991) the total dietary protein could be lowered from 30 to 25% with lysine supplementation to carp diets. The same findings have found by Viola et al. (1992) as well. Similarly, Cheng et al. (2003) observed that trout grew equally when fed 37% as opposed to 42% CP when the fish meal was reduced by 50% and lysine, methionine, threonine, and tryptophan were supplemented to be equivalent to the 42% crude protein control diet. Yamamoto et al. (2005) demonstrated that supplementation of all essential amino acids to 35% protein diet improved protein retention efficiency in trout from 35% in a 45% crude protein diet to 50% in a 35% crude protein diet. Bai and Gatlin III (1994) observed that under aquaria condition weight gain, feed efficiency, and protein conversion efficiency were improved when lysine was added to a 25% crude protein diet compared to a 30% crude protein diet. These studies suggested that protein levels can be reduced and optimal fish growth could be achieved, in so far the amino acid balance optimized.

In this study, as dietary intact protein reduced from 32 to 24% and EAA supplemented FCR and PER values were increased, while feed intake decreased significantly. As described in Nile tilapia by Abdel-Tawwab et al. (2010) protein retention efficiency increased with reducing the protein content with AA supplementation. Similarly, Yamamoto et al. (2005) showed that in trout fish which fed diets with higher levels of EAA supplements had higher nitrogen retention of 50 % compared to the control group at 35%. In rainbow trout, Gaylord and Barrows (2009) found that an increase in the feed conversion ratio in low crude protein 35% diet compared to 45CP, 45AA, 35AA, and 35AA-Gly. Protein retention efficiencies were improved in fish fed the 35AA and 35AA-Gly compared to the 45CP treatment. Also, Zhou et al. (2015) observed that protein retention of tilapia was increased with reducing dietary protein content from 33.5 to 30.3% with amino acid supplementation.NRC (2011) stated that the decrease in DP/DE ratio obtained by reducing the dietary protein level or by increasing the energy level will improve protein (nitrogen) utilization. However, the diets were iso-energetic but the diets with less dietary protein contents had higher

dietary starch contents. Wdale et al. (1995) demonstrated increasing the dietary content of non- protein digestible energy could increase nitrogen retention by decreasing nitrogen losses. Further, in our study, with reducing the dietary intact protein content to 24% fish feed intake decreased significantly, this could be the deficiency or imbalance of the amino acid. As suggested by, De la Higuera (2001) feed intake could be decreased when amino acid deficiency occurred.

In this study, fish growth was significantly lowered when dietary intact protein was lowered to 24%. However, the EAA profile was maintained at the required level stated in NRC (2011). This reduction in growth could be due to the dietary EAA limitation or deficiency. As explained by Righetti et al. (2011) as protein decreased in the diet, deficiency of amino acid may occur, which was not limiting in higher levels of protein. Wilson (2003) illustrated that in the case of AA imbalance, the utilization of the remaining amino acids will be decreased, and protein synthesis and another physiological process will be deprived. Moreover, it has been noticed by Zarate and Lovell (1997) that leaching loss of crystalline lysine is about 12%, while a 2% loss of protein-bound lysine, which clearly explains the lower efficiency of the crystalline form. Further, they illustrated that free lysine passes from the stomachs of channel catfish more rapidly than the protein-bound lysine. As reported by, Lumbard (1997) and Scheinmann et al. (1997) in trout and hybrid striped bass crystalline amino acids are absorbed quicker than intact proteins resulting in asynchronous circulation, then catabolized, the nitrogen excreted and subsequently impede protein synthesis. Consequently, all these factors could decrease and retard the bioavailability and the utilization of CAA and subsequently improper protein biosynthesis and limit fish growth.

Whole fish body proximate analysis

Whole fish body crude protein, moisture, ash, crude fiber, and amino acids percent (Table 4) were not significantly affected by reducing the dietary intact protein from 32 to 24%, However, the values were increased numerically as dietary intact protein increased. While whole fish body fat content significantly increased as the dietary intact protein was reduced to 24% with EAA supplementation. This effect is usually associated with diets containing very low levels of dietary protein. This is because with decreasing the dietary protein level, the DE/P ratio will be increased than the required level, increasing body fat content. Also, in case of amino acid deficiency or imbalance which maybe happened when intact protein reduced to 24%, the unutilized amino acids will be catabolized (oxidation of the carbon skeleton) and deposited as body fat. As demonstrated by, Cheng et al. (2003) feeding diets with amino acid imbalance for rainbow trout, resulted in altered protein deposition and excess energy deposition as fat in the liver, fillet or peritoneal cavity. As observed by, Robinson and Li (2011) as dietary protein levels increased to 26 or 28%, the fillet protein and moisture increased and fillet fat decreased.

The body taurine content reduced significantly compared to 32 and 30 % IP diets. In this study, the experimental diets were formulated by using protein sources other than fish meal. And it has found that fish meal is rich in taurine but absent from plants. In our study, the dietary methionine level decreased from 0.55 to 0.42% as protein was lowered from 32 to 24% respectively. This levels of methionine (0.42%) maybe not enough to support taurine synthesis, which correlated with the growth reduction at that low protein level 24%. As reported by Wilson (2003) and Gaylord et al. (2007) methionine can be converted to taurine in fish by cysteine dioxygenase. This process is up controlled by dietary methionine levels.

Serum and blood health parameters

In the present study catfish serum levels of total protein, albumin, total immunoglobulins, and glucose were not significantly affected with the reduction of protein level from 32 to 24%, however, the values increased numerically at the high level of protein (32%), (Table 5). Abdel-Tawwab (2012) have found that plasma proteins and glucose were significantly affected by dietary protein level. Also, increasing the serum protein level with an increase in the dietary protein level has been observed in European eel, and Nile tilapia (Suárez et al., 1995), and Abdel-Tawwab et al. (2010). The rise of serum protein with increasing the dietary protein content could be due to the enhancement of digested protein (Lundstedt et al., 2002)

Serum triglycerides, ALT, AST, and ALT concentration demonstrated a clear increase with the reduction of dietary protein to 24% (Table 5). Abdel-Tawwab (2012) has observed that plasma lipids, AST, and ALT were significantly affected by dietary protein level. Asin case of amino acid deficiency or imbalance which could be happened with reducing the dietary protein level, other amino acids will not be utilized and will be catabolized, deaminated and converted into energetic compounds (Stone et al., 2003). The enzymes control the metabolism of amino acids, which involves the deamination and transamination reactions are called "amino acid-metabolizing enzymes. Metón et al. (2003) indicated that the expression of enzymes of intermediary metabolism is up-regulated by nutritional status in fish. Particularly, the levels of amino acid and protein metabolizing enzymes and nitrogen excretion are considered strong evidence of dietary protein status in terms of quantity and quality. The rise of ALT and AST activities may reflect the use of the hydrocarbons from the catabolized amino acids to supply energy. Serum triglycerides increased significantly, it may be because the muscle tissue is responsible for amino acid turnover; protein synthesis or breakdown of those molecules as energetic substrates.

In our work, serum total hemoglobin concentration significantly decreased in the fish group fed on low protein diet 24% and higher value showed in fish fed on high protein level diet. As suggested by, Pulsford et al. (1994) the splenic activity and the production of hemoglobin from the storage pool in the spleen are increased by increasing dietary protein level. Serum lysozyme concentration also decreased as protein decreased to 24%. These results may have resulted from the low level of whole-body taurine content in fish fed 24% protein diet, which adversely affected the immune response. As found by Qiang et al. (2013) the optimal dietary protein and generally the nutritional status of the fish could affect the non-specific immune response. As in the case of, amino acids imbalance, the immune function will be decreased. Kiron et al. (1995) observed serum lysozyme activity and C-reactive proteins of rainbow trout were reduced when fed 10% protein-deficient diet compared to 35% and 50% protein. Also, Lim and Klesius (1998 a) found that macrophage migration was higher in catfish fed 28% protein diet compared to fish fed diets with lower percentages of protein. So, from these results, it can be concluded that the optimal protein level and amino acid balance is needed to maintain a non specific defense mechanism.

CONCLUSION

It can be concluded that in channel catfish practical diets the dietary intact protein could be reduced from 32 to 26% with supplementation of commercial lysine and histidine to maintain the amino acid balance at the recommended level by NRC (2011). But, fish growth was adversely decreased, while whole body fat content increased by lowering of protein to 24%.Additionally, fish immune response and blood

hemoglobin concentration deteriorated in catfish fed 24% protein diet.

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