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Comparison of the efficiency of utilization of amino acids from intact protein and amino acids in crystalline form by channel catfish, (Ictaluras punctatus)

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COMPARISON OF THE EFFICIENCY OF UTILIZATION OF AMINO ACIDS FROM INTACT PROTEIN AND AMINO ACIDS IN CRYSTALLINE FORM BY CHANNEL CATFISH, (*ICTALURUS PUNCTATUS*)

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ABSTRACT

Two laboratory feeding trials were conducted to quantify differences in the digestibility and absorption of dietary amino acids provided in intact protein or in purified amino acid mixtures. In the first experiment, 36 channel catfish (346 ± 47 g) were force-fed one of six practical feed ingredients (blood meal, corn meal, fish meal, meat and bone meal, soybean meal or wheat middlings), or a mixture of crystalline amino acids designed to duplicate the amino acid composition of each of the practical ingredients. Apparent digestibility coefficients (ADC) were calculated for 15 amino acids in each practical ingredient and in each amino acid mixture. An average ADC of all amino acids in each treatment also was calculated. Very few differences in the ADCs of individual amino acids were observed. However, in all ingredients but two, the mean ADCs of amino acids in fish fed purified amino acids were significantly higher than the mean ADCs of amino acids in fish fed intact protein. In the second experiment, 468 channel catfish (396 ± 49 g) were force-fed the same ingredients and amino acid mixtures used in the digestibility trial, and blood was collected from the hepatic portal vein at 1-h intervals for 12 h after feeding. In four of the six ingredients tested, postprandial concentrations of 2-8 amino acids in blood plasma were significantly higher and reached peak levels earlier (1-3 h after feeding) in fish fed purified amino acids than in fish fed intact protein. Results indicated that protein was well digested by channel catfish regardless of its source. However, soybean protein appeared to be digested more slowly than the other proteins tested. The efficiency with which supplemental amino acids are utilized might be reduced if slowly digested proteins compose a major portion of the diet, because of temporal differences in the absorption of amino acids from different dietary sources. If so, the effectiveness of amino acid supplementation could vary with the ingredient composition of the diet to a greater extent than has previously been recognized.

CHAPTER 1

INTRODUCTION

Channel catfish, *Ictalurus punctatus,* production is the largest aquaculture industry in North America (Lovell 1992). Arkansas, Alabama, Mississippi and Louisiana are the four largest channel catfish producing states, contributing over 90% of US production (NASS 2004). Commercial production of channel catfish developed in the southern U.S.A. during the late 1950s and early 1960s.

Initially, channel catfish were raised in ponds at low stocking densities and fed low-cost, incomplete diets. As stocking densities increased, development of nutritionally balanced diets became an economic necessity. Commercially produced, complete catfish feeds were based on the nutrient requirements of other animals, such as poultry and salmonids (Wilson and Lovell 1993). In the early 1970s, development of a fish meal shortage emphasized the need to determine the nutrient requirements of channel catfish, which have since been published (Wilson 1991).

The use of plant proteins to replace fish meal in commercial feeds also required greater attention to satisfying dietary amino acid requirements (Wilson et al. 1981). Amino acids can be provided in the form of intact protein in feed ingredients or in purified, crystalline form as dietary supplements. The increased use of plant proteins in catfish diets in recent years has led to increased use of purified amino acids to avoid deficiencies caused by high levels of plant products. For example, dietary L-lysine can be supplemented in purified form to eliminate the lysine deficiency in soybean meal, (El-Saidy and Gaber 2002). Evidence from fish other than catfish (Cowey and Sargent 1979, Thebault 1985, Lumbard 1997) suggests that amino acids in intact protein are utilized more efficiently than those provided in crystalline form, possibly

because crystalline amino acids are absorbed and catabolized more rapidly than amino acids from intact proteins.

Fish require amino acids for maintenance and growth, and naturally occurring proteins are the primary source of amino acids in fish diets. The level of protein needed by fish varies with the species and the amino acid composition of the protein fed. Philips et al. (1957) reported that trout required 28% protein. Salmon require 40-55% protein within the temperature range of 8.3-12.8^{o}C (Delong et al. 1958). Approximately 25% protein is needed for optimum growth of channel catfish (Nail 1962). Currently, 28-32% protein feeds are available for grow-out of catfish (Robinson et al. 1994). Gaylord and Gatlin (2001) reported the growth rate of channel catfish increased when they were fed with high protein and high energy diets. Protein requirements of channel catfish fry are higher than those of fingerling or adult fish. Protein levels of 52% and 40% are required for catfish fry and fingerling weighing 0.02 g and 20 g, respectively (Robinson et al. 1994). The availability of dietary protein can vary considerably among different sources. Apparent crude protein digestibility of feedstuffs for catfish range from 65% to 92% among plant and animal sources of protein, respectively (Brown and Strange 1985, Gaylord and Gatlin 1996, Hossain et al. 1997).

Fish, like other animals, have specific requirements for dietary essential amino acids. Ingested proteins are hydrolyzed to release amino acids that are used for synthesis of tissue proteins or used for energy. Studies have determined the essential amino acid requirements of catfish per gram of dietary protein consumed. The arginine requirement is approximately 4.3% of dietary protein in channel catfish (Robinson et al. 1980a). The lysine requirement is 5.0% of protein (Robinson et al. 1980b). Harding et al. (1977) reported the sulfur amino acid (methionine plus cystine) requirement to be 2.3% of dietary protein. Wilson et al. (1980)

reported requirements for histidine (1.5%), isoleucine (2.6%), leucine (3.5%), and valine (3%). Phenylalanine and tyrosine requirements are 4.5% of dietary protein (Robinson et al. 1979). The threonine requirement is 2.2% of dietary protein and tryptophan is 0.5% of dietary protein (Wilson et al. 1978). Harding et al. (1977) and Page (1978) concluded that the sulfur amino acids requirement of channel catfish fed 24% crude protein was 0.5-0.75% of the diet, and cystine could replace 60% of the dietary methionine requirement.

Fish meal is desirable in the diet of channel catfish because it provides all of the dietary essential amino acids required for growth, in favorable ratios. However, the high cost of fish meal has led to the use of alternative protein sources like soybean meal in catfish feeds. Lysine, one of the most limiting dietary essential amino acids in plant products, is relatively abundant in soybean meal (Rumsey and Ketola 1975), but supplementation of soybean meal with crystalline lysine has been shown to enhance the growth and feed efficiency of channel catfish juveniles (Andrews and Page 1974). Bai and Gatlin (1994) concluded that the level and source of dietary protein significantly influenced the performance characteristics of channel catfish, and the benefits of supplemental lysine increased as the protein content of the diet declined.

Amino acid requirements can be met by feeding mixtures of complementary proteins and by supplementing deficient proteins with crystalline amino acids. The dietary non-essential amino acids supplied in a diet, while not required, help to promote growth by reducing the need for amino acid synthesis, which saves energy (Tucker and Robinson 1991). The nutritive value of dietary protein depends on its amino acid content and the extent to which the amino acids it contains are absorbed into the bloodstream. The rate of release and absorption of amino acids from protein during digestion influence the dietary value of a protein source. For example, the average protein digestibility of cottonseed meal is 86% whereas lysine availability in cottonseed

meal is only 66% in channel catfish. The practice of using supplemental amino acids to improve the quality of inferior protein sources in catfish diets raises some questions. Studies have shown that crystalline amino acids may not be absorbed from the intestine in a manner that promotes effective utilization. Several researchers (Yamada et al. 1981, Murai et al. 1987, Schuhmacher et al. 1993) have demonstrated that amino acid concentrations in blood plasma increased more rapidly, and reached peak levels more quickly, after feeding crystalline amino acids than after feeding intact proteins. Cowey and Walton (1988), made similar observations when they measured the increase in radioactivity in the blood of rainbow trout after feeding pellets containing either ^{14}C-labeled amino acids or ^{14}C-labeled protein. The inferior growth rates and feed conversion ratios of rainbow trout fed crystalline amino acids have been attributed to the rapid uptake of amino acids from the gut into the peripheral blood, leading to deamination of the excess amino acids and high rates of nitrogen excretion (Yamada et al. 1981, Cowey and Walton 1988). The best method to provide adequate amino acid nutrition to the channel catfish may be to use a mixture of complementary proteins, in which different protein sources are mixed effectively to meet the dietary amino acid requirements. However, in some diet formulations, amino acids might have to be supplemented in purified form to meet minimum requirements.

The objectives of the present study were (1) to measure and compare the apparent availability of amino acids in six practical feed ingredients with the apparent amino acid availability of crystalline amino acids in mixtures designed to match the concentrations of amino acids in each of the feed ingredients, and (2) to measure the differences in the rates of uptake of amino acids from the intestine into the blood of the HPV of channel catfish fed intact protein or crystalline amino acid mixtures. This research was designed to measure the efficiency of digestion and absorption of amino acids from intact proteins and crystalline amino acid

supplements to determine the magnitude of the differences in the utilization of amino acids from natural (protein-bound) and crystalline sources.

CHAPTER 2

MATERIALS AND METHODS

2.1 Experimental Design and Diets

Channel catfish (346.6 $\pm$ 47.2 g) were subjected to a five-week acclimation period in a recirculating culture system before the experiments were initiated. Two recirculation systems were used in the experiments. Each consisted of twenty, 120-L, rectangular glass aquaria connected to a 0.1 m^3 bead-bed biofilter, 0.25 hp pump and a 40 W ultraviolet-light disinfection unit. Aeration was provided to all the tanks via air stones connected to a low-pressure blower. Styrofoam sheets were placed over the aquaria to prevent fish from jumping out.

Ambient temperatures were between 25-30^o C. Water temperatures ranged from 24-25^o C. Dissolved oxygen levels were maintained above 5 mg/L. Total ammonia-nitrogen and nitrite nitrogen were measured using test kits (Hach Co., Loveland, Colorado). An electric pH meter (Model 330 Orion Research, Inc., Boston, Massachusetts) was used to monitor pH in the culture systems. Ammonia nitrogen and nitrite nitrogen were maintained at levels below 0.03 mg/L and 0.33 mg/L, respectively. Hardness and pH were maintained at levels of 170 mg/L and 8.2, respectively. Biofilters were backflushed every day. Waste in the tanks was siphoned out every two days.

Twelve experimental diets were formulated as indicated in Table 1. Six of the diets contained intact protein from a practical ingredient commonly used in channel catfish feeds (Table 2) and six contained mixtures of amino acids (Table 3) formulated to match the amino acid composition of each practical ingredient. The six ingredients, three of which were plant protein sources— soybean meal (SBC), corn meal (CM) and wheat middlings (WM)— and three

TABLE 1. Composition of the test diets. Each of the feedstuffs composed 99% of the test diet with 0.5% binder and 0.5% indicator.

Ingredients	Diet											
	1	2	3	4	5	6	7	8	9	10	11	12
Blood meal	X											
Fish meal, menhaden		X										
Meat and bone meal			X									
Corn meal				X								
Soybean meal					X							
Wheat middlings						X						
Blood meal CAAM[1]							X					
Fish meal CAAM[1]								X				
Meat and bone meal CAAM[1]									X			
Corn meal CAAM[1]										X		
Soybean meal CAAM[1]											X	
Wheat middlings CAAM[1]												X
Binder[2]	X	X	X	X	X	X	X	X	X	X	X	X
Indicator[3]	X	X	X	X	X	X	X	X	X	X	X	X

[1] Crystalline amino acid mixture (CAAM) formulated to match the amino acid composition of the intact protein source, as determined by analysis. CAAM was delivered in a carrier designed to simulate the proximate composition (crude protein, lipid, fiber, ash and NFE content) of the practical feed ingredient.
[2] Carboxymethylcellulose, 0.5% of diet.
[3] Chromic oxide, 0.5% of diet.

TABLE 2. Proximate composition and amino acid composition of the feed ingredients (%).

	Blood meal	Fish meal	Meat and Bone meal	Corn meal	Soybean meal	Wheat middlings
Moisture	12.86	16.49	8.40	11.38	9.19	11.76
Crude Protein	84.63	61.45	55.79	7.00	48.19	15.10
Lipid	0.08	4.26	9.45	1.26	1.71	3.46
Fiber	0.02	0.11	3.03	0.01	2.77	5.48
Ash	2.36	17.66	23.32	0.55	6.91	3.80
NFE	0.00	0.00	0.00	79.79	31.23	60.40
Alanine	8.58	7.65	9.12	17.00	5.29	6.09
Arginine	4.83	7.94	9.56	3.72	9.80	10.40
Aspartic acid	8.59	8.11	7.27	8.31	11.53	8.49
Glutamic acid	7.10	13.22	12.31	*	17.82	25.24
Glycine	5.71	12.93	23.93	9.39	6.45	7.75
Histidine	13.29	3.42	2.41	3.32	4.37	5.34
Isoleucine	1.33	4.27	3.07	5.82	4.96	4.64
Leucine	11.49	7.39	6.43	21.59	8.50	7.82
Lysine	16.12	14.39	9.08	*	10.69	4.74
Methionine	1.20	3.07	1.41	1.45	0.82	0.53
Phenylalanine	5.87	3.87	3.37	6.20	4.99	4.87
Serine	2.77	2.45	2.68	6.89	3.77	3.52
Threonine	3.08	4.02	3.08	5.42	3.73	3.70
Tyrosine	1.20	1.78	1.33	2.27	1.67	0.95
Valine	8.77	5.43	4.96	8.64	5.59	5.90

*Not detected

TABLE 3. Proximate composition of the diets and the amino acid composition of the crystalline amino acid mixtures (%).

	Blood meal	Fish meal	Meat and bone meal	Corn Meal	Wheat middlings	Soybean meal
Moisture	12.73	16.33	8.32	11.27	11.64	9.09
Protein	83.80	60.85	55.25	6.93	14.95	47.71
Lipid	0.08	4.22	9.36	1.25	3.42	1.69
Fiber	0.02	0.11	3.00	0.01	5.42	2.74
Ash	2.36	17.48	23.09	0.55	3.76	6.84
Starch	0.00	0.00	0.00	79.01	59.80	30.92
CMC	0.49	0.49	0.49	0.50	0.49	0.49
Chromic oxide	0.49	0.49	0.49	0.50	0.49	0.49
Alanine	8.58	7.65	9.12	17.00	6.09	5.29
Arginine [1]	4.83	7.94	9.56	3.72	10.40	9.80
Aspartic acid	8.59	8.11	7.27	8.31	8.49	11.53
Glutamic acid	7.10	13.22	12.31	0.00	25.24	17.82
Glycine	5.71	12.93	23.93	9.39	7.75	6.45
Histidine [2]	13.29	3.42	2.41	3.32	5.34	4.37
Isoleucine	1.33	4.27	3.07	5.82	4.64	4.96
Leucine	11.49	7.39	6.43	21.59	7.82	8.50
Lysine [3]	16.12	14.39	9.08	0.00	4.74	10.69
Methionine	1.20	3.07	1.41	1.45	0.53	0.82
Phenylalanine	5.87	3.87	3.37	6.20	4.87	4.99
Serine	2.77	2.45	2.68	6.89	3.52	3.77
Threonine	3.08	4.02	3.08	5.42	3.70	3.73
Tyrosine	1.20	1.78	1.33	2.27	0.95	1.67
Valine	8.77	5.43	4.96	8.64	5.90	5.59

[1] Arginine was added in the form of arginine monohydrochloride in the following amounts: blood meal, 4.90%; fish meal, 5.84%; meat and bone meal, 6.38%; soybean meal, 5.65%; wheat middlings, 1.88%; corn meal, 0.3%.
[2] Histidine was added in the form of histidine monohydrochloride in the following amounts: blood meal, 15.05 %; fish meal, 2.81%; meat and bone meal, 1.79%; soybean meal, 2.81%; wheat middlings, 1.07%; corn meal, 0.3%.
[3] Lysine was added in the form of lysine monohydrochloride in the following amounts: blood meal, 16.88%; fish meal, 10.94%; meat and bone meal, 6.26%; soybean meal, 6.37%; wheat middlings, 0.9%; corn meal, not supplemented.

of which were animal protein sources— menhaden fish meal (FM), meat and bone meal (MB) and blood meal (BM), were fed individually as the sole protein-bearing components of the diet. The practical diets were analyzed with standard methods (AOAC 2000) to determine crude protein, lipid, fiber, and ash content. The amino acid composition of each ingredient was determined by high performance liquid chromatography (Table 2.) The crude lipid and crude fiber content of the ingredients are shown in Table 4.

The proximate analyses of the intact ingredients and their amino acid compositions were used to create six artificial ingredients that were used as test diets. These ingredients were prepared by mixing purified, crystalline amino acids, (Sigma-Aldrich Co., St. Louis, Missouri) with cellulose (fiber), starch (NFE), menhaden fish oil (lipid), and dicalcium phosphate (ash). The crystalline amino acid mixtures (CAAM) for soybean meal (SBC), corn meal (CMC), wheat middlings (WMC), fish meal (FMC), blood meal (BMC) and meat and bone meal (MBC) were prepared to duplicate the proximate composition of each of those ingredients. Lysine, arginine and histidine were provided in the form of lysine monohydrochloride, arginine monohydrochloride, and histidine monohydrochloride, respectively. The amount of each compound that was required to provide the desired quantity of amino acid was calculated (Table 5).

The pH of the amino acid mixtures was adjusted to 7 with sodium hydroxide to avoid potential problems associated with the use of crystalline amino acids in previous experiments with channel catfish (Nose et al. 1974, Wilson et al. 1977). The neutralization factor (Table 6) for each of the artificial ingredients was determined using a solution of 1 M sodium hydroxide.

TABLE 4. Crude fiber and crude lipid content of the six ingredients (%)

Ingredients	Crude lipid[1]	Crude fiber[1]
Blood meal	0.09	0.02
Fish meal	4.29	0.12
Meat and bone meal	9.51	3.05
Corn meal	1.26	0.01
Soybean meal	1.71	2.77
Wheat middlings	3.46	5.48

[1]Department of Agricultural Chemistry, Louisiana State University Agricultural Center, Baton Rouge, Louisiana, USA

TABLE 5. Amino acid composition of the arginine, histidine and lysine supplements.

Compound	Formula	Mol. Wt.	Mol. Wt. HCl	Mol. Wt. H_20	Mol. Wt. AA
L-Arginine mono hydrochloride	$C_6H_{14}N_4O_2.HCl$	210.6	36.46	---	174.14
L-Histidine mono hydrochloride	$C_6H_5N_3O_2.HCl.H_2O$	209.6	36.46	18.02	155.12
L-Lysine mono hydrochloride	$C_6H_{14}N_2O_2.HCl$	182.6	36.46	---	146.14

TABLE 6. Sodium hydroxide neutralization factor, expressed in milliliters per gram of CAAM for each ingredient.

Blood meal	Fish meal	Meat and bone meal	Corn meal	Soybean meal	Wheat middlings
0.98	0.90	0.48	0.07	0.65	0.35

2.2 Sample Collection for Digestibility Study

Fish were fed daily (1% of body weight) and the fecal samples were collected approximately 6 h after feeding (Table 7). The daily procedure was as follows. The fish were removed from the tanks and anesthetized in a 200-ppm solution of clove oil. Clove oil was used because it is less harmful to the nervous system than some other commercial anesthetics used in fish studies (Anderson et al. 1997). Anesthetized fish were force-fed with a 3-cc syringe. A length of plastic tubing that reached the stomach of the fish was attached to the syringe for force-feeding. The food was provided in slurry form which was pushed past the esophagus to the stomach. Approximately 6 h after feeding, feces were collected by physically stripping fecal material from the rectal area of each fish. Stripping was used to avoid leaching of nutrients from the feces into the water. The samples were obtained by manually pushing the feces from the rectal intestine of the fish with gentle thumb pressure on the lower third of the abdomen. The fish were then dipped in a fresh water rinse with vigorous aeration and returned to the culture tanks. The feces were collected in aluminum drying pans and dried overnight at 100^{o}C in a forced-air oven (Thermolyne Corp.,Dubuque, Iowa). Feces were pooled, by tank (fish), until a 20 – 50 mg sample was accumulated. Partially dried samples were stored in a cooler (4^{o}C) until analyzed.

Triplicate samples of the test diets and feces were subjected to chemical analysis. Chromium concentrations in the test diets and fecal samples were determined by inductively coupled plasma emission spectroscopy (ICP). Amino acid concentrations in the test diets and feces were determined by HPLC. The diets and feces were subjected to acid hydrolysis during this procedure (Gehrke et al. 1985). Tryptophan was destroyed by the acid hydrolysis procedure used to prepare the samples for HPLC analysis, so concentrations of tryptophan could not be

determined. Apparent digestibility coefficients for amino acids hereafter referred to as ADCs were calculated, as a percentage of amino acids present (Wilson et al. 1981).

2.3 Statistical Analysis I

ADCs for the amino acids from the six practical ingredients and six amino acid mixtures were compared with the analysis of variance procedure. When significant differences were indicated, Tukey's studentized range test was used to identify significantly different means ($\alpha = 0.05$).

2.4 Blood Sampling Studies

Four hundred and sixty eight channel catfish, (396.47 ± 49.2 g), were used to measure amino acid uptake study. The fish were stocked in large fiberglass pools for a one-week acclimatization period before the experiment began. Thirteen fish were used each day for blood sampling, with three replicates per experimental diet. Randomly selected fish were placed in 90-L aquaria and fed 1% of body weight per day. Blood was collected following the schedule in Table 8.

The diets were the same as those used in the digestibility study. The fish were force-fed by stomach intubation as previously described (Schumacher et al. 1997). In order to get an acceptable food slurry, all diets were mixed with two parts water. A 3-ml, plastic disposable syringe was used to deliver the slurry into the stomach of the fish, as described for the digestibility study. The slurry was mixed thoroughly before drawing it into the syringe. Approximately 5 min before blood sampling began, the fish were anesthetized in 200-ppm bath of clove oil. The abdomen of each fish was opened and blood was collected from the HPV with a heparinized syringe. A preliminary study undertaken to establish the optimum time intervals for blood sampling indicated that concentrations of plasma free amino acids in a 200 ppm bath

TABLE 7. Fecal sample collection schedule for measurement of amino acid availability
(F = feeding, C = collection)

Ingredients	replicates	0h	1h	2h	3h	4h	5h	6h	7h	8h
BM[1]	1	F						C		
	2	F						C		
	3	F						C		
FM[1]	1	F						C		
	2	F						C		
	3	F						C		
MB[1]	1	F						C		
	2	F						C		
	3	F						C		
CM[1]	1	F						C		
	2	F						C		
	3	F						C		
SB[1]	1		F						C	
	2		F						C	
	3		F						C	
WM[1]	1		F						C	
	2		F						C	
	3		F						C	
BMC[2]	1		F						C	
	2		F						C	
	3		F						C	
FMC[b]	1		F						C	
	2		F						C	
	3		F						C	
MBC[2]	1			F						C
	2			F						C
	3			F						C
CMC[2]	1			F						C
	2			F						C
	3			F						C
SBC[2]	1			F						C
	2			F						C
	3			F						C
WMC[2]	1			F						C
	2			F						C
	3			F						C

[1] BM, blood meal; FM, fish meal; MB, meat and bone meal; SB, soybean meal; CM, corn meal and WM, wheat middlings.
[2]Crystalline mixtures: BMC, blood meal; FMC, fish meal; MBC, meat and bone meal; SBC, soybean meal; CMC, corn meal and WMC, wheat middlings.

of clove oil. The abdomen of each fish was opened and blood was collected from the hepatic portal vein with a heparinized syringe. A preliminary study undertaken to establish the optimum time intervals for the blood sampling indicated the concentrations of plasma free amino acids from soybean meal reached their peak in 5-6 h, then dropped down to baseline levels in 10 h. Samples were taken immediately before feeding at (0 h) and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h after force-feeding. The blood samples were centrifuged at 10,000 rpm (Model SC110 AR, Savant Instruments Inc, Holbrook, New York), after which the plasma was deproteinized by centrifuging at 5,000 rpm in a Centrifree Micropartition device, (Millipore Corporation, Billerica, Massachusetts). The protein-free liquid was labeled and stored at -62^{o}C until analyzed.

Plasma free amino acids were separated and quantified by HPLC (Model 1100 Agilent Technologies, Palo Alto, California) with automated online derivatization using o-phthalaldehyde (OPA) and fluorenylmethyl chloroformate (FMOC).

2.5 Statistical Analysis II

Individual amino acid concentration was expressed as the mean of three observations. Significant differences ($P \leq 0.05$) between the effects of the experimental diets (intact protein vs. purified amino acids) on amino acid concentrations in HPV blood at 1-h time intervals were analyzed statistically using repeated measures of analysis of variance according to the Restricted Maximum Likelihood (REML) methods of estimation. A Saxton's macro was applied to identify pair-wise differences that were used to create groups of similar means whose relationships to each other could be identified by shared letters, similar to the results of a typical means separation test.

TABLE 8. Hourly blood collection schedule (6 days per week) for a 12-h period after force-feeding during week 1 and week 2[a].

Ingredient	0h	1h	2h	3h	4h	5h	6h	7h	8h	9h	10h	11h	12h
BM[b]	F_1	Th_1	W_2	F_1	Th_2	Sa_1	F_2	M_1	Sa_2	Tu_1	M_2	W_1	Tu_2
FM[b]	Th_1	F_1	Th_2	Sa_1	F_2	M_1	Sa_2	Tu_1	M_2	W_1	Tu_2	Th_1	W_2
MB[b]	Sa_1	W_1	Tu_2	Th_1	W_2	F_1	Th2	Sa_1	F_2	M_1	Sa_2	Tu_1	M_2
CM[b]	W_1	Sa_1	Sa_2	M_1	M_2	Tu_1	Tu_2	W_1	W_2	Th_1	Th_2	F_1	F_2
SB[b]	M_1	Tu_1	M_2	W_1	Tu_2	Th_1	W_2	F_1	Th_2	Sa_1	F_2	M_1	Sa_2
WM[b]	Tu_1	M_1	F_2	Tu_1	Sa_2	W_1	M_2	Th_1	Tu_2	F_1	W_2	S_1	Th_2
BMC[c]	F_1	Th_1	W_2	F_1	Th_2	SA_1	F_2	M_1	SA_2	Tu_1	M_2	W_1	Tu_2
FMC[c]	Th_1	F_1	Th_2	Sa_1	F_2	M_1	Sa_2	Tu_1	M_2	W_1	Tu_2	Th_1	W_2
MBC[c]	Sa_1	W_1	Tu_2	Th_1	W_2	F_1	Th2	Sa_1	F_2	M_1	Sa_2	Tu_1	M_2
CMC[c]	W_1	Sa_1	Sa_2	M_1	M_2	Tu_1	Tu_2	W_1	W_2	Th_1	Th_2	F_1	F_2
SBC[c]	M_1	Tu_1	M_2	W_1	Tu_2	Th_1	W_2	F_1	Th_2	Sa_1	F_2	M_1	Sa_2
WMC[c]	Tu_1	M_1	F_2	Tu_1	Sa_2	W_1	M_2	Th_1	Tu_2	F_1	W_2	S_1	Th_2

[a] Subscript 1 indicates samples taken in week 1, subscript 2 indicates samples taken in week 2

[b]BM, blood meal; FM, fish meal; MB, meat and bone meal; SB, soybean meal; CM, corn meal and WM, wheat middlings.

[c]Crystalline mixtures: BMC, blood meal; FMC, fish meal; MBC, meat and bone meal; SBC, soybean meal; CMC, corn meal and WMC, wheat middlings.

CHAPTER 3

RESULTS

The manner in which an amino acid is transported across the intestinal mucosa is determined by its molecular structure. There are different active transport systems for different groups of chemically similar amino acids. Amino acids with similar structures have been grouped together here to show trends among related groups of compounds. The amino acid groupings are (1) the acidic series – aspartic acid and glutamic acid; (2) the aliphatic series – alanine, glycine, isoleucine, leucine, serine, threonine, and valine; (3) the aromatic series – phenylalanine and tyrosine; (4) the basic series – arginine, histidine, and lysine; (5) the heterocyclic series – hydroxyproline, proline, and tryptophan; and (6) the sulfur-containing series – cysteine, cystine, and methionine.

Not all of the amino acids listed here were detected in all samples. For example, tryptophan, another member of the heterocyclic series, was destroyed by acid hydrolysis which is the standard procedure used to prepare diet and fecal samples for amino acid analysis and thus could not be measured in any of the samples used to determine digestibility. Tryptophan was measured in blood samples. Hydroxyproline and proline, two other members of heterocyclic series, could not be measured in the feed ingredients; with the derivatization process used at the time and is not reported here. Cystine also could not be measured and is not reported here. Concentrations of other amino acids varied among samples and occasionally fell below detectable levels.

3.1 Digestibility

Apparent amino acid digestibility (i.e., availability) coefficients, hereafter referred to as ADCs, indicate the amount (%) of each amino acid that was absorbed from a feedstuff as it

passed through the digestive tract. ADCs are not adjusted for endogenous sources of amino acids — i.e., sloughed cells and intestinal secretions — and thus represent "apparent" digestibility. ADCs are always numerically lower than "true" digestibility coefficients, which are adjusted for non-dietary sources of amino acids in fecal samples. Among fishes, the difference between apparent and true digestibility coefficients is typically less than three percentage points (R.C. Reigh, Louisiana State University, personal communication).

3.1.1 Blood Meal (BM and BMC)

The ADCs of individual acidic, aromatic, and basic amino acids did not differ significantly between BM and BMC (Table 9). The apparent digestibility of most aliphatic amino acids also did not differ significantly between BM and BMC, however, the ADCs of alanine and serine were higher ($P \leq 0.05$) among fish fed BMC than among fish fed BM (Table 9). Methionine (sulfur-containing series) had a significantly higher ADC among fish fed BMC than among those fed BM (Table 9). The average ADC of amino acids in BM was 67.5%, which was significantly lower than the 93.9% average ADC of amino acids in BMC.

3.1.2. Corn Meal (CM and CMC)

The ADCs of individual acidic, aromatic, basic, and sulfur-containing amino acids were not different ($P > 0.05$) between CM and CMC (Table 9). Similarly, the ADCs of most aliphatic amino acids did not differ ($P > 0.05$) between CM and CMC, with the exception of serine and threonine, which had significantly higher apparent digestibility among fish fed CM than among fish fed CMC (Table 9). The average ADC of amino acids in CM, 91.2%, was higher ($P \leq 0.05$) than the average ADC of amino acids in CMC, 79.6%.

TABLE 9. Apparent digestibility coefficients (ADC ± SD, n=3) of amino acids in intact blood meal (BM) and corn meal (CM), and in mixtures of crystalline amino acids (BMC and CMC) designed to duplicate the amino acid composition of the intact ingredient. Within each ingredient, means in the same row with different letters are significantly different ($P \leq 0.05$). Within each ingredient, mean ADCs (average of all amino acids) with different letters are significantly different ($P \leq 0.05$).

Amino acid	BM %	BMC %	CM %	CMC %
Acidic series				
Aspartic acid	64.4 ± 16.8	94.9 ± 4.4	90.8 ±13.2	70.0 ± 6.6
Glutamic acid	72.0 ± 12.9	94.6 ± 3.7	96.5 ± 4.9	85.2 ± 7.7
Aliphatic series				
Alanine	58.6 ± 19.1[b]	93.5 ± 5.3[a]	93.3 ± 5.0	83.7 ± 2.5
Glycine	50.2 ± 24.1	85.5 ± 10.5	86.9 ± 16.9	61.6 ± 2.3
Isoleucine	44.6 ± 24.5	82.9 ± 13.3	91.8 ± 7.2	89.6 ± 9.1
Leucine	65.8 ± 16.2	96.2 ± 2.9	95.8 ± 3.6	92.1 ± 1.9
Serine	45.6 ± 23.2[b]	91.6 ± 5.3[a]	91.8 ± 10.3[a]	72.5 ± 4.2[b]
Threonine	54.1 ± 22.1	88.4 ± 7.5	87.1 ± 10.5[a]	63.5 ± 12.1[b]
Valine	63.8 ± 17.2	94.6 ± 2.5	90.3 ± 8.4	73.1 ± 6.6
Aromatic series				
Phenylalanine	71.8 ± 13.3	96.4 ± 2.4	97.0 ± 5.3	94.0 ± 5.2
Tyrosine	66.1 ± 14.4	94.1 ± 3.1	81.0 ± 9.9	81.2 ± 4.3
Basic series				
Arginine	78.4 ± 10.0	95.0 ± 3.0	88.0 ± 8.6	73.4 ± 3.8
Histidine	87.5 ± 6.7	99.4 ± 1.1	92.4 ± 13.1	79.5 ± 3.2
Lysine	84.1 ± 13.7	95.3 ± 4.2	*	*
Sulfur-containing series				
Methionine	77.2 ± 8.2[b]	100.0 ± 0.0[a]	100.0 ± 0.0	100.0 ± 0.0
Mean ADC	67.5 ± 15.3[b]	93.9 ± 4.4[a]	91.2 ± 8.6[a]	79.6 ± 4.8[b]

*Undetected.

3.1.3. Fish Meal (FM and FMC)

The apparent digestibility of individual acidic, aromatic, basic, and sulfur-containing amino acids did not differ ($P > 0.05$) between FM and FMC (Table 10). Again, the ADCs of most aliphatic amino acids did not differ ($P > 0.05$) between the two treatments, although glycine and serine had significantly higher apparent digestibility among fish fed FMC than among those fed FM (Table 10). The average apparent digestibility of amino acids in FM was significantly lower than the average apparent digestibility of amino acids in FMC (83.5% vs. 98.9%, respectively).

3.1.4. Meat and Bone Meal (MB and MBC)

There were no differences ($P > 0.05$) between treatments in the ADCs of individual acidic, aliphatic, aromatic, basic, or sulfur-containing amino acids (Table 10). However, when the ADCs of all amino acids were averaged by treatment, the mean ADC of amino acids in MB (70.4%) was significantly lower than the mean ADC of amino acids in MBC (86.5%).

3.1.5. Soybean Meal (SB and SBC)

The ADCs of individual acidic, aliphatic, aromatic, basic, and sulfur-containing amino acids did not differ significantly between treatments (Table 11). However, the average ADC of amino acids in SB, 87.9%, was lower ($P \leq 0.05$) than the average ADC of amino acids in SBC, 96.6%.

3.1.6. Wheat Middlings (WM and WMC)

There were no differences ($P > 0.05$) between the WM and WMC treatments in the ADCs of individual acidic, aliphatic, aromatic, basic, or sulfur-containing amino acids (Table 11). Nor was there a difference ($P > 0.05$) between the average ADCs of amino acids in WM and WMC (69.4% and 59.2%, respectively).

3.1.7. Summary

The ADCs of individual amino acids in half of the ingredients tested — meat and bone meal, soybean meal, and wheat middlings — were not significantly changed by the form (i.e., intact protein or purified mixture) in which the amino acids were provided in the diet. In those ingredients where significant differences in ADCs were observed between treatments — blood meal, corn meal, and fish meal — only a few aliphatic amino acids (glycine, serine, or threonine) ADCs were involved in two of the three occurrences (i.e., corn meal and fish meal). In the third case (blood meal), changes in the ADCs of two aliphatic amino acids (alanine and serine) and a sulfur-containing amino acid (methionine) accounted for the significant differences between treatments. In blood meal and fish meal, significantly higher ADCs were associated with two aliphatic amino acids among fish fed the crystalline amino acid mixtures, and in each case serine was one of the two amino acids involved. In corn meal, significantly higher ADCs were associated with serine and leucine among fish fed the intact protein source. In one case, methionine exhibited a significantly higher ADC among fish fed a mixture of purified amino acids. When the ADCs of all amino acids were averaged for each ingredient, in four of six ingredients — blood meal, fish meal, meat and bone meal, and soybean meal — the average ADC of amino acids in each of the purified amino acid mixtures was significantly higher than the average ADC of amino acids in the corresponding intact protein. In corn meal the opposite was true, and in wheat middlings there was no difference ($P > 0.05$) between the average ADC of amino acids in intact protein and the average ADC of amino acids in the purified mixture.

TABLE 10. Apparent digestibility coefficients (ADC ± SD, n=3) of amino acids in intact fish meal (FM) and meat and bone meal (MB), and in mixtures of crystalline amino acids (FMC and MBC) designed to duplicate the amino acid composition of the intact ingredient. Within each ingredient, means in the same row with different letters are significantly different ($P \leq 0.05$). Within each ingredient, mean ADCs (average of all amino acids) with different letters are significantly different ($P \leq 0.05$).

Amino acid	FM %	FMC %	MB %	MBC %
Acidic series				
Aspartic acid	72.0 ± 19.5	99.1 ± 0.3	62.7 ± 8.7	81.3 ± 3.6
Glutamic acid	99.5 ± 0.5	100.0 ± 0.0	79.1 ± 3.1	89.2 ± 1.6
Aliphatic series				
Alanine	78.2 ± 13.7	98.2 ± 0.6	58.3 ± 26.5	81.8 ± 2.7
Glycine	66.0 ± 23.5[b]	99.2 ± 0.5[a]	49.5 ± 15.6	88.5 ± 1.9
Isoleucine	84.8 ± 8.8	98.3 ± 0.3	73.7 ± 4.7	80.8 ± 3.6
Leucine	85.9 ± 7.6	97.9 ± 0.4	71.5 ± 6.5	81.4 ± 6.0
Serine	66.5 ± 19.9[b]	98.7 ± 0.5[a]	34.5 ± 20.4	72.6 ± 7.8
Threonine	81.5 ± 11.6	97.8 ± 0.5	58.1 ± 10.5	77.7 ± 6.3
Valine	84.3 ± 9.5	97.6 ± 0.5	66.1 ± 7.7	79.3 ± 3.9
Aromatic series				
Phenylalanine	84.0 ± 12.9	98.8 ± 0.3	75.6 ± 6.1	84.1 ± 2.9
Tyrosine	92.0 ± 4.6	98.3 ± 0.4	80.4 ± 7.1	90.9 ± 1.1
Basic series				
Arginine	92.0 ± 5.0	99.4 ± 0.2	75.7 ± 6.5	93.5 ± 2.6
Histidine	86.4 ± 10.5	100.0 ± 0.0	84.7 ± 4.2	88.1 ± 6.5
Lysine	87.0 ± 7.4	100.0 ± 0.0	83.1 ± 6.6	99.1 ± 1.5
Sulfur-containing series				
Methionine	92.8 ± 5.6	100.0 ± 0.0	81.8 ± 6.7	99.1 ± 1.6
Mean ADC	83.5 ± 10.7[b]	98.9 ± 0.3[a]	70.4 ± 9.2[b]	86.5 ± 3.5[a]

TABLE 11. Apparent digestibility coefficients (ADC ± SD, n=3) of amino acids in intact soybean meal (SB) and wheat middlings (WM), and in mixtures of crystalline amino acids (SBC and WMC) designed to duplicate the amino acid composition of the intact ingredient. Within each ingredient, means in the same row with different letters are significantly different ($P \leq 0.05$). Within each ingredient, mean ADCs (average of all amino acids) with different letters are significantly different ($P \leq 0.05$).

Amino acid	SB %	SBC %	WM %	WMC %
Acidic series				
Aspartic acid	85.1 ± 6.3	96.9 ± 2.5	77.0 ± 16.3	64.4 ± 8.5
Glutamic acid	91.3 ± 4.7	98.2 ± 1.2	83.3 ± 2.4	80.3 ± 5.4
Aliphatic series				
Alanine	81.3 ± 9.9	93.3 ± 5.3	64.4 ± 19.6	2.0 ± 78.6
Glycine	79.0 ± 8.8	92.8 ± 5.7	55.1 ± 26.5	46.9 ± 12.3
Isoleucine	86.0 ± 7.5	96.2 ± 3.0	74.2 ± 12.7	57.2 ± 8.6
Leucine	87.0 ± 6.5	96.1 ± 3.0	80.7 ± 16.8	60.1 ± 6.7
Serine	81.5 ± 7.0	95.3 ± 3.7	54.9 ± 24.6	60.2 ± 12.1
Threonine	83.1 ± 7.7	94.8 ± 4.0	61.4 ± 16.3	40.2 ± 7.0
Valine	83.4 ± 7.4	94.9 ± 4.0	68.8 ± 15.0	48.6 ± 7.5
Aromatic series				
Phenylalanine	88.4 ± 5.3	96.7 ± 2.3	77.9 ± 8.1	69.3 ± 4.9
Tyrosine	86.7 ± 4.6	97.5 ± 1.7	57.4 ± 15.3	30.3 ± 23.1
Basic series				
Arginine	95.0 ± 2.6	98.6 ± 1.0	90.5 ± 3.9	87.2 ± 1.6
Histidine	93.9 ± 2.5	98.2 ± 1.1	84.7 ± 7.0	85.5 ± 4.2
Lysine	92.2 ± 7.1	97.9 ± 1.6	*	35.6 ± 5.9
Sulfur-containing series				
Methionine	95.5 ± 4.8	99.5 ± 0.9	100.0 ± 0.0	100.0 ± 0.0
Mean ADC	87.9 ± 5.9[b]	96.6 ± 2.7[a]	69.4 ± 15.4	59.2 ± 12.1

* Undetected

3.2. Plasma Free Amino Acids

3.2.1 Blood Meal (BM and BMC)

Statistically significant differences between treatments, at one hour intervals for 12 h after feeding, were as follows:

1) Acidic series– aspartic acid (Table 12) and glutamic acid (Table 13): No difference between BM and BMC at any time during the 12-h sampling period.

2) Aliphatic series– alanine (Table 12), glycine (Table 13), isoleucine (Fig. 1), serine (Table 14), threonine (Fig. 3) and valine (Fig. 4): No difference between BM and BMC at any time during the 12-h sampling period.

3) Aliphatic series– leucine (Fig. 2): Significantly higher in HPV (HPV) blood of fish fed BMC, 3 h after feeding, than in blood of fish fed BM.

4) Aromatic series– phenylalanine (Fig. 5) and tyrosine (Table 14): No difference between BM and BMC at any time during the 12-h sampling period.

5) Basic series– arginine (Fig. 6): Significantly higher in HPV blood of fish fed BMC, 3 h after feeding, than in blood of fish fed BM.

6) Basic series– histidine (Fig. 7) and lysine (Fig. 8): No difference between BM and BMC at any time during the 12-h sampling period.

7) Heterocyclic series– tryptophan (Fig. 9): No difference between BM and BMC at any time during the 12-h sampling period.

8) Sulfur-containing series– methionine: Not detected at any time, in either treatment group, during the 12-h sampling period.

Three hours after feeding, concentrations of two amino acids, arginine and leucine, were significantly higher in the HPV blood of fish fed the purified amino acid mixture (BMC) than in

the blood of fish fed intact protein (BM). Blood levels of these two amino acids did not differ significantly between treatments at any of the other sampling times. Concentrations of 13 amino acids in HPV blood did not differ significantly between treatments at any time during the 12-h postprandial sampling period, and one amino acid was not detected in any of the samples. There were no significant differences in the concentrations of individual acidic, aliphatic, aromatic, basic, and heterocyclic amino acids in the plasma during the 12-h sampling period within the BM and BMC treatments.

3.2.2 Corn Meal (CM and CMC)

Statistically significant differences between treatments, at one hour intervals for 12 h after feeding, were as follows:

1) Acidic series– aspartic acid (Table 15) and glutamic acid (Table 16): No difference between CM and CMC at any time during the 12-h sampling period.
2) Aliphatic series– alanine (Table 15), glycine (Table 16), isoleucine (Fig. 10), leucine (Fig. 11), serine (Table 17), threonine (Fig. 12) and valine (Fig. 13)]: No difference between CM and CMC at any time during the 12-h sampling period.
3) Aromatic series– phenylalanine (Fig. 14) and tyrosine (Table 17): No difference between CM and CMC at any time during the 12-h sampling period.
4) Basic series– arginine (Fig. 15), histidine (Fig. 16) and lysine (Fig. 17): No difference between CM and CMC at any time during the 12-h sampling period.
5) Heterocyclic series– tryptophan (Fig. 18): Significantly higher in HPV blood of fish fed CM, 8 h after feeding, than in blood of fish fed CMC.
6) Sulfur-containing series– methionine: Not detected at any time, in either treatment group, during the 12-h sampling period.

TABLE 12. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, alanine and aspartic acid, in the HPV blood of channel catfish during a 12-h period after force-feeding of BM or BMC. No significant differences were observed between treatments.

Time in h	Alanine		Aspartic acid	
	BM	BMC	BM	BMC
	nmol/μL	nmol/μL	nmol/μL	nmol/μL
0	231.9 ± 75.9	211.8 ± 64.2	29.3 ± 15.8	26.1 ± 12.0
1	595.4 ± 168.8	3518.2 ± 1929.5	74.7 ± 24.7	1457.2 ± 113.0
2	675.1 ± 355.7	5730.4 ± 7268.6	88.8 ± 21.6	2474.2 ± 3684.2
3	394.1 ± 13.1	3871.2 ± 2235.4	46.7 ± 19.7	2127.8 ± 2869.7
4	521.5 ± 102.8	2015.9 ± 1481.8	75.7 ± 28.8	1169.5 ± 1864.8
5	1715.5 ± 2132.3	1515.8 ± 626.3	65.8 ± 13.4	1601.5 ± 2336.0
6	524.4 ± 186.3	888.1 ± 392.8	161.9 ± 199.8	260.2 ± 121.4
7	481.7 ± 217.3	764.0 ± 280.0	85.2 ± 50.2	90.0 ± 30.7
8	284.1 ± 137.7	509.0 ± 101.0	42.7 ± 11.0	45.5 ± 14.9
9	523.0 ± 433.4	602.3 ± 195.0	79.8 ± 31.3	62.3 ± 15.9
10	347.3 ± 151.1	456.0 ± 183.1	71.4 ± 45.5	79.7 ± 77.4
11	416.0 ± 119.6	368.2 ± 120.6	72.5 ± 33.4	45.2 ± 22.9
12	463.1 ± 294.9	922.6 ± 595.2	62.6 ± 28.7	92.9 ± 83.1

TABLE 13. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, glutamic acid and glycine, in the HPV blood of channel catfish during a 12-h period after force-feeding of BM or BMC. No significant differences were observed between treatments.

Time in h	Glutamic acid		Glycine	
	BM	BMC	BM	BMC
	nmol/μL	nmol/μL	nmol/μL	nmol/μL
0	64.5 ± 3.1	57.7 ± 30.8	321.2 ± 62.3	238.1 ± 51.3
1	132.1 ± 21.9	1492.1 ± 1061.1	444.8 ± 78.3	6032.8 ± 8039.8
2	300.4 ± 99.5	1879.0 ± 2479.9	487.8 ± 146.5	20477.2 ± 27313.3
3	135.3 ± 50.1	1620.3 ± 1785.9	328.8 ± 28.3	17164.7 ± 12670.9
4	218.6 ± 79.1	946.4 ± 1267.8	469.3 ± 90.6	7842.3 ± 9625.4
5	2810.3 ± 4602.6	1722.2 ± 2420.7	6517.8 ± 10670.6	5088.8 ± 3588.1
6	233.4 ± 187.2	586.8 ± 85.0	588.9 ± 338.1	2273.3 ± 815.7
7	129.0 ± 27.8	187.8 ± 88.8	313.4 ± 98.7	2057.8 ± 1801.5
8	92.3 ± 14.4	167.1 ± 108.2	244.2 ± 97.1	715.2 ± 182.0
9	144.3 ± 107.6	168.7 ± 46.8	371.1 ± 254.7	1280.6 ± 1098.7
10	172.0 ± 146.4	224.4 ± 225.8	311.8 ± 45.2	609.3 ± 455.7
11	148.3 ± 89.6	135.9 ± 118.6	281.3 ± 55.1	505.4 ± 250.3
12	159.2 ± 131.0	237.1 ± 196.0	366.8 ± 160.9	1923.8 ± 1155.5

TABLE 14. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, serine and tyrosine, in the HPV blood of channel catfish during a 12-h period after force-feeding of BM or BMC. No significant differences were observed between treatments.

Time in h	Serine		Tyrosine	
	BM	BMC	BM	BMC
	nmol/µL	nmol/µL	nmol/µL	nmol/µL
0	374.4 ± 48.1	191.2 ± 44.4	186.0 ± 50.9	83.1 ± 18.7
1	418.0 ± 73.6	2068.2 ± 695.0	156.7 ± 60.7	397.0 ± 107.9
2	478.0 ± 144.8	3185.2 ± 3219.0	234.3 ± 77.4	522.0 ± 290.8
3	344.7 ± 25.1	2561.4 ± 1564.5	192.5 ± 61.4	510.4 ± 72.7
4	447.5 ± 41.3	2219.8 ± 2006.7	244.9 ± 96.2	316.4 ± 210.5
5	2569.2 ± 3721.4	1746.4 ± 921.4	1529.9 ± 2293.3	333.6 ± 136.6
6	477.9 ± 183.3	1342.7 ± 375.9	170.0 ± 63.4	337.3 ± 106.7
7	429.8 ± 161.8	1283.0 ± 659.4	187.3 ± 97.0	318.4 ± 88.5
8	304.1 ± 123.0	603.5 ± 171.1	143.1 ± 67.3	242.3 ± 67.2
9	534.8 ± 269.0	875.1 ± 193.0	242.0 ± 156.3	292.7 ± 72.0
10	345.0 ± 51.5	600.2 ± 422.3	179.4 ± 29.2	237.3 ± 150.7
11	334.7 ± 50.6	462.9 ± 170.2	181.4 ± 48.8	182.0 ± 38.9
12	418.3 ± 253.8	1085.9 ± 517.5	214.9 ± 153.8	221.8 ± 123.7

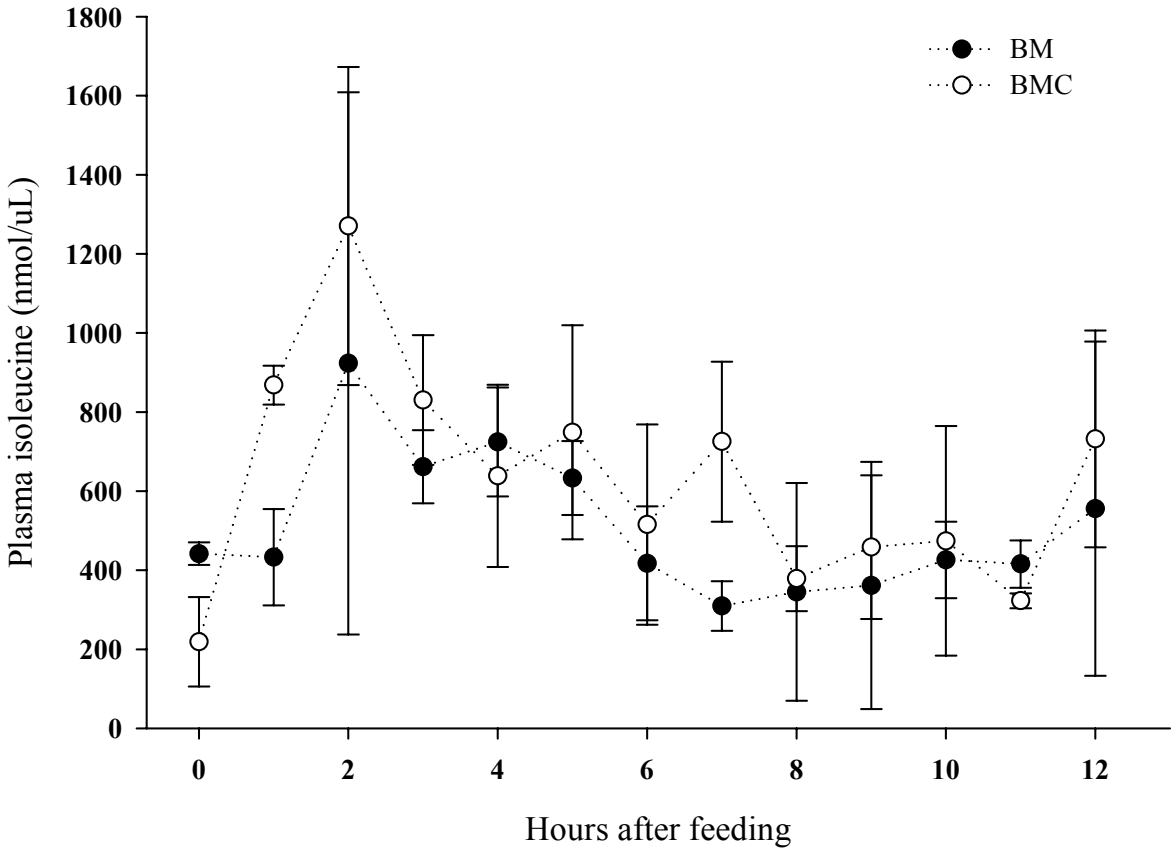

FIGURE 1. Concentrations of isoleucine in the HPV blood of channel catfish during the 12-h period after force-feeding of BM or BMC. There were no significant differences between or within treatments.

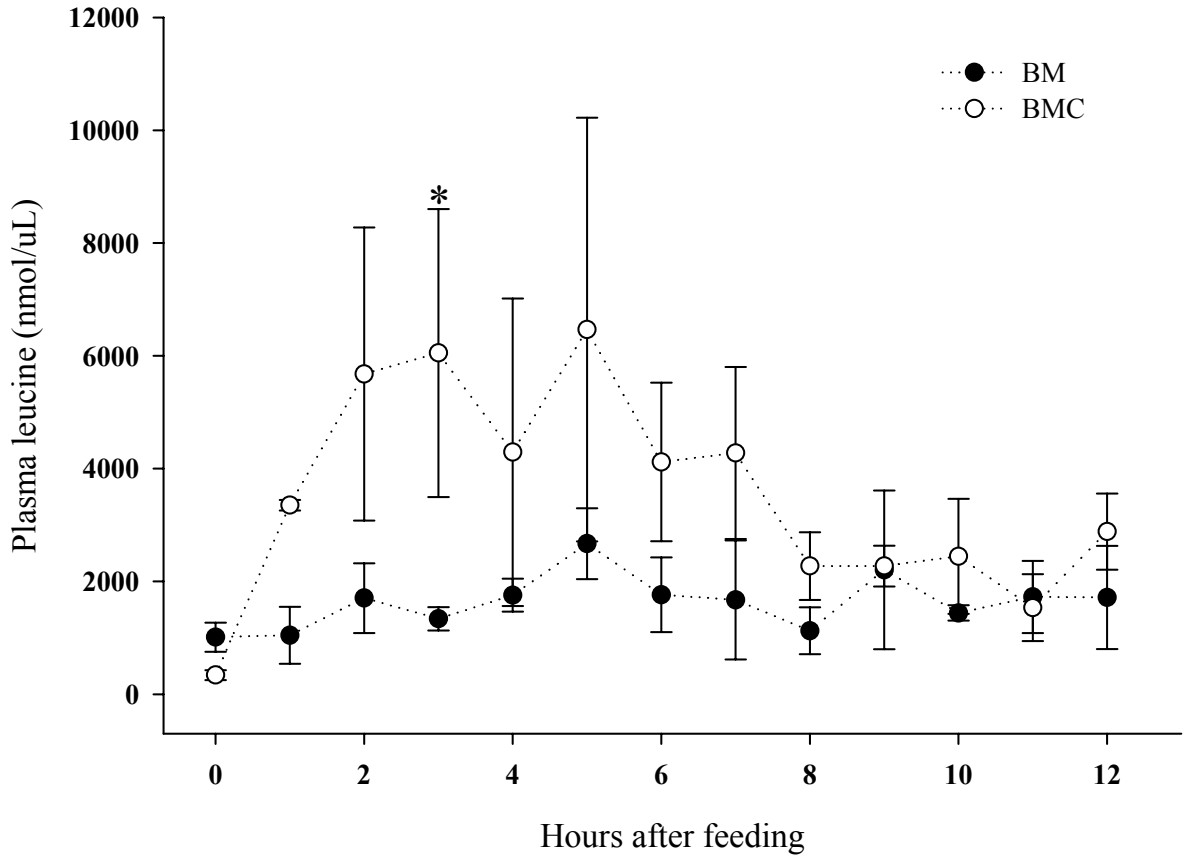

FIGURE 2. Concentrations of leucine in the HPV blood of channel catfish during the 12-h period after force-feeding of BM or BMC. An asterisk indicates a significant difference between treatments. There were no significant differences within treatments.

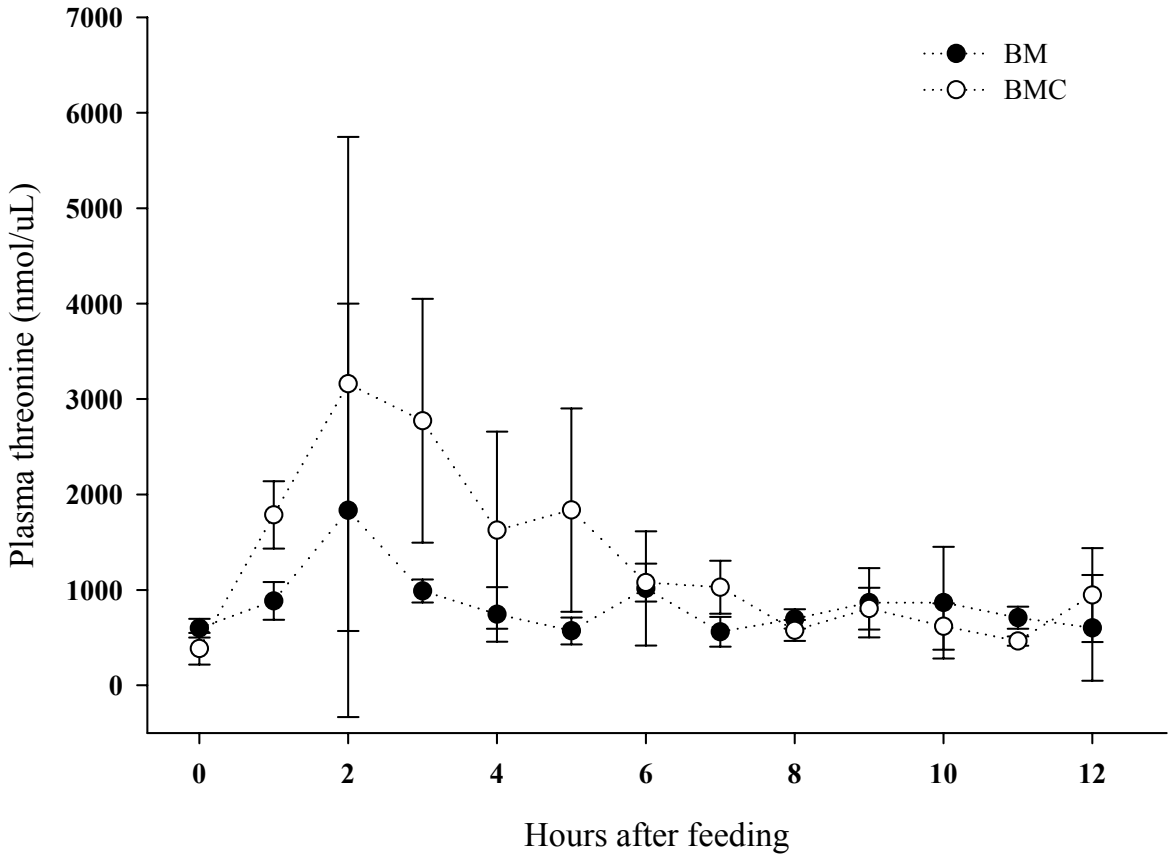

FIGURE 3. Concentrations of threonine in the HPV blood of channel catfish during the 12-h period after force-feeding of BM or BMC. There were no significant differences between or within treatments.

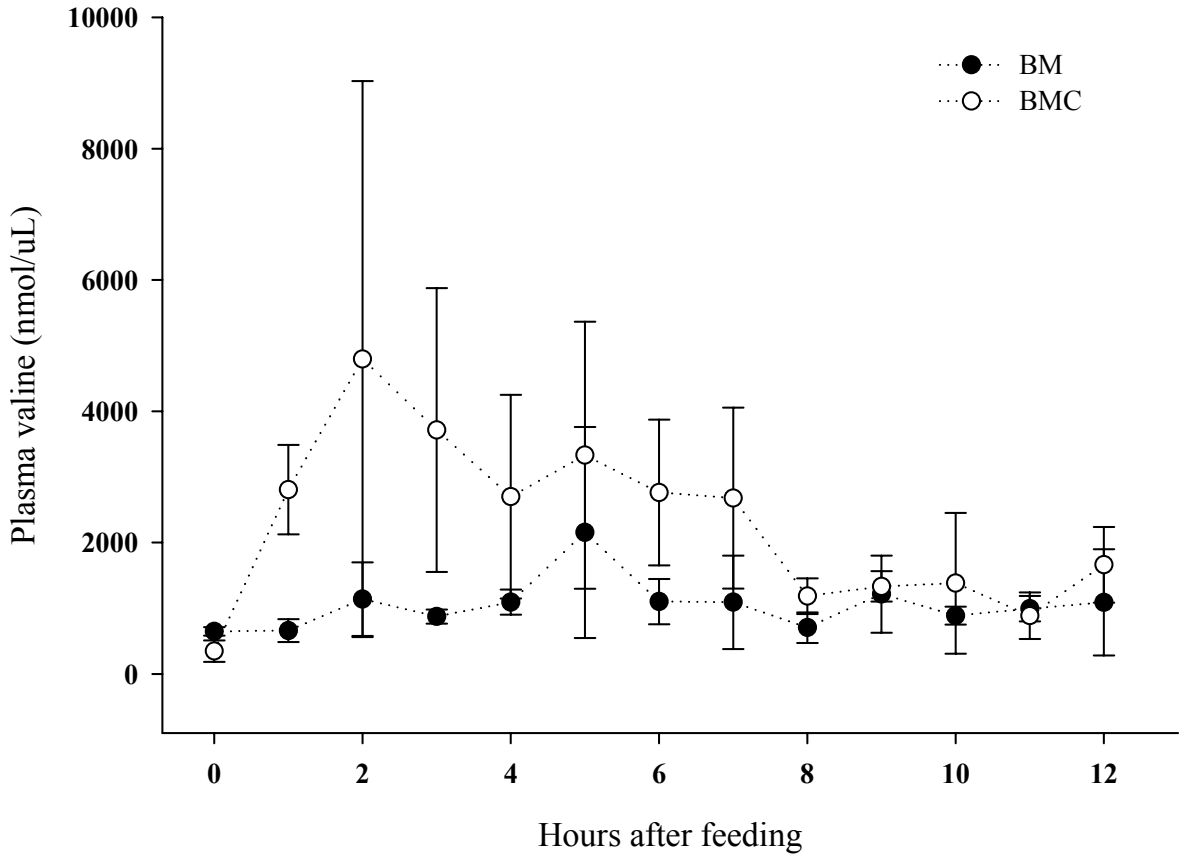

FIGURE 4. Concentrations of valine in the HPV blood of channel catfish during the 12-h period after force-feeding of BM or BMC. There were no significant differences between or within treatments.

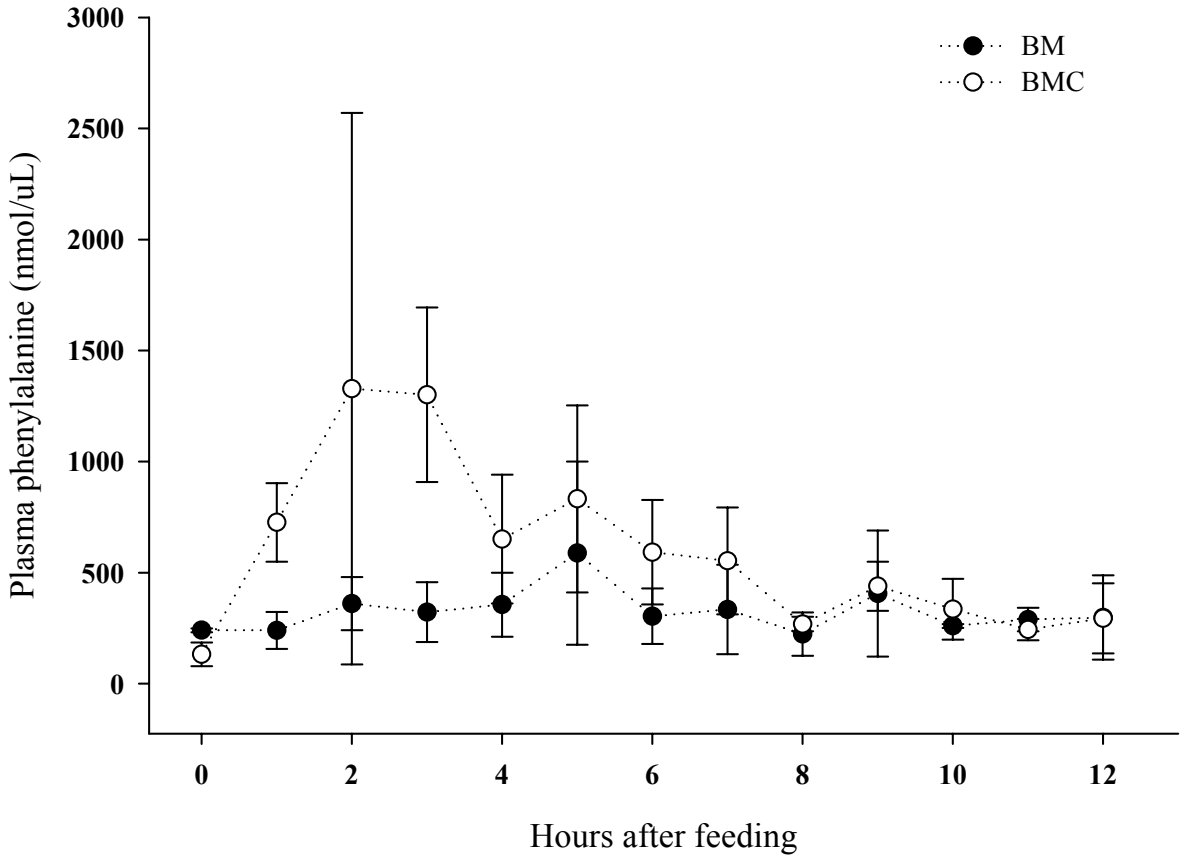

FIGURE 5. Concentrations of phenylalanine in the HPV blood of channel catfish during the 12-h period after force-feeding of BM or BMC There were no significant differences between or within treatments.

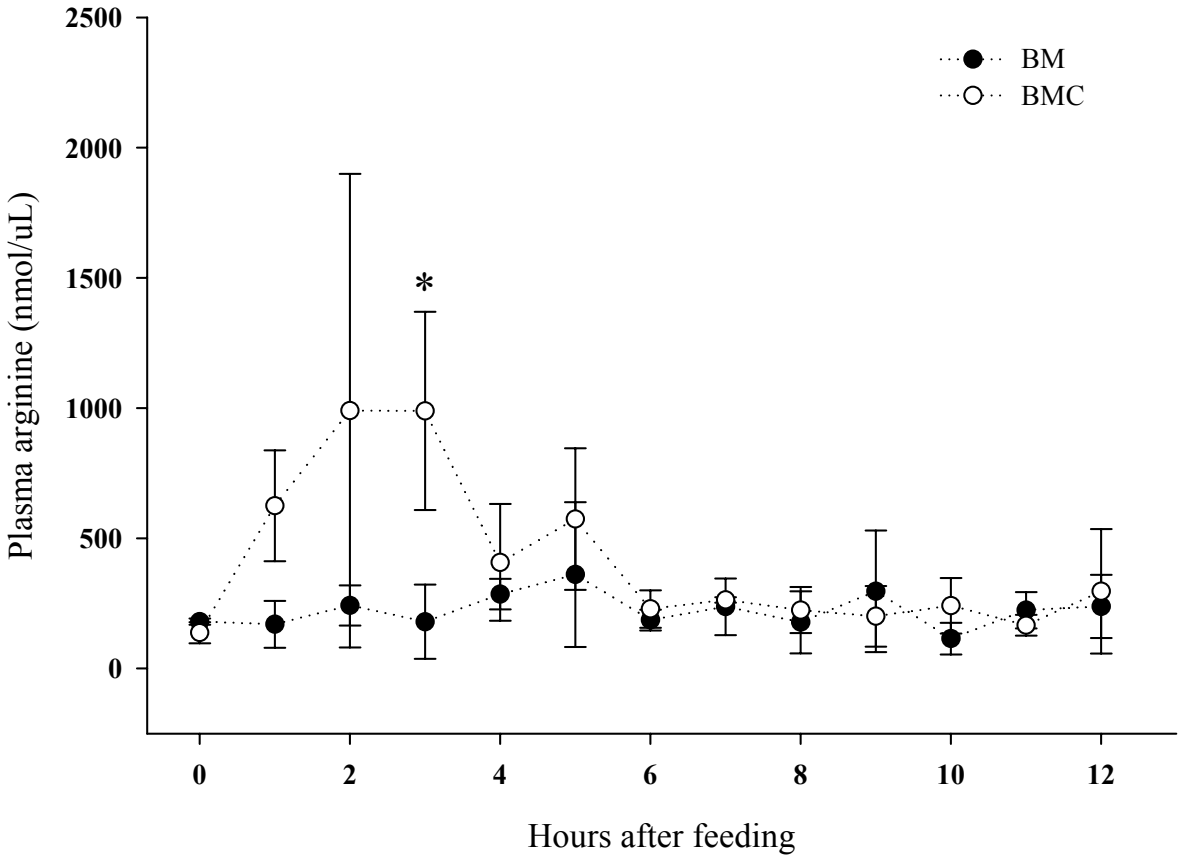

FIGURE 6. Concentrations of arginine in the HPV blood of channel catfish during the 12-h period after force-feeding of BM or BMC. An asterisk indicates a significant difference between treatments. There were no significant differences within treatments.

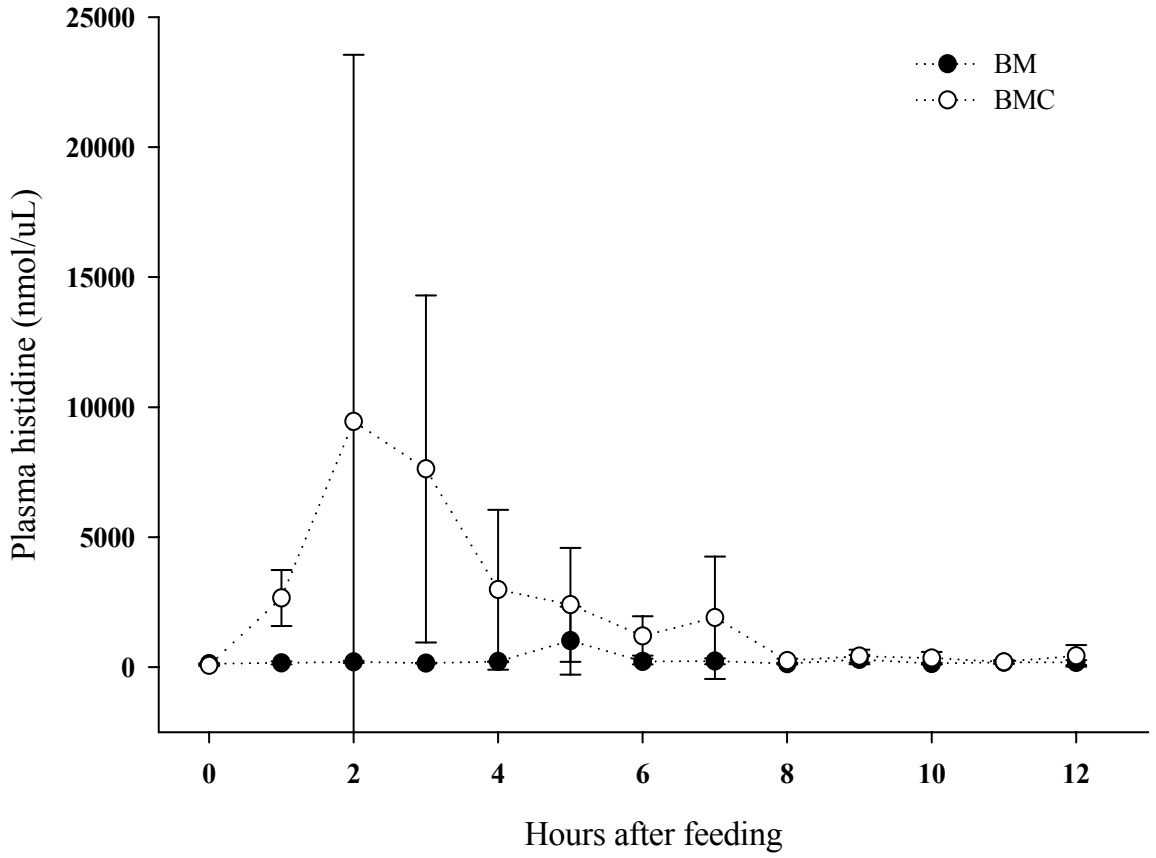

FIGURE 7. Concentrations of histidine in the HPV blood of channel catfish during the 12-h period after force-feeding of BM or BMC. There were no significant differences between or within treatments.

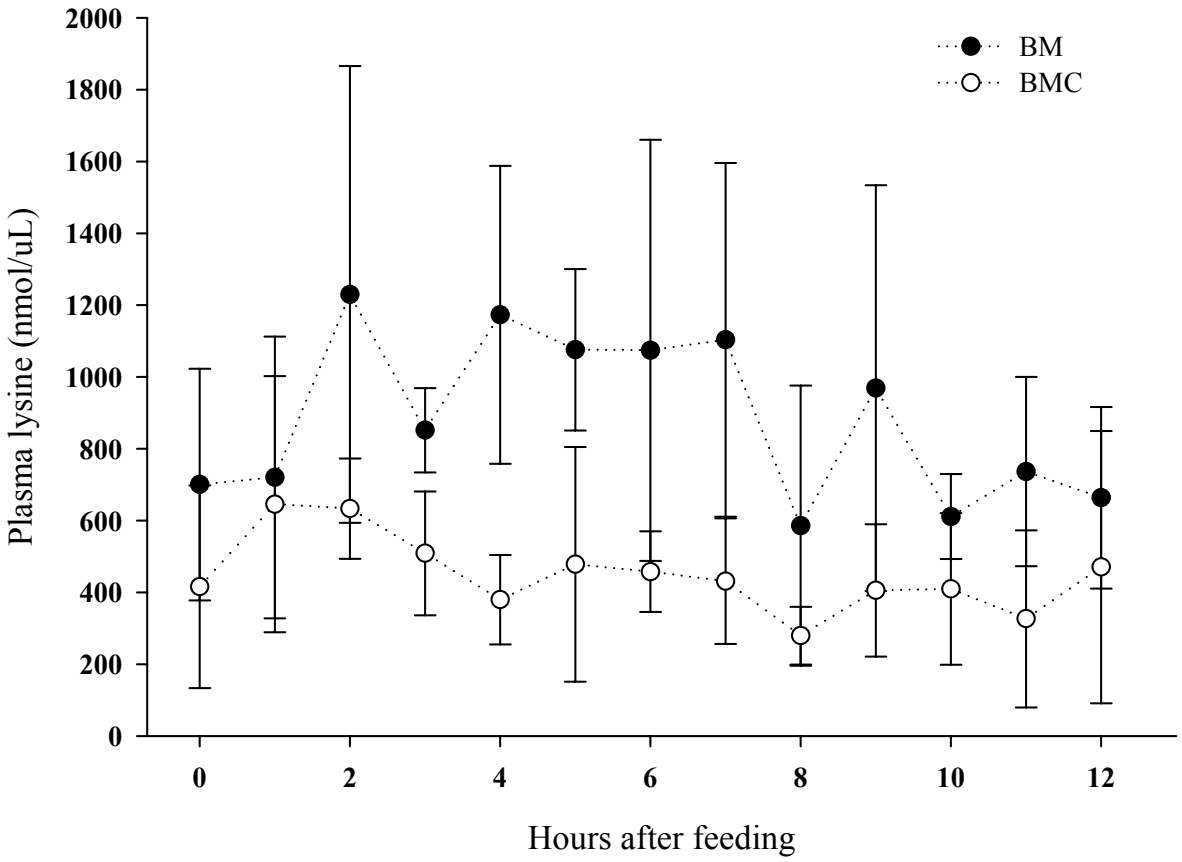

FIGURE 8. Concentrations of lysine in the HPV blood of channel catfish during the 12-h period after force-feeding of BM or BMC. There were no significant differences between or within treatments.

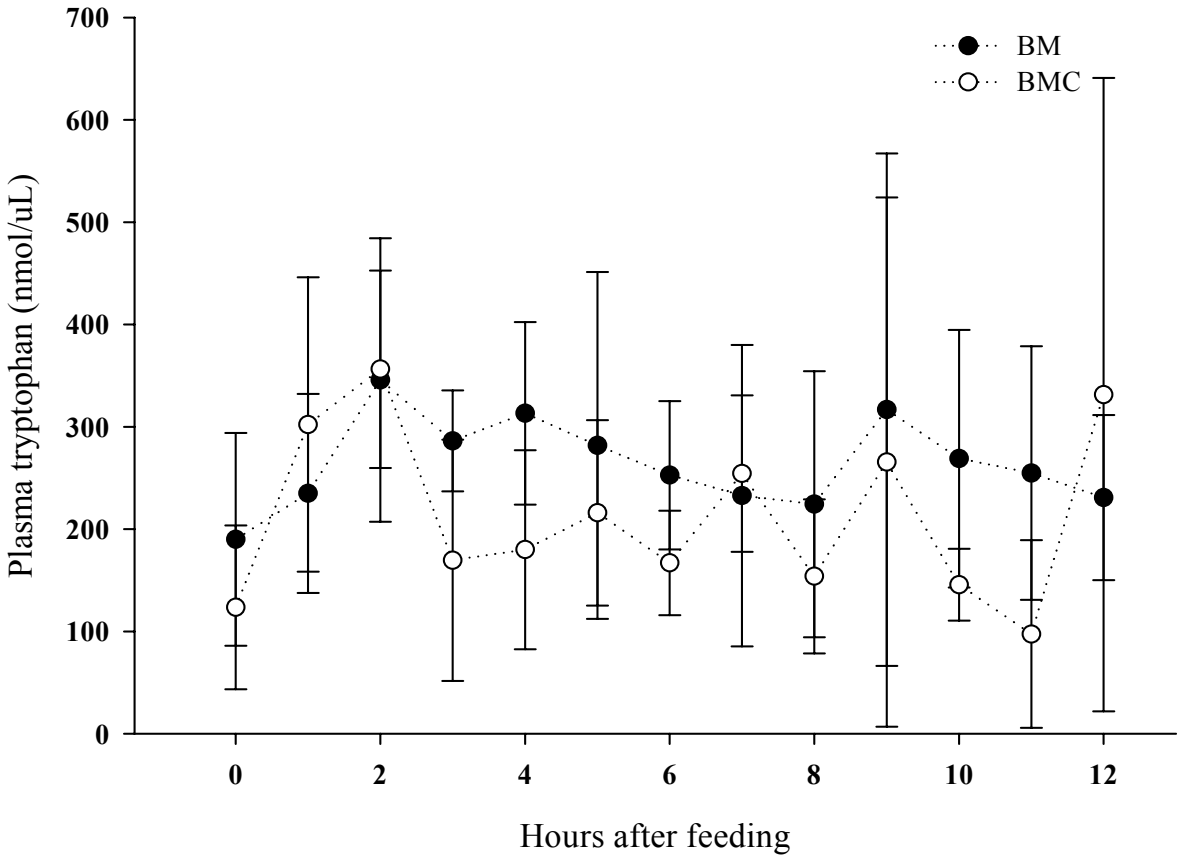

FIGURE 9. Concentrations of tryptophan in the HPV blood of channel catfish during the 12-h period after force-feeding of BM or BMC. There were no significant differences between or within treatments.

Eight hours after feeding, the concentration of one amino acid, tryptophan, was significantly higher in the HPV blood of fish fed intact protein (CM) than in the blood of fish fed the purified amino acid mixture (CMC). Blood levels of tryptophan did not differ significantly between treatments at any of the other sampling times. Concentrations of 14 amino acids in HPV blood did not differ significantly between treatments at any time during the 12-h postprandial sampling period, and one amino acid was not detected in any of the samples.

There were no significant differences in the concentrations of individual acidic, aliphatic, aromatic, basic, and heterocyclic amino acids in the plasma during the 12-h sampling period within the CM and CMC treatments.

3.2.3 Fish Meal (FM and FMC)

Statistically significant differences between treatments, at one hour intervals for 12 h after feeding, were as follows:

1) Acidic series– aspartic acid (Table 18) and glutamic acid (Table 19): Significantly higher in HPV blood of fish fed FMC, 1 h after feeding, than in blood of fish fed FM.
2) Aliphatic series– alanine (Table 18), glycine (Table 19) and leucine (Fig. 20): Alanine and glycine concentrations were significantly higher in HPV blood of fish fed FMC, 1 h after feeding, than in blood of fish fed FM. Leucine was significantly higher in HPV blood of fish fed FMC, 4 h and 8 h after feeding, than in blood of fish fed FM.
3) Aliphatic series– isoleucine (Fig. 19), serine (Table 20), threonine (Fig. 21) and valine (Fig. 22): No difference between FM and FMC at any time during the 12-h sampling period.
4) Aromatic series– phenylalanine (Fig. 23) and tyrosine (Table 20): No difference between FM and FMC at any time during the 12-h sampling period.

TABLE 15. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, alanine and aspartic acid, in the HPV blood of channel catfish during a 12-h period after force-feeding of CM or CMC. No significant differences were observed between treatments.

Time in h	Alanine		Aspartic acid	
	CM	CMC	CM	CMC
	nmol/μL	nmol/μL	nmol/μL	nmol/μL
0	301.9 ± 49.8	559.5 ± 107.8	34.2 ± 3.8	25.4 ± 7.5
1	442.3 ± 147.1	544.9 ± 165.8	58.8 ± 21.6	24.7 ± 9.3
2	406.8 ± 88.6	799.9 ± 191.5	32.6 ± 11.1	29.0 ± 4.4
3	467.0 ± 303.4	745.6 ± 179.2	24.5 ± 4.8	28.6 ± 9.0
4	428.5 ± 220.3	667.1 ± 345.5	39.1 ± 8.0	38.5 ± 15.2
5	741.3 ± 396.9	829.5 ± 477.3	59.7 ± 42.5	35.6 ± 8.2
6	684.2 ± 535.0	618.3 ± 111.5	49.4 ± 29.2	39.0 ± 15.5
7	447.0 ± 87.2	403.7 ± 20.9	27.5 ± 4.0	29.0 ± 5.6
8	1009.2 ± 1019.9	617.4 ± 126.2	175.6 ± 220.7	30.6 ± 8.7
9	642.7 ± 521.2	636.7 ± 231.5	45.8 ± 24.9	29.4 ± 6.2
10	439.5 ± 137.5	584.5 ± 176.6	46.6 ± 21.8	32.2 ± 6.3
11	438.5 ± 168.2	603.7 ± 118.3	35.9 ± 10.6	36.0 ± 10.3
12	473.5 ± 92.2	701.5 ± 324.2	43.1 ± 6.8	26.6 ± 7.3

TABLE 16. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, glutamic acid and glycine, in the HPV blood of channel catfish during a 12-h period after force-feeding of CM or CMC. No significant differences were observed between treatments.

Time in h	Glutamic acid		Glycine	
	CM	CMC	CM	CMC
	nmol/µL	nmol/µL	nmol/µL	nmol/µL
0	97.7 ± 18.7	66.1 ± 4.9	325.5 ± 24.2	322.9 ± 53.9
1	122.5 ± 33.4	41.7 ± 32.5	244.5 ± 55.1	346.2 ± 137.8
2	101.4 ± 67.6	58.8 ± 20.1	317.1 ± 22.0	413.2 ± 10.5
3	58.7 ± 17.3	65.9 ± 11.6	271.7 ± 115.0	439.7 ± 15.3
4	87.8 ± 14.8	98.5 ± 33.3	246.3 ± 59.4	530.9 ± 288.8
5	116.4 ± 41.5	100.8 ± 39.0	468.6 ± 324.2	615.6 ± 419.0
6	252.8 ± 205.1	112.9 ± 15.2	410.3 ± 258.4	424.0 ± 146.4
7	83.6 ± 33.2	76.6 ± 7.2	306.4 ± 48.3	333.1 ± 115.5
8	83.9 ± 20.4	91.1 ± 19.3	715.8 ± 646.9	410.4 ± 126.7
9	188.0 ± 174.5	82.8 ± 19.4	492.7 ± 384.9	395.3 ± 153.7
10	127.4 ± 57.8	82.5 ± 17.7	336.6 ± 73.2	409.5 ± 25.0
11	111.2 ± 21.7	127.7 ± 56.5	319.7 ± 89.3	408.1 ± 153.5
12	117.6 ± 30.2	91.9 ± 35.1	384.7 ± 34.8	437.2 ± 203.5

TABLE 17. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, serine and tyrosine, in the HPV blood of channel catfish during a 12-h period after force-feeding of CM or CMC. No significant differences were observed between treatments.

Time in h	Serine		Tyrosine	
	CM	CMC	CM	CMC
	nmol/µL	nmol/µL	nmol/µL	nmol/µL
0	333.7 ± 59.4	314.1 ± 65.0	110.7 ± 26.4	133.1 ± 27.3
1	273.3 ± 86.3	386.9 ± 165.9	202.0 ± 57.9	143.1 ± 26.2
2	336.2 ± 38.5	466.6 ± 147.0	165.9 ± 18.2	173.0 ± 35.7
3	339.6 ± 148.0	514.0 ± 121.3	129.7 ± 47.2	162.9 ± 36.5
4	250.5 ± 189.1	641.7 ± 420.9	193.2 ± 93.7	174.4 ± 98.8
5	432.5 ± 100.7	669.2 ± 353.3	268.1 ± 224.7	197.1 ± 117.1
6	462.8 ± 387.3	542.7 ± 187.7	236.3 ± 173.9	186.8 ± 93.3
7	325.5 ± 55.4	362.5 ± 96.1	173.7 ± 36.8	130.0 ± 17.8
8	552.2 ± 281.3	433.2 ± 89.2	607.6 ± 663.1	179.9 ± 44.7
9	459.9 ± 311.9	412.1 ± 180.1	181.9 ± 58.9	144.7 ± 19.9
10	403.3 ± 48.1	357.6 ± 66.1	185.4 ± 46.1	128.9 ± 8.7
11	352.9 ± 103.1	449.5 ± 173.7	154.1 ± 66.6	178.8 ± 63.3
12	397.6 ± 107.2	485.9 ± 201.3	165.2 ± 57.6	158.6 ± 48.1

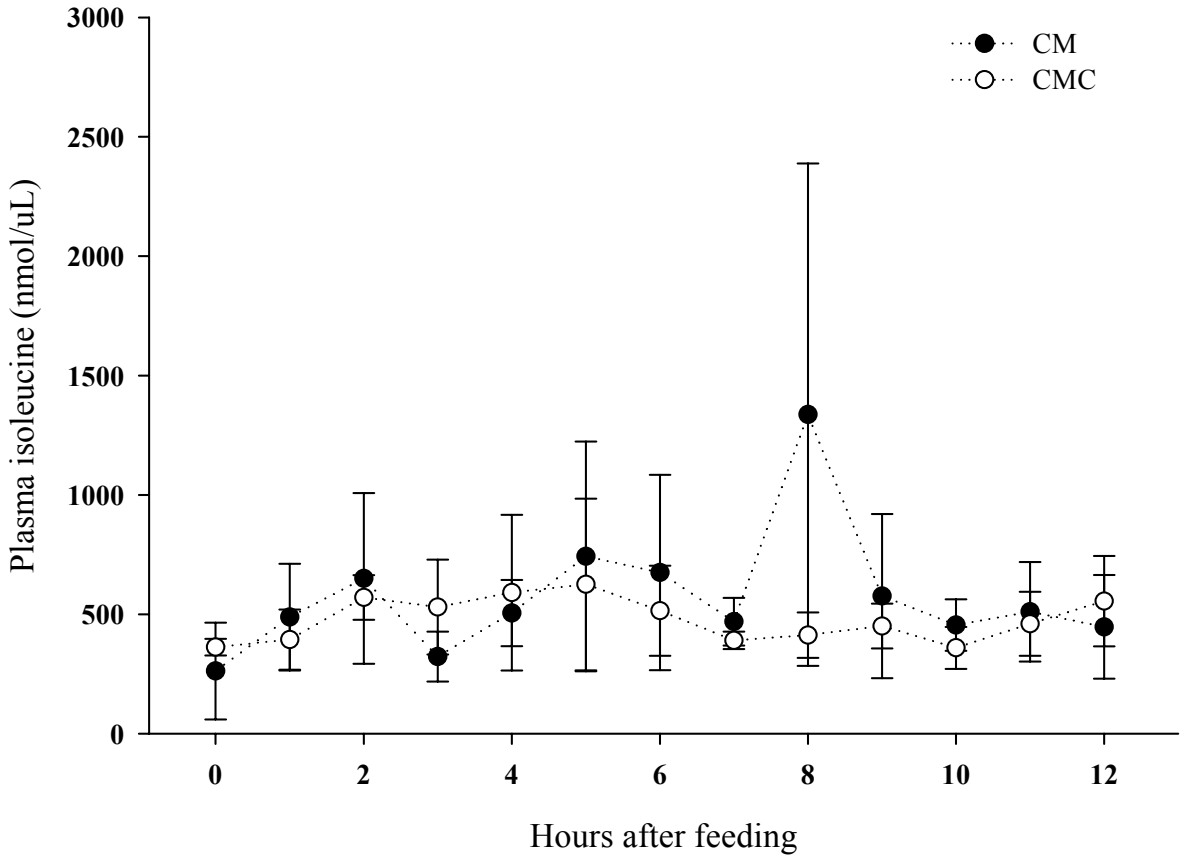

FIGURE 10. Concentrations of isoleucine in the HPV blood of channel catfish during the 12-h period after force-feeding of CM or CMC. There were no significant differences between or within treatments.

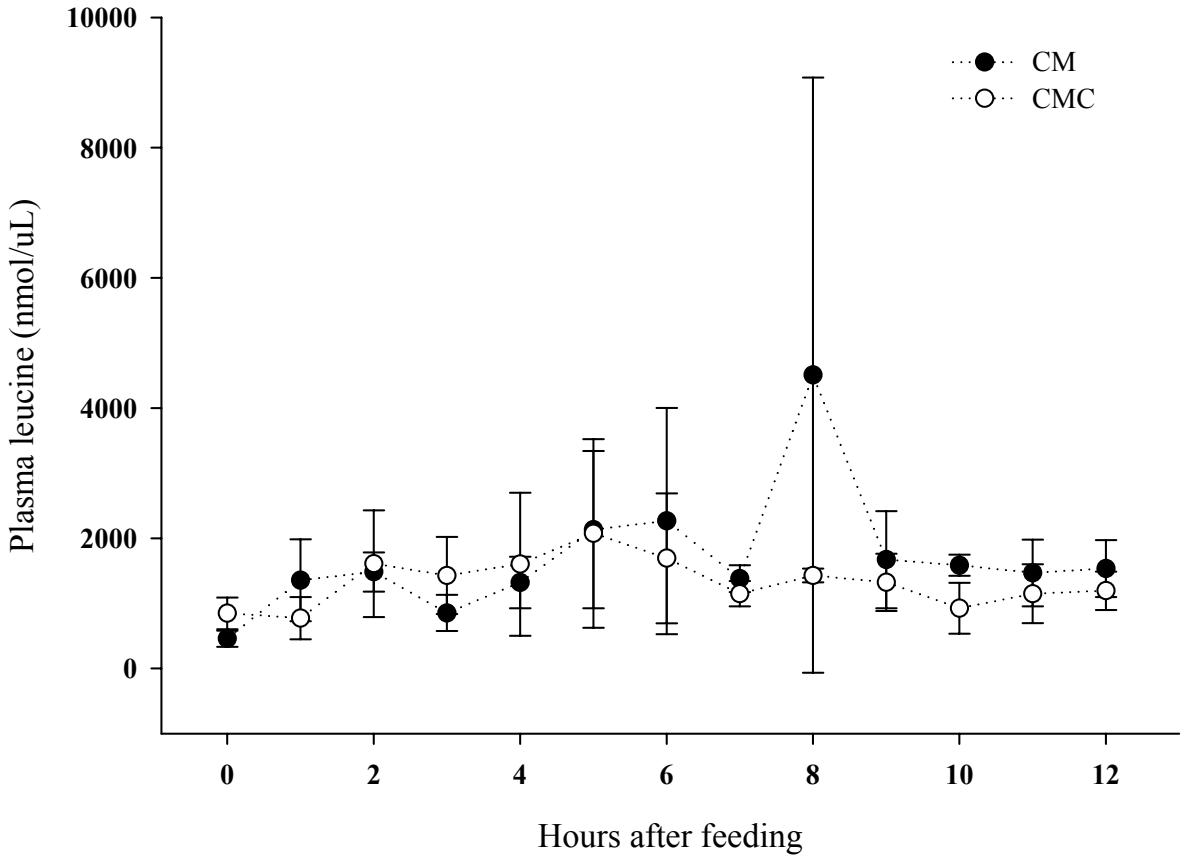

FIGURE 11. Concentrations of leucine in the HPV blood of channel catfish during the 12-h period after force-feeding of CM or CMC. There were no significant differences between or within treatments.

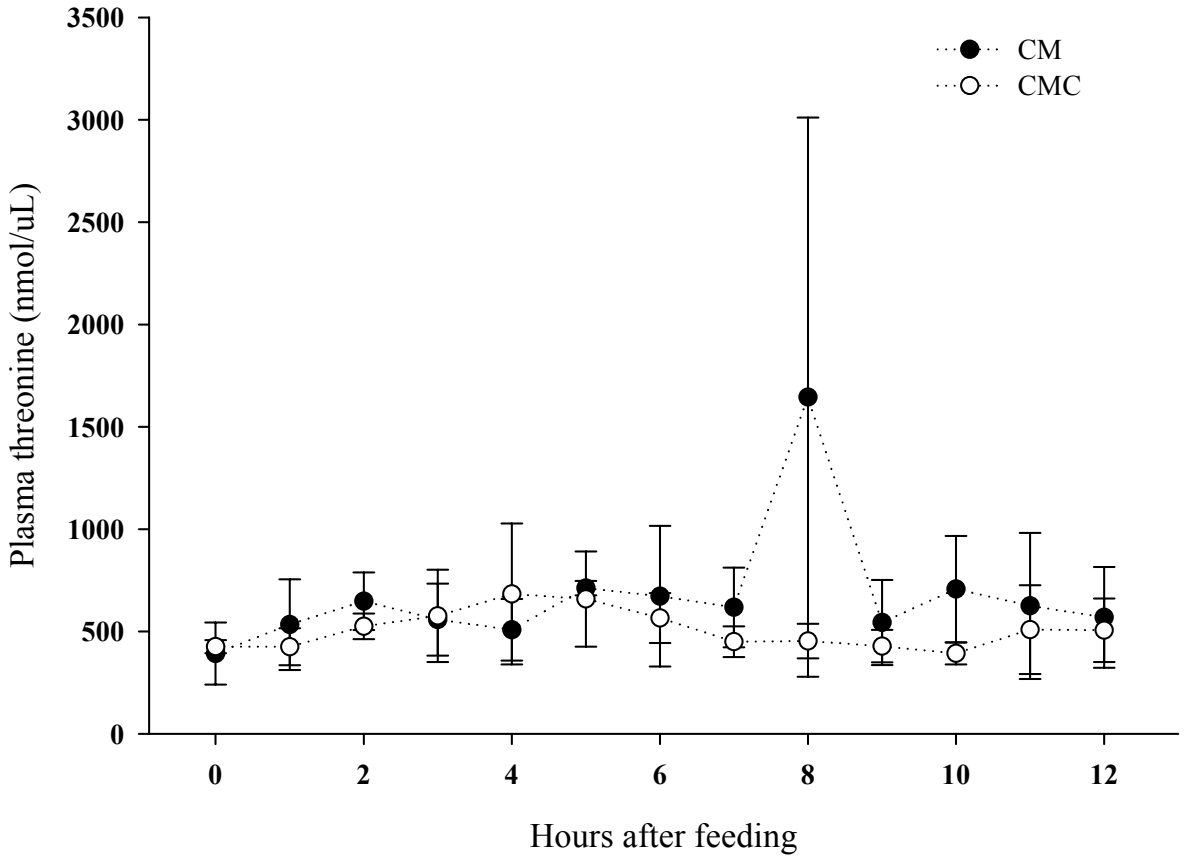

FIGURE 12. Concentrations of threonine in the HPV blood of channel catfish during the 12-h period after force-feeding of CM or CMC. There were no significant differences between or within treatments.

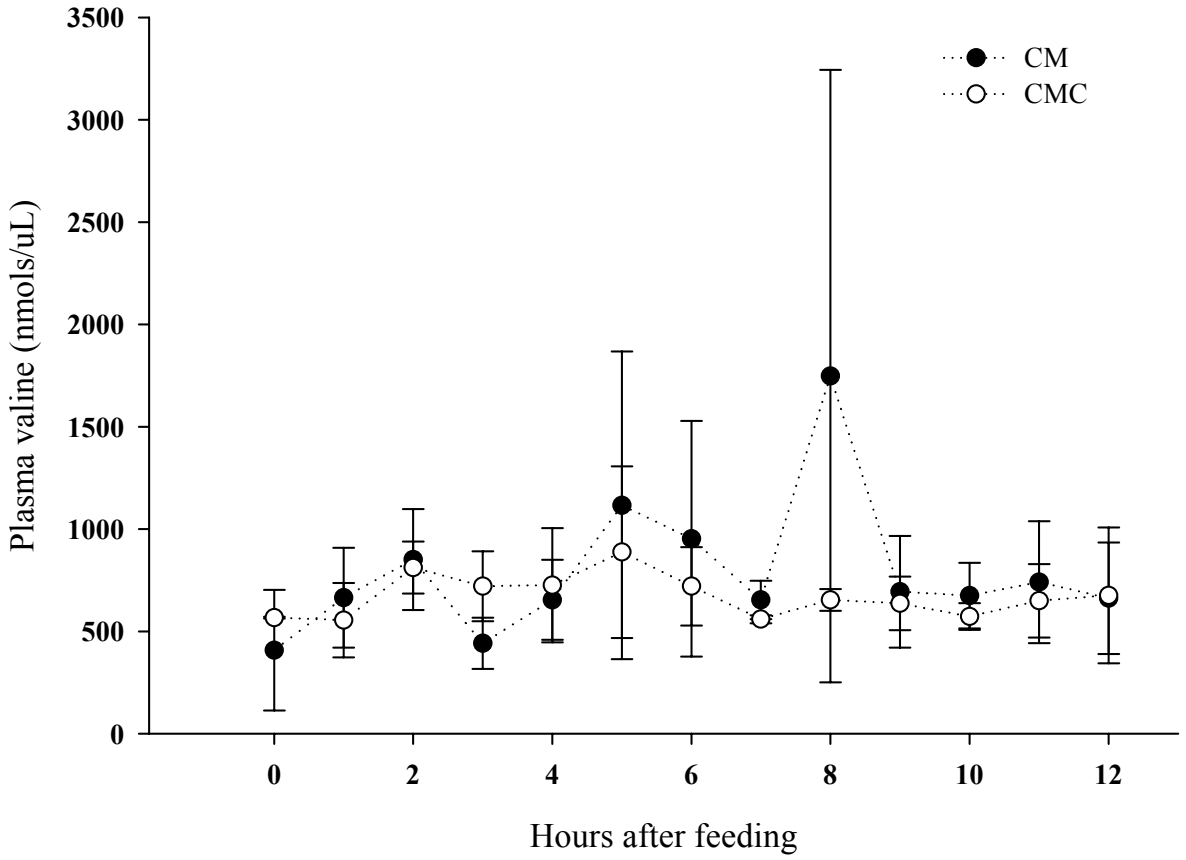

FIGURE 13. Concentrations of valine in the HPV blood of channel catfish during the 12-h period after force-feeding of CM or CMC. There were no significant differences between or within treatments.

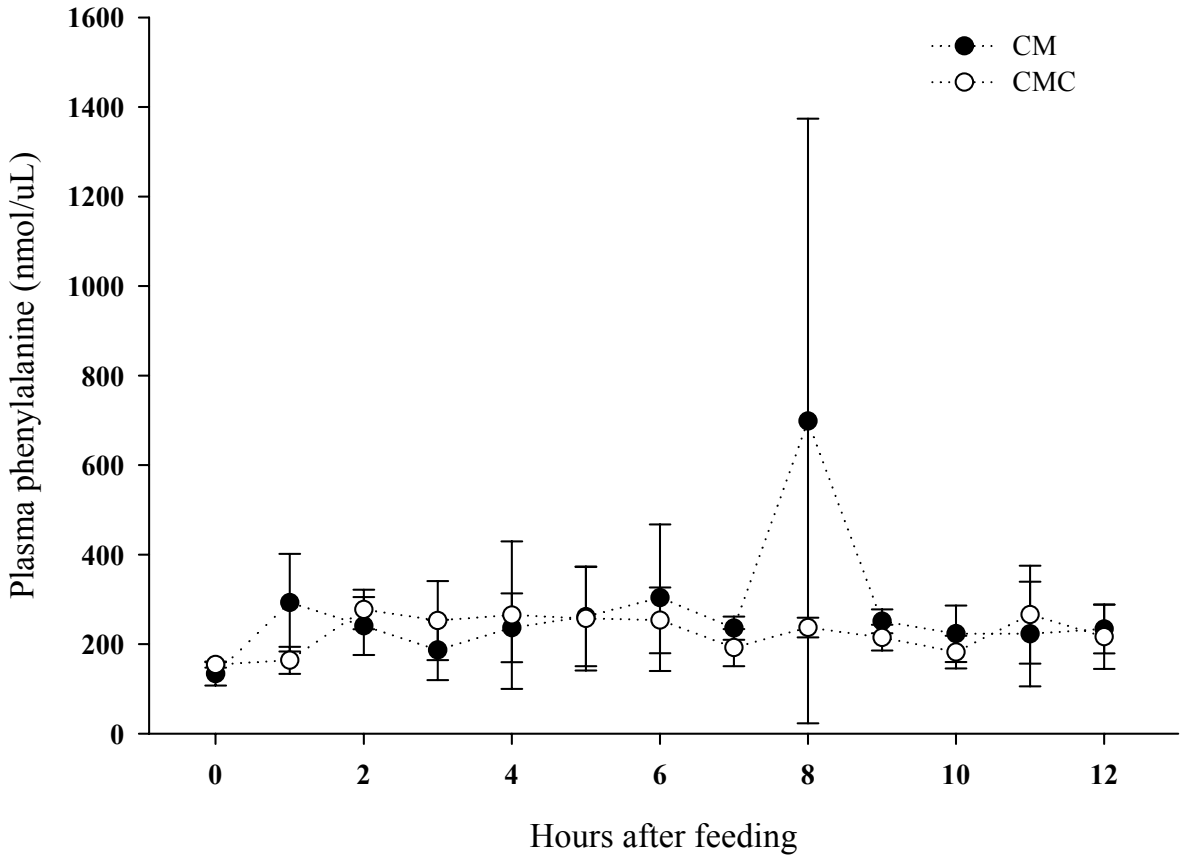

FIGURE 14. Concentrations of phenylalanine in the HPV blood of channel catfish during the 12-h period after force-feeding of CM or CMC. There were no significant differences between or within treatments.

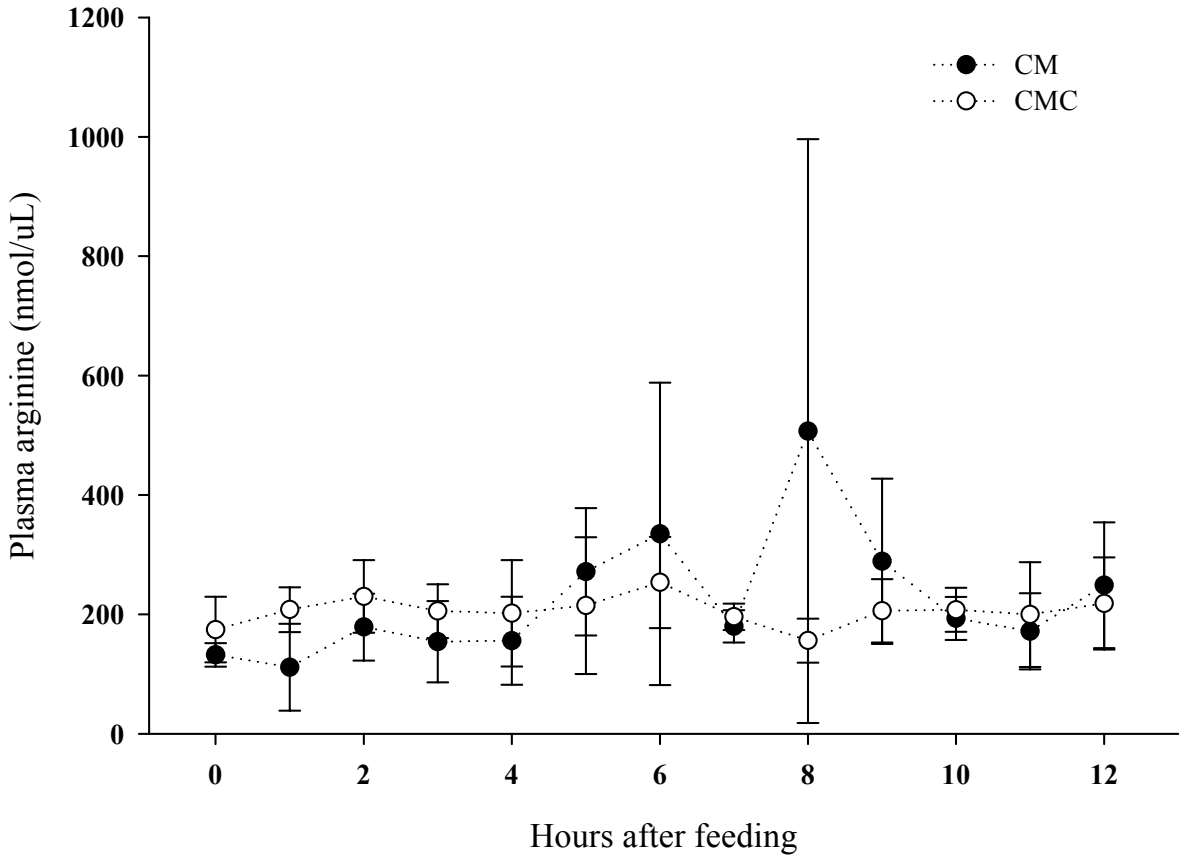

FIGURE 15. Concentrations of arginine in the HPV blood of channel catfish during the 12-h period after force-feeding of CM or CMC. There were no significant differences between or within treatments.

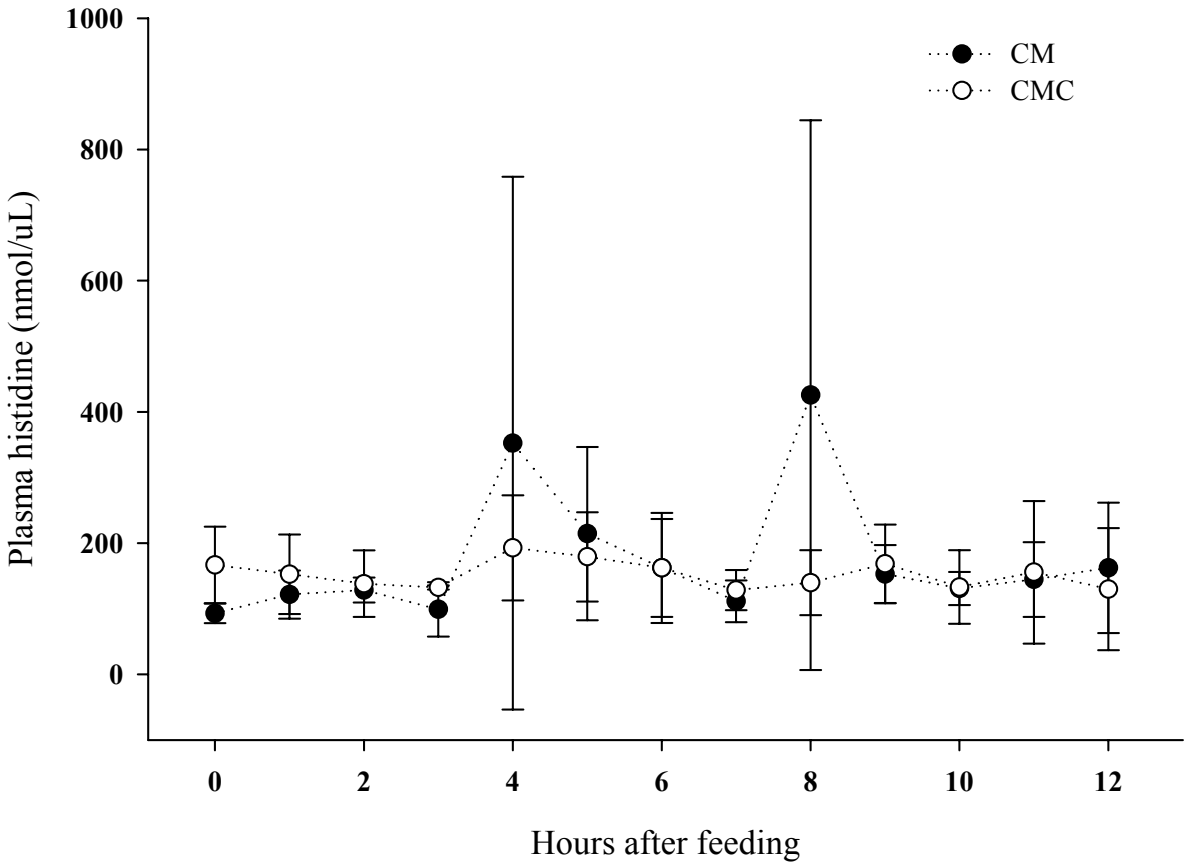

FIGURE 16. Concentrations of histidine in the HPV blood of channel catfish during the 12-h period after force-feeding of CM or CMC. There were no significant differences between or within treatments.

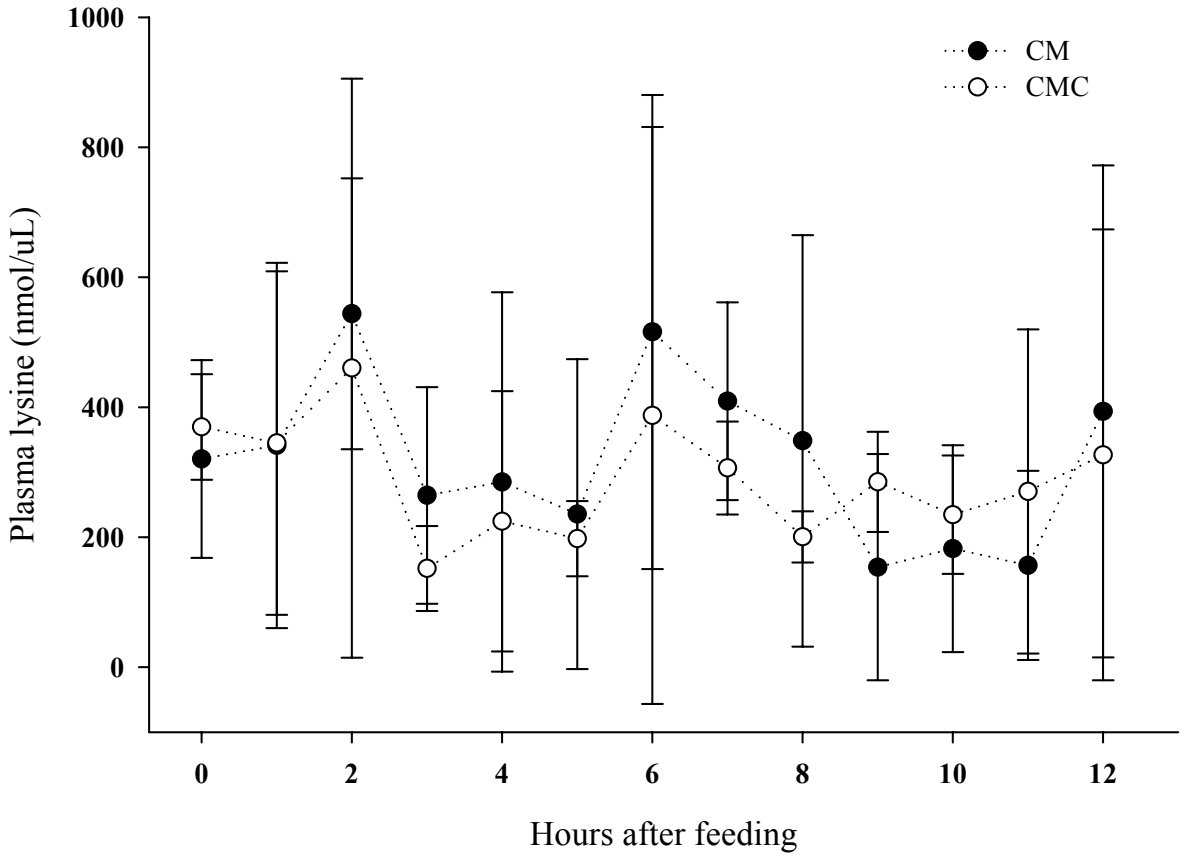

FIGURE 17. Concentrations of lysine in the HPV blood of channel catfish during the 12-h period after force-feeding of CM or CMC. There were no significant differences between or within treatments.

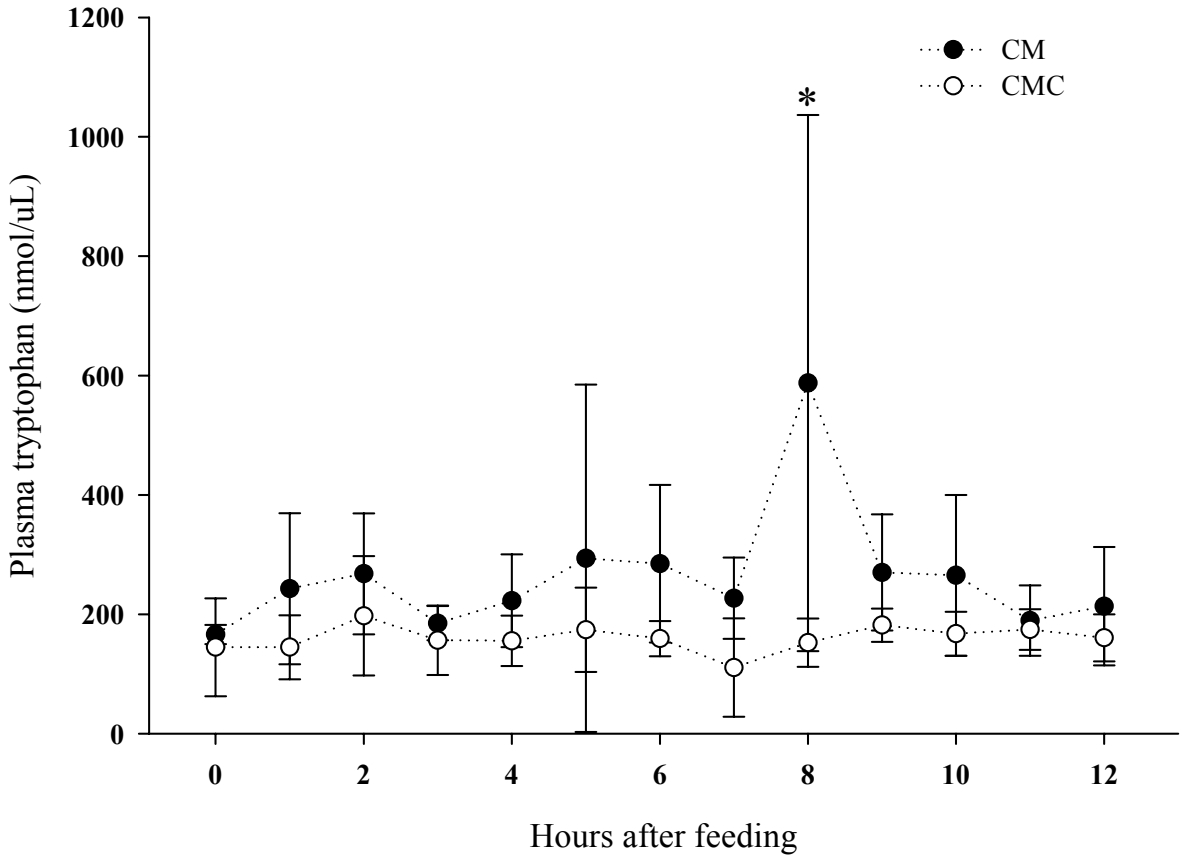

FIGURE 18. Concentrations of tryptophan in the HPV blood of channel catfish during the 12-h period after force-feeding of CM or CMC. An asterisk indicates a significant difference between treatments. There were no significant differences within treatments.

5) Basic series– arginine (Fig. 24), histidine (Fig. 25) and lysine (Fig. 26): Significantly higher in HPV blood of fish fed FMC, 1 h after feeding, than in blood of fish fed FM.

6) Heterocyclic series– tryptophan (Fig. 27): No difference between FM and FMC at any time during the 12-h sampling period.

7) Sulfur-containing series– methionine: Not detected at any time, in either treatment group, during the 12-h sampling period.

One hour after feeding, concentrations of the acidic amino acids, aspartic acid and glutamic acid; the aliphatic amino acids, alanine and glycine; and the basic amino acids, arginine, histidine and lysine, were significantly higher in the HPV blood of fish fed the purified amino acid mixture (FMC) than in the blood of fish fed intact protein (FM). Concentrations of the aliphatic amino acid, leucine, in the HPV blood of fish fed FMC were higher at 4 h and 8 h after feeding, than in the blood of fish fed FM. Blood levels of these amino acids did not differ significantly between treatments at any of the other sampling times. Concentrations of seven amino acids in HPV blood did not differ significantly between treatments at any time during the 12-h postprandial sampling period, and one amino acid was not detected in any of the samples.

There were no significant differences in the concentrations of individual aromatic and heterocyclic amino acids in the plasma during the 12-h sampling period within the FM and FMC treatments.

A significant difference in the lysine content of the HPV blood of fish fed FMC, occurred 1 h after feeding. Fish fed the purified amino acid mixture had a significantly higher concentration of lysine in the blood 1 h after feeding than fish sampled before feeding (0 h) or 2-12 h after feeding. Fourteen other amino acids did not vary significantly in concentration in HPV blood

during the 12-h period following ingestion of FMC. No significant differences in the concentrations of any amino acid occurred with time among fish fed FM.

3.2.4 Meat and Bone Meal (MB and MBC)

Statistically significant differences between treatments, at one hour intervals for 12 h after feeding, were as follows:

1) Acidic series– aspartic acid (Table 21) and glutamic acid (Table 22): No difference between MB and MBC at any time during the 12-h sampling period.

2) Aliphatic series– alanine (Table 21), glycine (Table 22), isoleucine (Fig. 28), leucine (Fig. 29), serine (Table 23), threonine (Fig. 30) and valine (Fig. 31): No difference between MB and MBC at any time during the 12-h sampling period.

3) Aromatic series– phenylalanine (Fig. 32) and tyrosine (Table 23): No difference between MB and MBC at any time during the 12-h sampling period.

4) Basic series– arginine (Fig. 33) and lysine (Fig. 35): Significantly higher in HPV blood of fish fed BMC, 4 h after feeding, than in blood of fish fed BM.

5) Basic series– histidine (Fig. 34): No difference between MB and MBC at any time during the 12-h sampling period.

6) Heterocyclic series– tryptophan (Fig. 36): No difference between MB and MBC at any time during the 12-h sampling period.

7) Sulfur-containing series– methionine: Not detected at any time, in either treatment group, during the 12-h sampling period.

Four hours after feeding, concentrations of two amino acids, arginine and lysine, were significantly higher in the HPV blood of fish fed the purified amino acid mixture (MBC) than in the blood of fish fed intact protein (MB). Blood levels of these two amino acids did not differ

TABLE 18. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, alanine and aspartic acid, in the HPV blood of channel catfish during a 12-h period after force-feeding of FM or FMC. Within ingredients, means in the same row with different letters are significantly different ($P \leq 0.05$).

Time in h	Alanine		Aspartic acid	
	FM	FMC	FM	FMC
	nmol/μL	nmol/μL	nmol/μL	nmol/μL
0	1160.5 ± 506.5	199.9 ± 43.3	84.5 ± 71.2	41.6 ± 32.2
1	636.4 ± 128.4[b]	3494.7 ± 888.1[a]	66.1 ± 24.7[b]	1722.4 ± 314.8[a]
2	445.4 ± 82.9	698.2 ± 159.9	70.3 ± 27.0	177.7 ± 48.0
3	468.8 ± 200.0	554.8 ± 323.0	60.9 ± 28.9	142.2 ± 14.7
4	371.6 ± 200.4	474.7 ± 264.1	75.6 ± 41.1	169.9 ± 149.2
5	528.1 ± 116.7	379.6 ± 273.7	84.0 ± 54.6	38.4 ± 26.2
6	1075.9 ± 68.3	328.7 ± 179.0	84.0 ± 45.7	69.2 ± 66.7
7	592.2 ± 297.1	595.8 ± 448.5	55.6 ± 38.0	88.4 ± 67.0
8	547.2 ± 314.8	350.5 ± 168.8	65.5 ± 39.6	106.7 ± 69.7
9	556.4 ± 142.2	540.6 ± 267.2	84.8 ± 71.1	77.6 ± 33.1
10	538.1 ± 224.9	199.2 ± 32.6	64.0 ± 48.0	54.8 ± 18.7
11	712.5 ± 168.4	182.9 ± 73.3	52.2 ± 15.3	59.1 ± 43.4
12	723.8 ± 44.8	151.1 ± 135.6	66.8 ± 13.9	36.4 ± 24.6

TABLE 19. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, glutamic acid and glycine, in the HPV blood of channel catfish during a 12-h period after force-feeding of FM or FMC. Within ingredients, means in the same row with different letters are significantly different ($P \leq 0.05$).

Time in h	Glutamic acid		Glycine	
	FM	FMC	FM	FMC
	nmol/μL	nmol/μL	nmol/μL	nmol/μL
0	80.3 ± 20.2	42.5 ± 42.8	583.6 ± 366.5	220.3 ± 70.6
1	144.4 ± 37.0[b]	2176.7 ± 531.4[a]	646.3 ± 180.3[b]	5991.0 ± 2033.0[a]
2	117.4 ± 42.1	322.3 ± 216.8	428.0 ± 52.5	816.5 ± 205.8
3	87.3 ± 21.5	246.4 ± 13.2	273.7 ± 11.5	861.9 ± 267.8
4	145.2 ± 70.3	291.9 ± 197.5	275.7 ± 45.4	735.2 ± 431.3
5	155.3 ± 96.2	572.5 ± 869.8	444.8 ± 126.1	583.3 ± 544.6
6	143.2 ± 104.6	117.9 ± 59.8	543.7 ± 95.0	340.8 ± 164.7
7	188.9 ± 190.4	191.0 ± 133.4	277.6 ± 111.0	502.7 ± 234.6
8	118.8 ± 51.7	208.2 ± 116.7	333.4 ± 144.6	501.7 ± 327.2
9	139.0 ± 37.1	163.6 ± 84.8	502.6 ± 332.4	484.2 ± 242.6
10	281.7 ± 221.7	104.3 ± 67.6	420.7 ± 231.2	377.7 ± 53.1
11	197.0 ± 39.4	138.4 ± 75.3	311.4 ± 30.8	450.6 ± 443.0
12	270.5 ± 91.6	143.1 ± 120.8	352.7 ± 53.2	238.4 ± 177.1

TABLE 20. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, serine and tyrosine, in the HPV blood of channel catfish during a 12-h period after force-feeding of FM or FMC. No significant differences were observed between treatments.

Time in h	Serine		Tyrosine	
	FM	FMC	FM	FMC
	nmol/μL	nmol/μL	nmol/μL	nmol/μL
0	562.6 ± 305.7	256.9 ± 155.0	393.5 ± 341.2	111.3 ± 25.9
1	625.2 ± 189.8	1312.1 ± 217.7	188.2 ± 68.3	315.9 ± 32.1
2	548.0 ± 48.1	532.1 ± 79.3	194.4 ± 50.3	173.4 ± 18.4
3	431.5 ± 145.0	538.0 ± 229.9	192.6 ± 98.3	152.0 ± 54.8
4	306.9 ± 118.7	493.9 ± 199.3	159.7 ± 67.3	154.7 ± 35.0
5	292.2 ± 60.2	553.3 ± 410.1	155.0 ± 54.9	174.9 ± 114.5
6	681.6 ± 413.0	362.6 ± 269.3	211.9 ± 115.3	146.0 ± 98.6
7	294.4 ± 121.3	506.7 ± 224.2	165.8 ± 49.2	186.0 ± 96.5
8	364.3 ± 176.2	631.9 ± 388.2	153.6 ± 40.7	238.4 ± 24.2
9	319.7 ± 73.7	684.1 ± 402.4	172.5 ± 68.3	164.6 ± 50.6
10	350.8 ± 149.0	334.2 ± 93.1	145.8 ± 49.4	134.7 ± 39.1
11	521.4 ± 129.2	464.6 ± 365.5	205.7 ± 53.7	124.5 ± 49.4
12	467.8 ± 22.6	223.1 ± 159.0	320.3 ± 55.1	102.4 ± 74.0

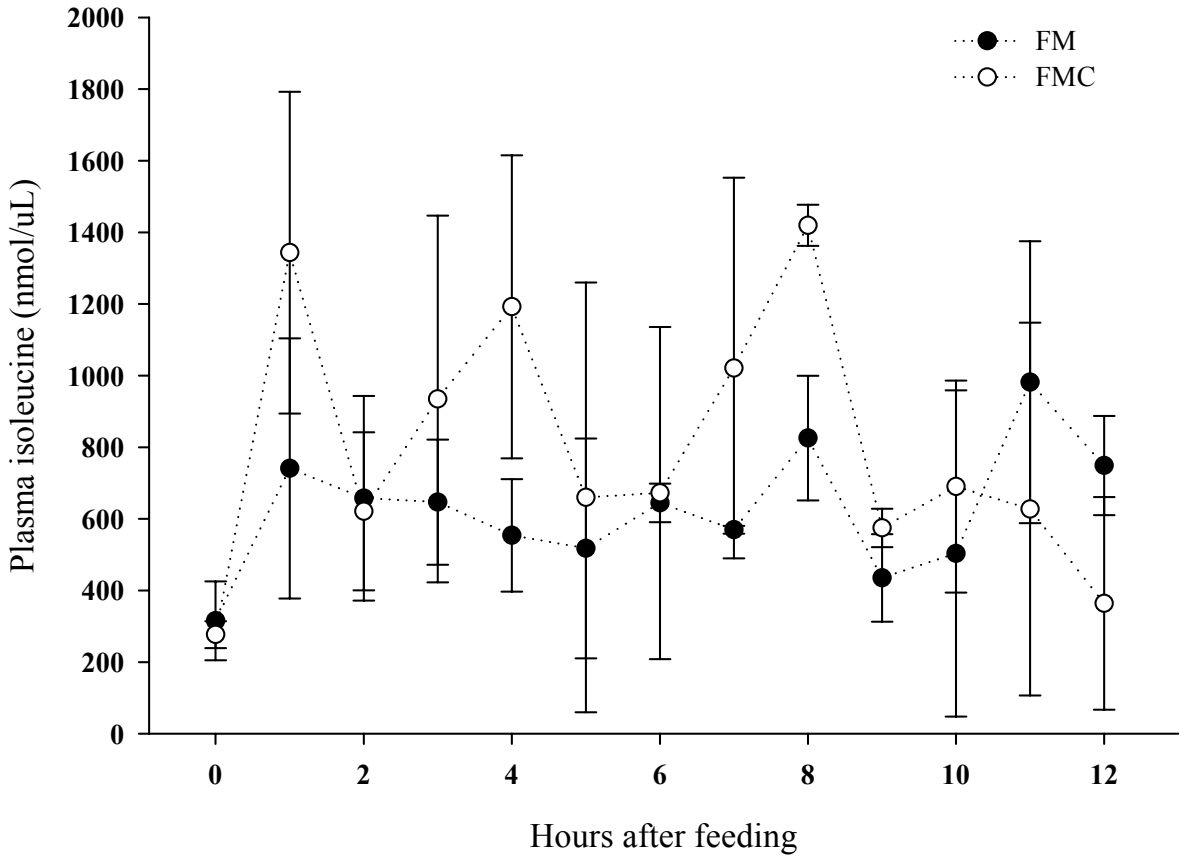

FIGURE 19. Concentrations of isoleucine in the HPV blood of channel catfish during the 12-h period after force-feeding of FM or FMC. There were no significant differences between or within treatments.

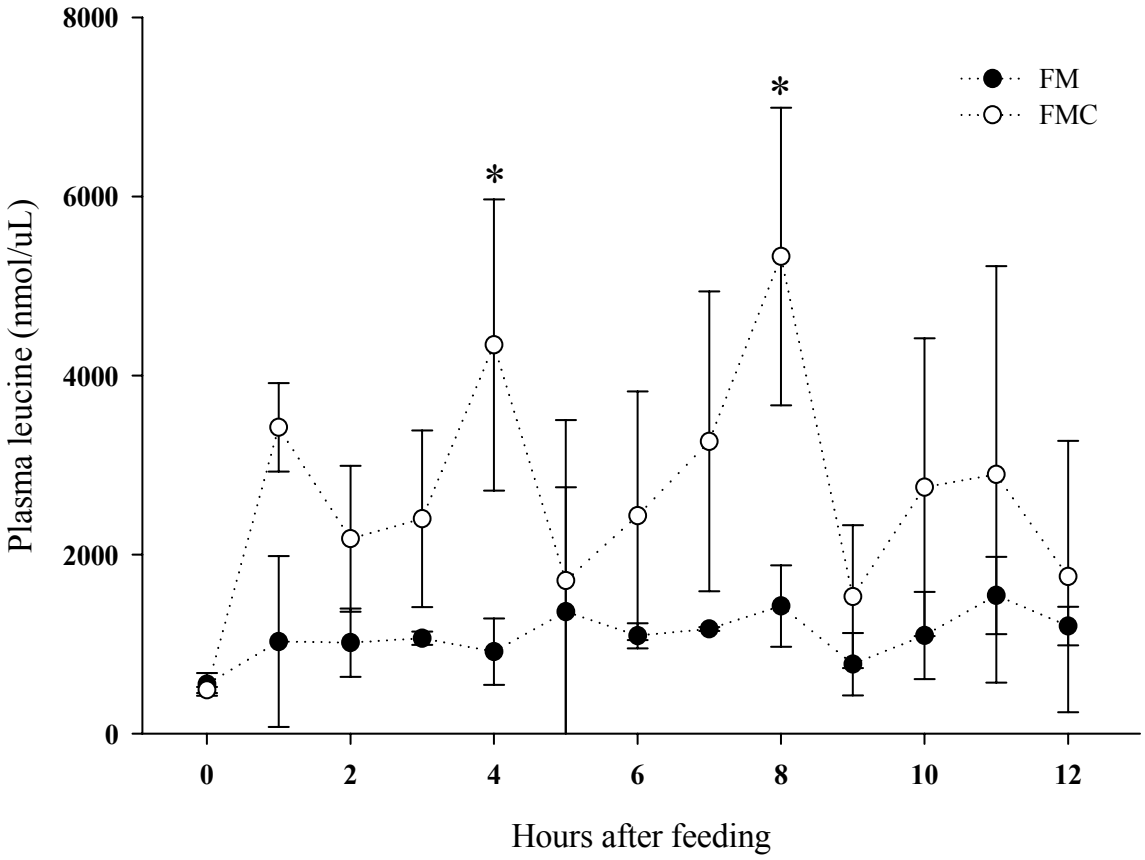

FIGURE 20. Concentrations of leucine in the HPV blood of channel catfish during the 12-h period after force-feeding of FM or FMC. An asterisk indicates a significant difference between treatments. There were no significant differences within treatments.

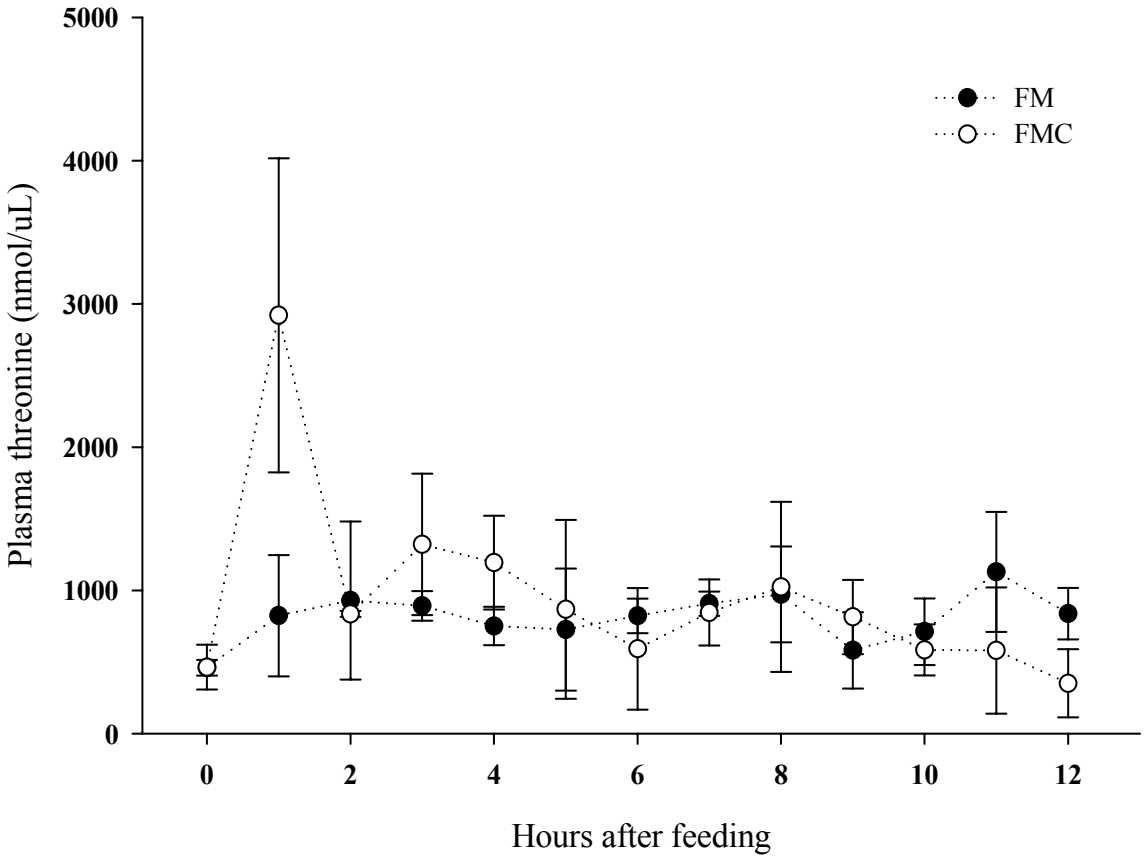

FIGURE 21. Concentrations of threonine in the HPV blood of channel catfish during the 12-h period after force-feeding of FM or FMC. There were no significant differences between or within treatments.

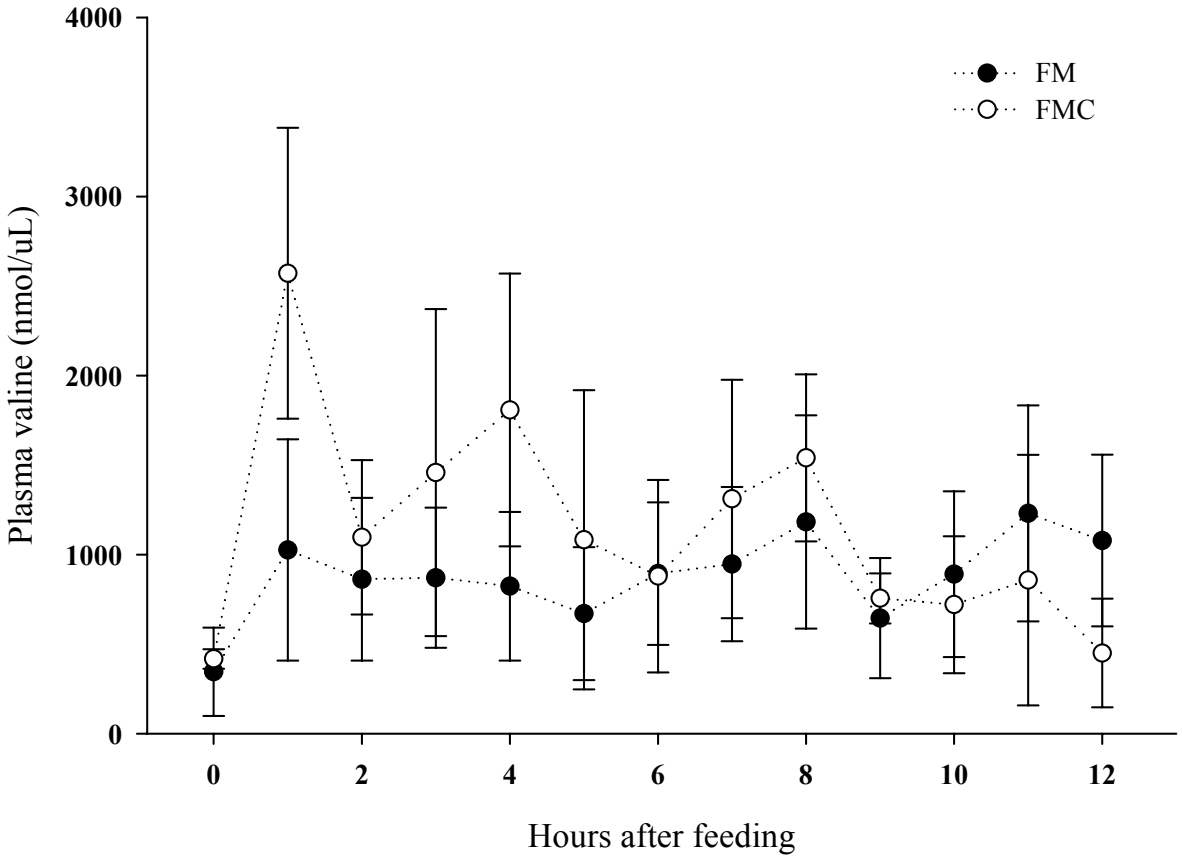

FIGURE 22. Concentrations of valine in the HPV blood of channel catfish during the 12-h period after force-feeding of FM or FMC. There were no significant differences between or within treatments.

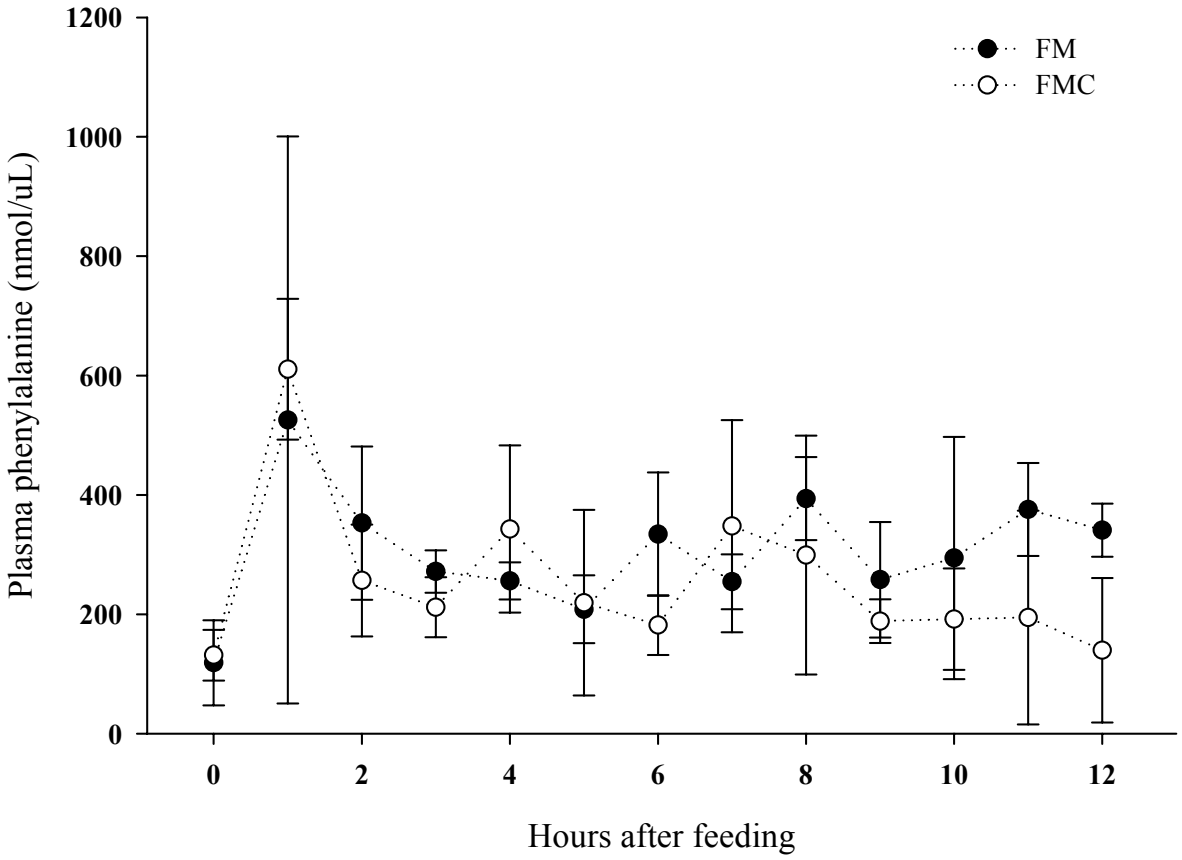

FIGURE 23. Concentrations of phenylalanine in the HPV blood of channel catfish during the 12-h period after force-feeding of FM or FMC. There were no significant differences between or within treatments.

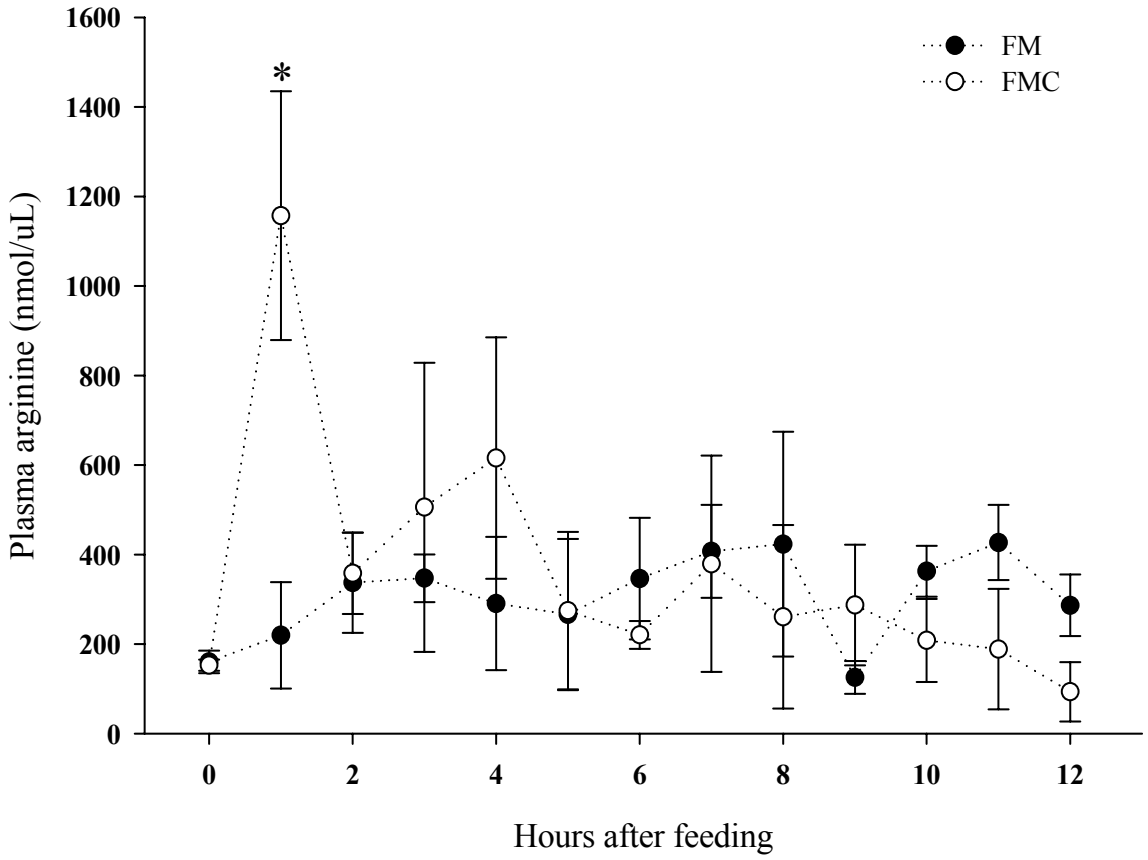

FIGURE 24. Concentrations of arginine in the HPV blood of channel catfish during the 12-h period after force-feeding of FM or FMC. An asterisk indicates a significant difference between treatments. There were no significant differences within treatments.

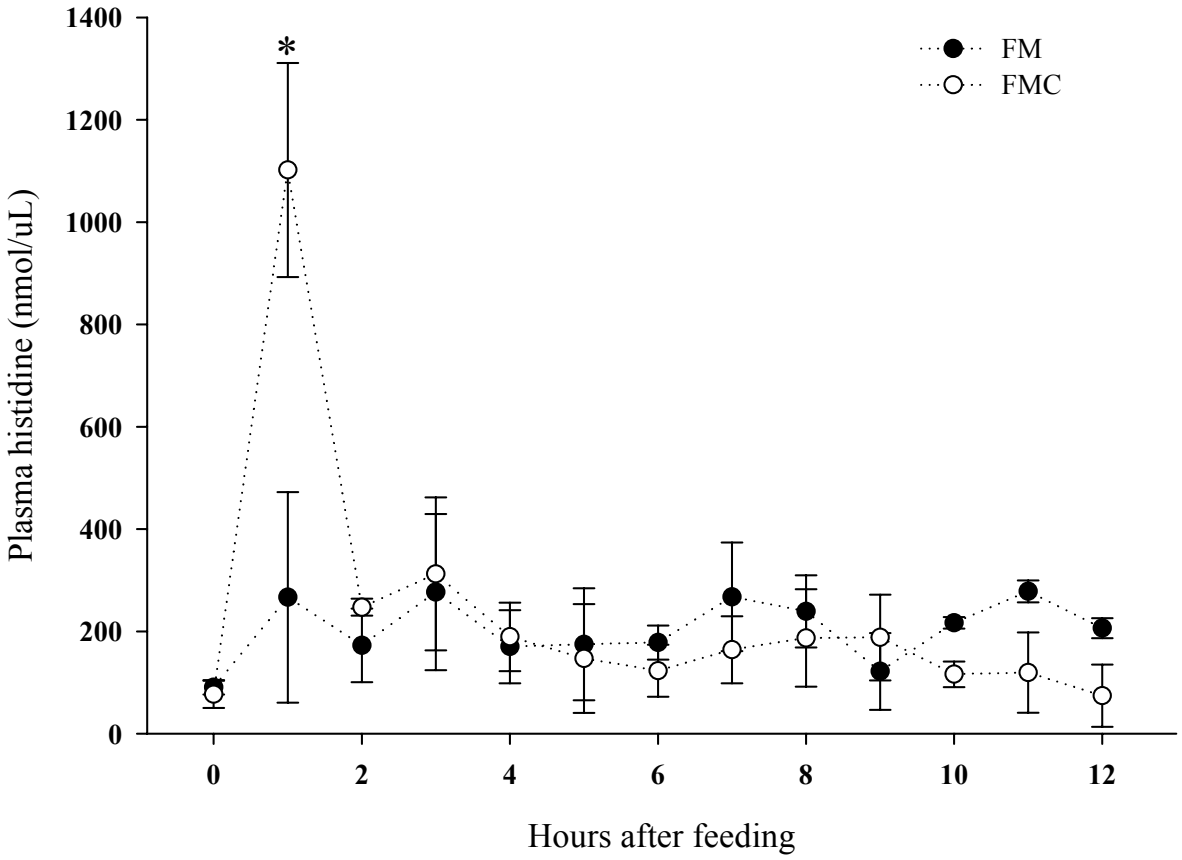

FIGURE 25. Concentrations of histidine in the HPV blood of channel catfish during the 12-h period after force-feeding of FM or FMC. An asterisk indicates a significant difference between treatments. There were no significant differences within treatments.

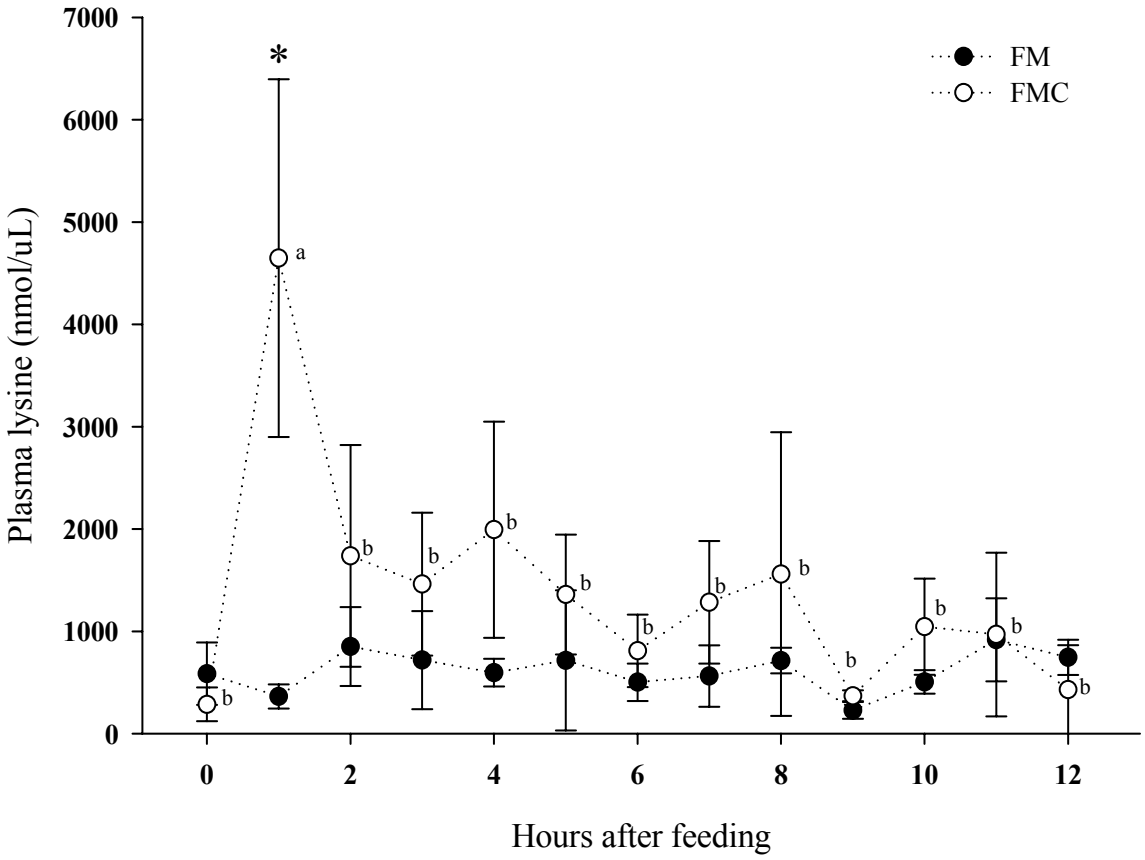

FIGURE 26. Concentrations of lysine in the HPV blood of channel catfish during the 12-h period after force-feeding of FM or FMC. An asterisk indicates a significant difference between treatments. Points on the FMC line with different letters are significantly different. There were no significant differences within FM treatment.

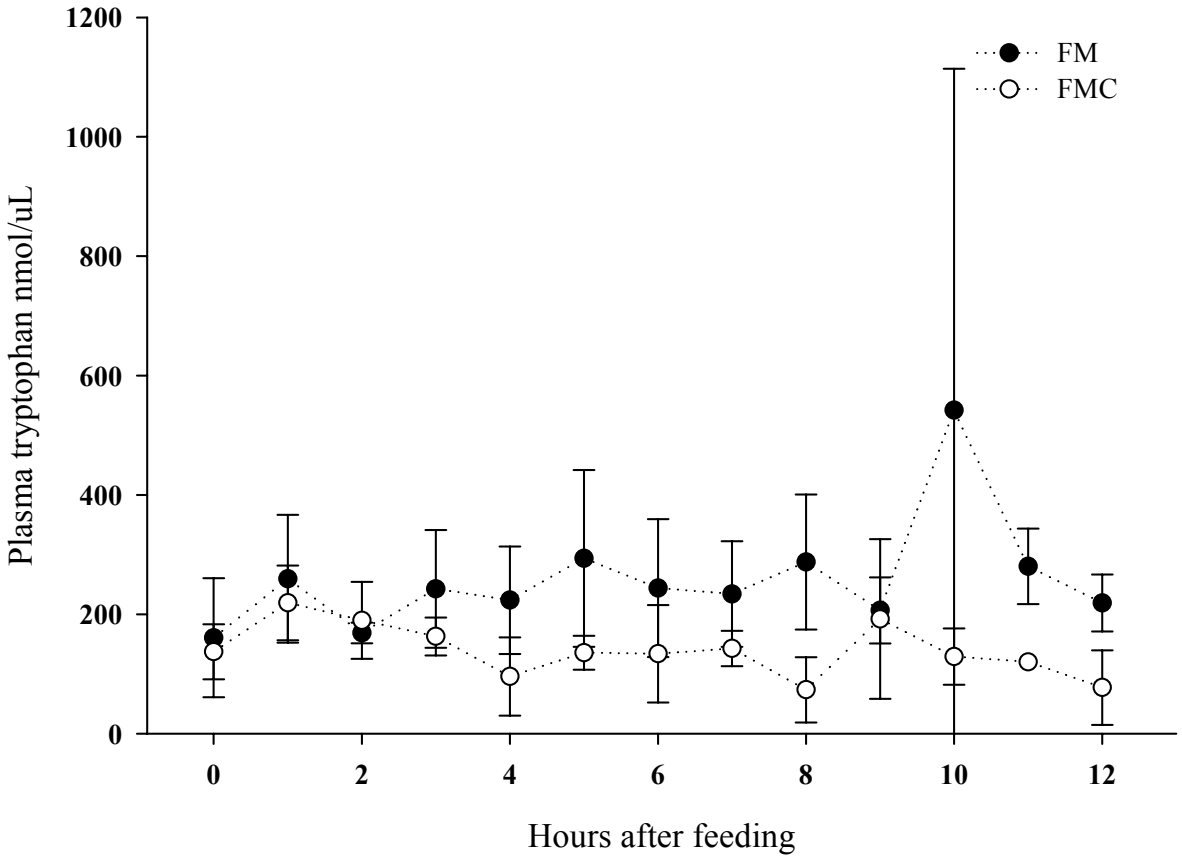

FIGURE 27. Concentrations of tryptophan in the HPV blood of channel catfish during the 12-h period after force-feeding of FM or FMC. There were no significant differences between or within treatments.

significantly between treatments at any of the other sampling times. Concentrations of 13 aminoacids in HPV blood did not differ significantly between treatments at any time during the 12-h postprandial sampling period, and one amino acid was not detected in any of the samples.

There were no significant differences, with the exception of leucine in MB, in the concentrations of individual acidic, aliphatic, aromatic, basic and heterocyclic amino acids in the plasma during the 12-h sampling period within the MB and MBC treatments.

Leucine content of HPV blood of catfish fed MB was slightly but significantly higher at 10 h after feeding.

3.2.5 Soybean Meal (SB and SBC)

Statistically significant differences between treatments, at one hour intervals for 12 h after feeding, were as follows:

1) Acidic series– aspartic acid (Table 24) and glutamic acid (Table 25): Significantly higher in HPV blood of fish fed SBC, 1 h after feeding, than in blood of fish fed SB.
2) Aliphatic series– isoleucine (Fig. 37) and alanine (Table 24): No difference between SB and SBC at any time during the 12-h sampling period.
3) Aliphatic series– glycine (Table 25), leucine (Fig. 38), and serine (Table 26), threonine (Fig. 39) and valine (Fig. 40): Significantly higher concentrations in HPV blood of fish fed SBC, 1 h after feeding (glycine and serine), 2 h after feeding (glycine) and 3 h after feeding (leucine, threonine and valine), than in blood of fish fed SB.
4) Aromatic series– phenylalanine (Fig. 41) and tyrosine (Table 26): No difference between SB and SBC at any time during the 12-h sampling period.
5) Basic series– arginine (Fig. 42): Significantly higher in HPV blood of fish fed SBC, 3 h after feeding, than in blood of fish fed SB.

TABLE 21. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, alanine and aspartic acid, in the HPV blood of channel catfish during a 12-h period after force-feeding of MB or MBC. No significant differences were observed between treatments.

Time in h	Alanine		Aspartic acid	
	MB	MBC	MB	MBC
	nmol/µL	nmol/µL	nmol/µL	nmol/µL
0	371.5 ± 77.4	166.5 ± 73.7	66.4 ± 28.1	19.0 ± 2.7
1	764.2 ± 177.8	3552.3 ± 1482.7	193.8 ± 197.1	925.7 ± 1018.7
2	829.4 ± 423.0	3626.1 ± 3027.1	75.3 ± 37.7	588.9 ± 725.5
3	771.1 ± 331.0	1760.3 ± 242.2	97.2 ± 52.0	524.0 ± 556.1
4	735.2 ± 58.9	2881.4 ± 879.2	72.4 ± 27.0	645.5 ± 945.0
5	749.6 ± 117.3	707.8 ± 92.6	76.2 ± 21.8	2511.4 ± 3928.7
6	739.8 ± 141.0	621.9 ± 226.5	84.9 ± 32.8	198.9 ± 129.5
7	606.0 ± 292.8	719.4 ± 297.7	124.0 ± 45.4	300.1 ± 399.3
8	609.6 ± 44.7	749.4 ± 143.7	65.7 ± 16.5	86.0 ± 4.6
9	982.8 ± 438.6	363.3 ± 92.7	156.4 ± 166.6	36.8 ± 11.6
10	499.2 ± 153.7	414.9 ± 123.4	78.0 ± 41.7	51.2 ± 16.8
11	760.3 ± 171.8	467.5 ± 164.5	61.1 ± 6.8	42.3 ±17.2
12	569.6 ± 290.0	585.8 ± 298.9	76.0 ± 54.3	69.8 ± 31.9

TABLE 22. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, glutamic acid and glycine, in the HPV blood of channel catfish during a 12-h period after force-feeding of MB or MBC. No significant differences were observed between treatments.

Time in h	Glutamic acid		Glycine	
	MB	MBC	MB	MBC
	nmol/μL	nmol/μL	nmol/μL	nmol/μL
0	102.4 ± 38.1	56.0 ± 42.9	354.7 ± 131.5	236.5 ± 115.3
1	442.3 ± 301.4	1392.2 ± 1410.3	554.0 ± 204.9	6360.1 ± 5230.3
2	248.9 ± 145.5	806.2 ± 1012.2	579.7 ± 267.5	5134.7 ± 5922.0
3	195.2 ± 107.1	1305.3 ± 1377.2	865.4 ± 318.4	2948.1 ± 1593.2
4	160.5 ± 71.4	866.2 ± 1004.1	717.6 ± 100.2	5564.4 ± 3455.9
5	196.2 ± 130.1	2341.2 ± 3095.8	901.8 ± 164.5	1921.1 ± 134.0
6	159.6 ± 2.9	326.2 ± 154.2	949.6 ± 272.6	1568.3 ± 757.5
7	110.7 ± 18.7	593.5 ± 609.8	703.1 ± 371.6	1431.1 ± 1318.6
8	215.2 ± 119.8	335.5 ± 173.9	583.6 ± 111.9	1821.0 ± 682.8
9	250.0 ± 221.7	144.4 ± 73.2	700.0 ± 147.4	1065.3 ± 520.0
10	168.6 ± 71.8	153.3 ± 54.3	535.1 ± 163.0	584.8 ± 241.9
11	160.2 ± 75.7	109.0 ± 30.1	642.4 ± 205.3	1223.4 ± 776.9
12	269.3 ± 232.3	162.9 ± 59.3	537.5 ± 166.0	687.1 ± 531.4

TABLE 23. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, serine and tyrosine, in the HPV blood of channel catfish during a 12-h period after force-feeding of MB or MBC. No significant differences were observed between treatments.

Time in h	Serine		Tyrosine	
	MB	MBC	MB	MBC
	nmol/µL	nmol/µL	nmol/µL	nmol/µL
0	417.4 ± 267.6	207.6 ± 114.6	156.6 ± 37.2	102.8 ± 53.0
1	679.0 ± 427.0	1204.1 ± 631.4	264.8 ± 61.6	195.4 ± 65.2
2	373.6 ± 280.5	836.5 ± 573.1	281.1 ± 144.3	206.8 ± 107.1
3	667.4 ± 185.0	1140.6 ± 617.5	227.7 ± 10.6	282.0 ± 201.9
4	592.6 ± 61.5	1700.1 ± 968.5	174.4 ± 15.7	267.9 ± 124.7
5	649.6 ± 132.6	2106.8 ± 2341.4	193.3 ± 19.2	241.5 ± 184.0
6	729.0 ± 191.0	658.0 ± 161.2	182.2 ± 18.9	180.5 ± 17.9
7	720.2 ± 398.7	756.7 ± 354.1	205.4 ± 13.2	169.5 ± 31.2
8	524.0 ± 54.8	1015.7 ± 303.9	176.9 ± 34.2	244.9 ± 102.6
9	688.1 ± 274.6	717.1 ± 278.9	233.4 ± 125.2	160.5 ± 27.8
10	487.8 ± 147.8	616.6 ± 307.3	175.7 ± 50.2	160.2 ± 19.4
11	681.7 ± 155.6	961.5 ± 247.1	197.4 ± 55.5	177.1 ± 68.8
12	539.9 ± 156.3	623.4 ± 462.3	195.1 ± 103.5	185.7 ± 99.8

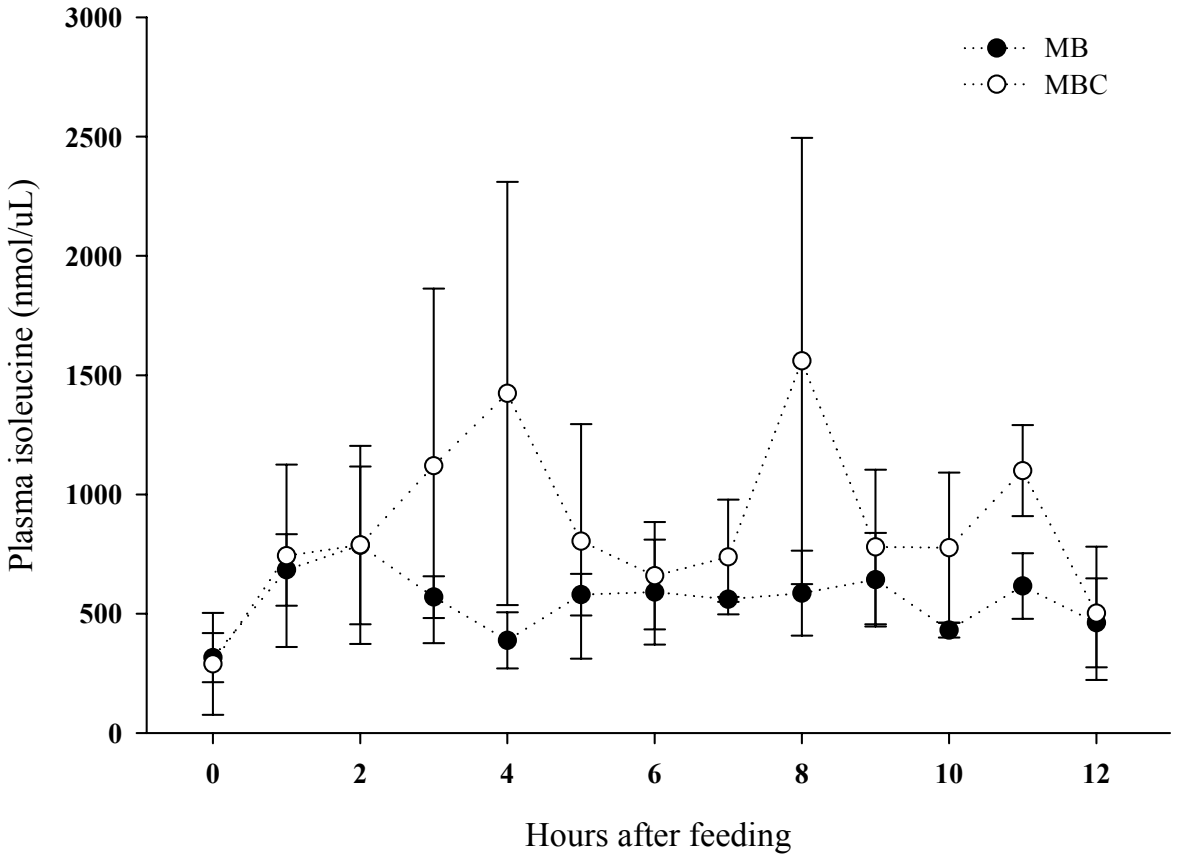

FIGURE 28. Concentrations of isoleucine in the HPV blood of channel catfish during the 12-h period after force-feeding of MB or MBC. There were no significant differences between or within treatments.

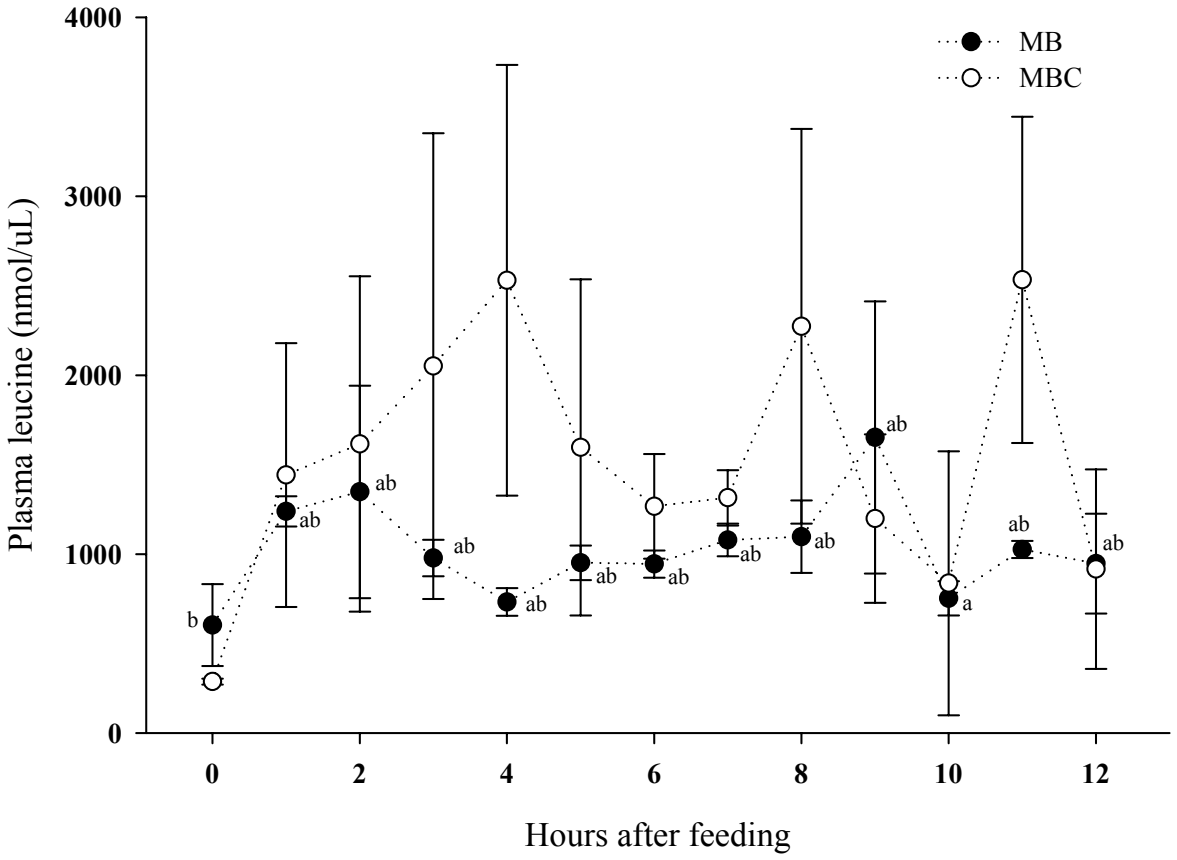

FIGURE 29. Concentrations of leucine in the HPV blood of channel catfish during the 12-h period after force-feeding of MB or MBC. There were no significant differences between or within treatments. Points on MB line with different letters are significantly different. There were no significant differences within MBC treatment.

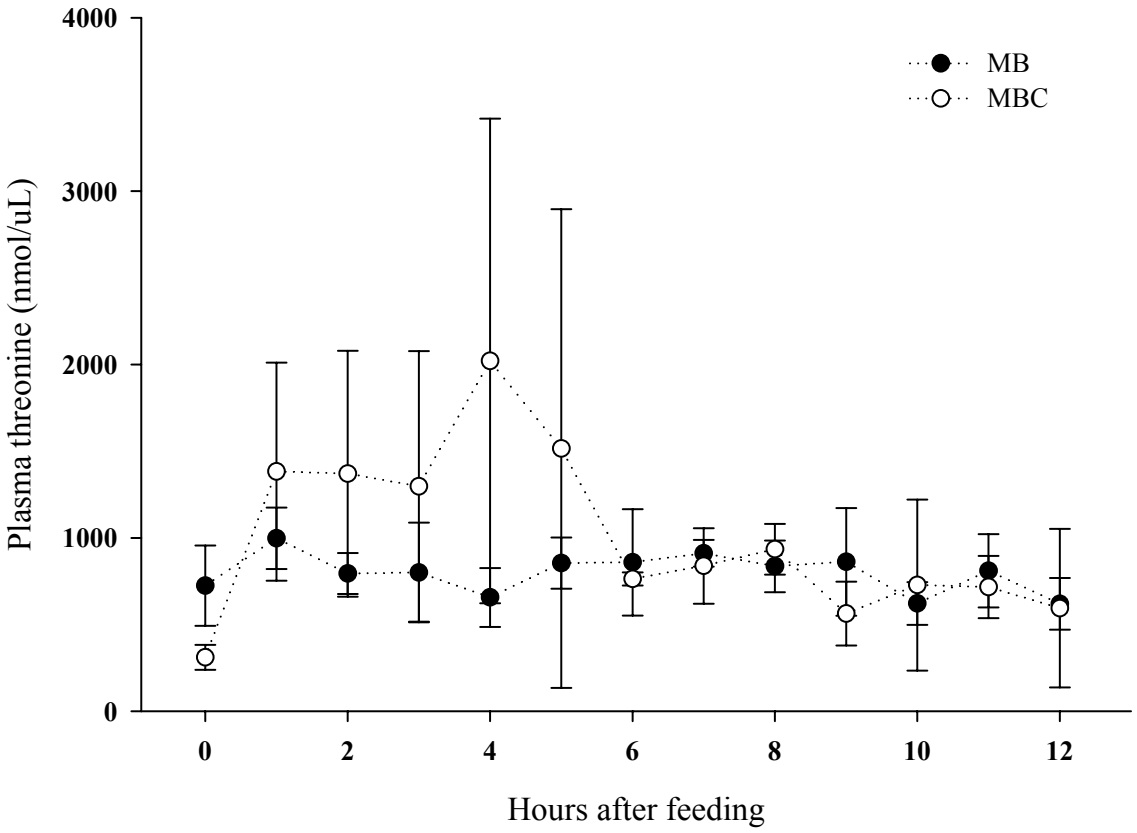

FIGURE 30. Concentrations of threonine in the HPV blood of channel catfish during the 12-h period after force-feeding of MB or MBC. There were no significant differences between or within treatments.

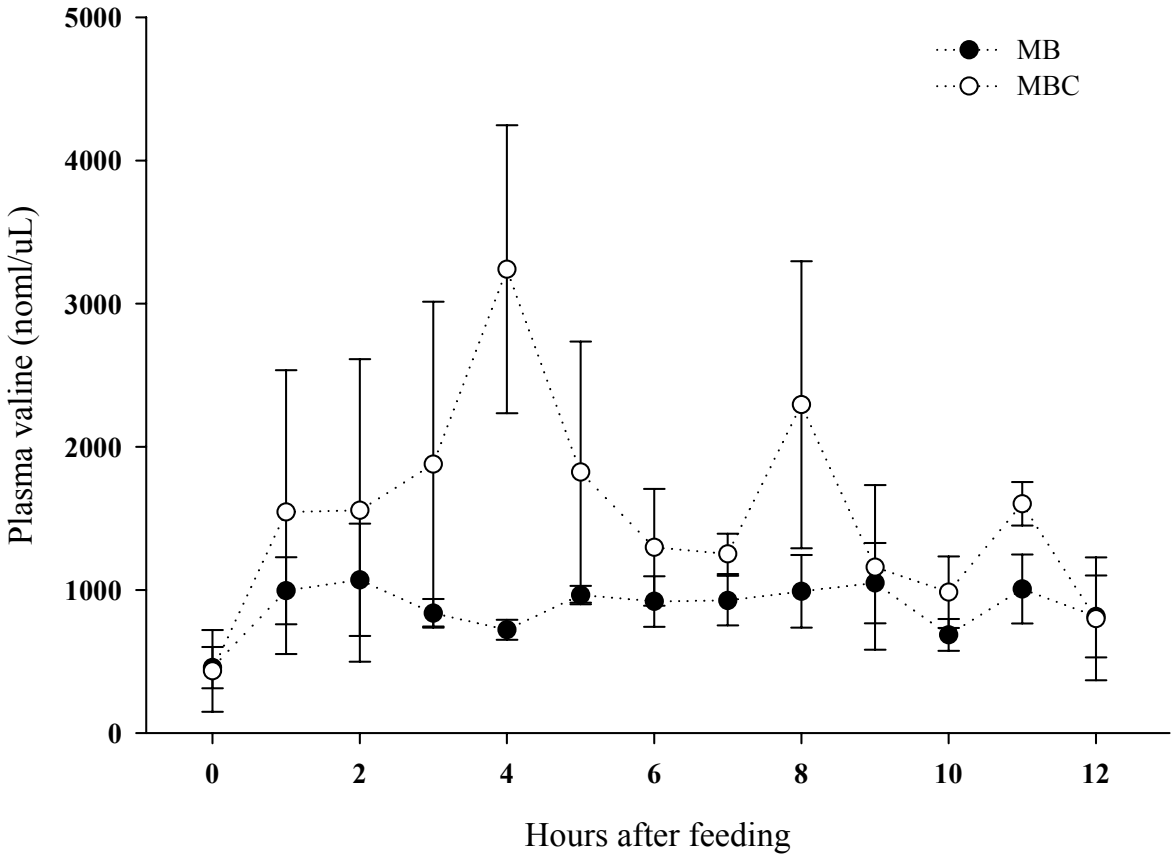

FIGURE 31. Concentrations of valine in the HPV blood of channel catfish during the 12-h period after force-feeding of MB or MBC. There were no significant differences between or within treatments.

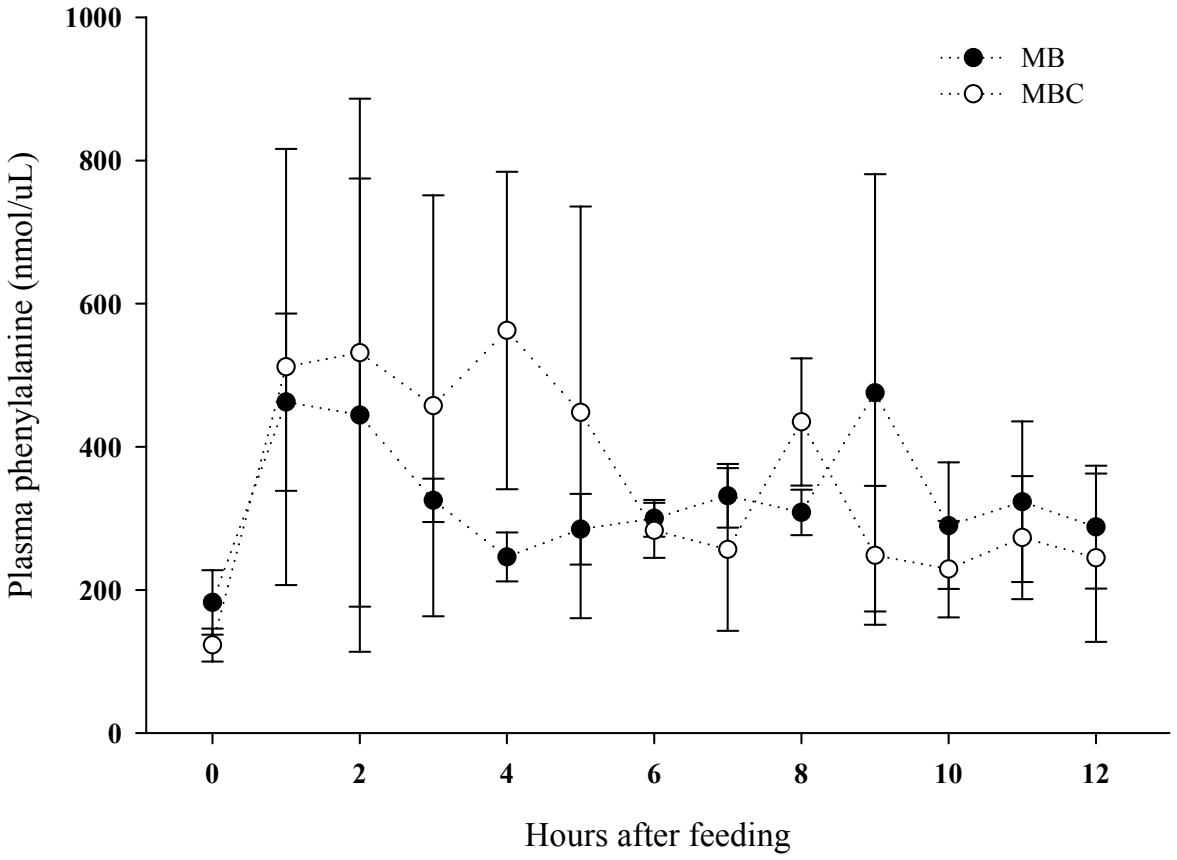

FIGURE 32. Concentrations of phenylalanine in the HPV blood of channel catfish during the 12-h period after force-feeding of MB or MBC. There were no significant differences between or within treatments.

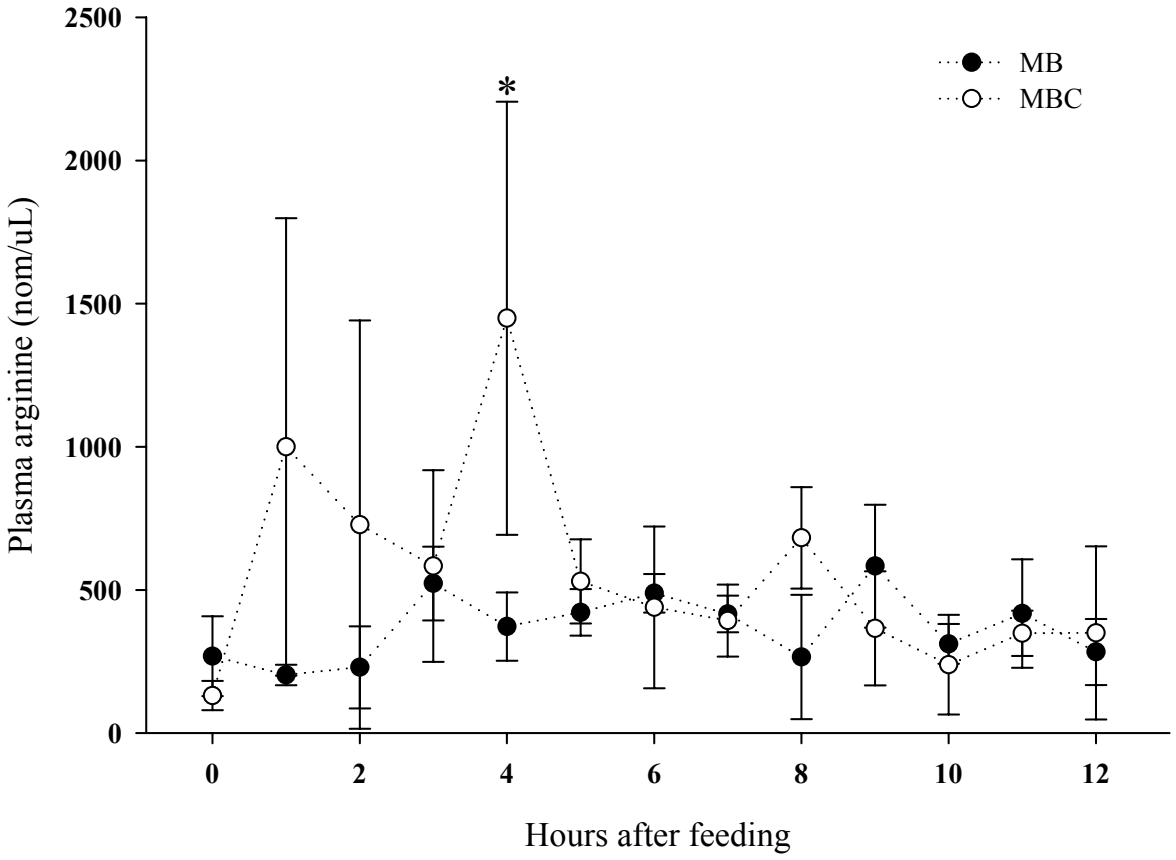

FIGURE 33. Concentrations of arginine in the HPV blood of channel catfish during the 12-h period after force-feeding of MB or MBC. An asterisk indicates a significant difference between treatments. There were no significant differences within treatments.

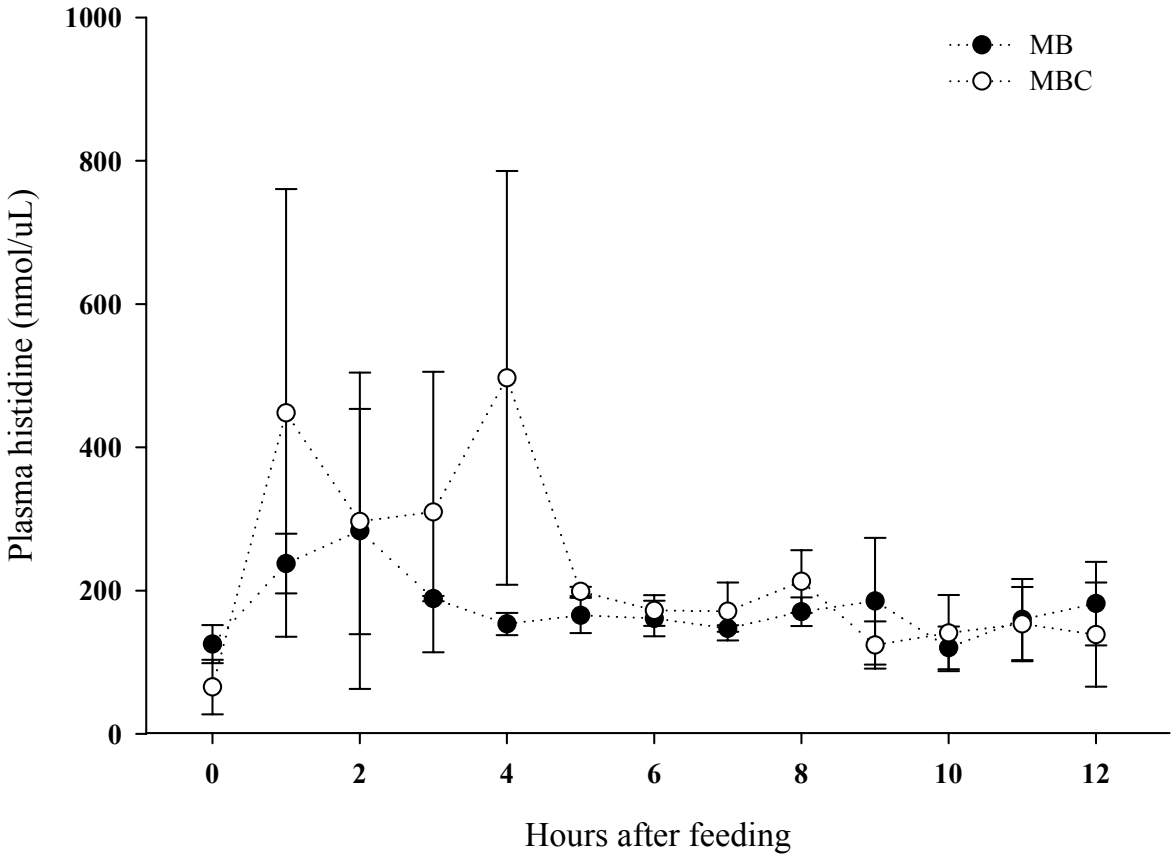

FIGURE 34. Concentrations of histidine in the HPV blood of channel catfish during the 12-h period after force-feeding of MB or MBC. There were no significant differences between or within treatments.

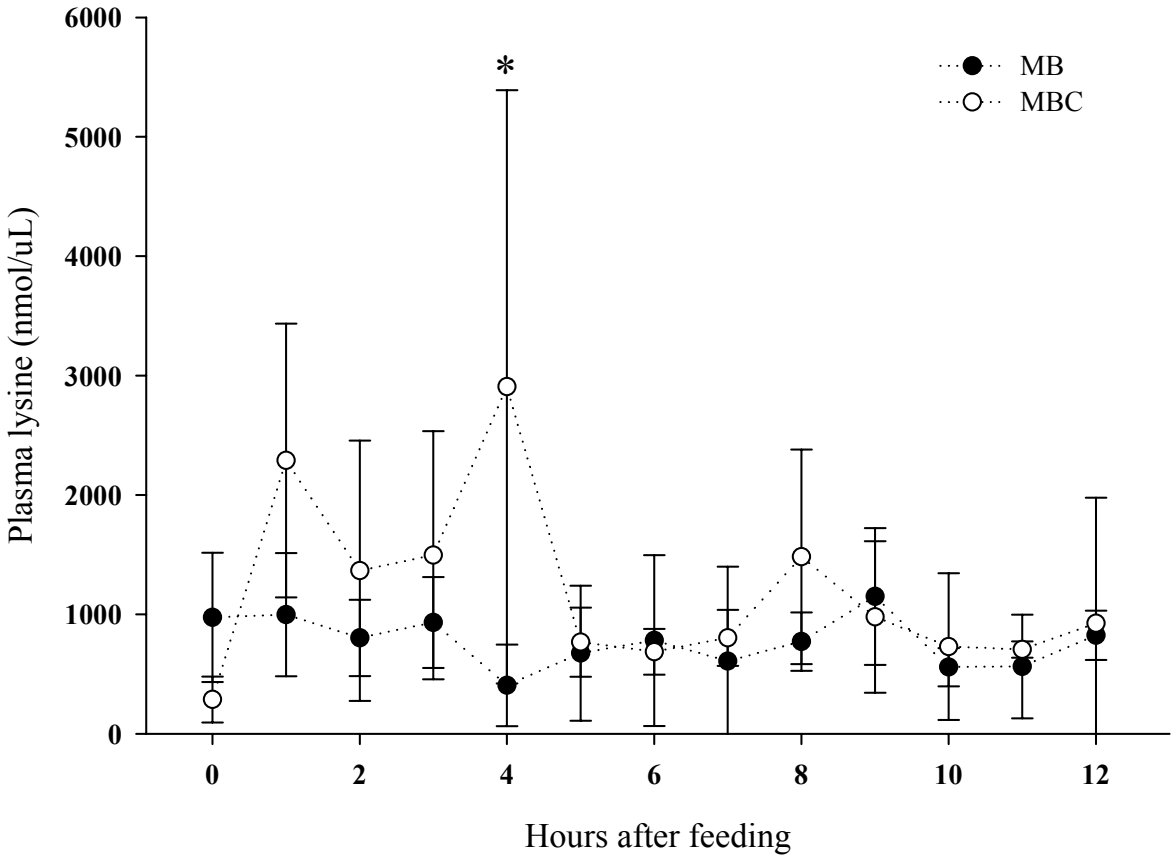

FIGURE 35. Concentrations of lysine in the HPV blood of channel catfish during the 12-h period after force-feeding of MB or MBC. An asterisk indicates a significant difference between the treatments. There were no significant differences within treatments.

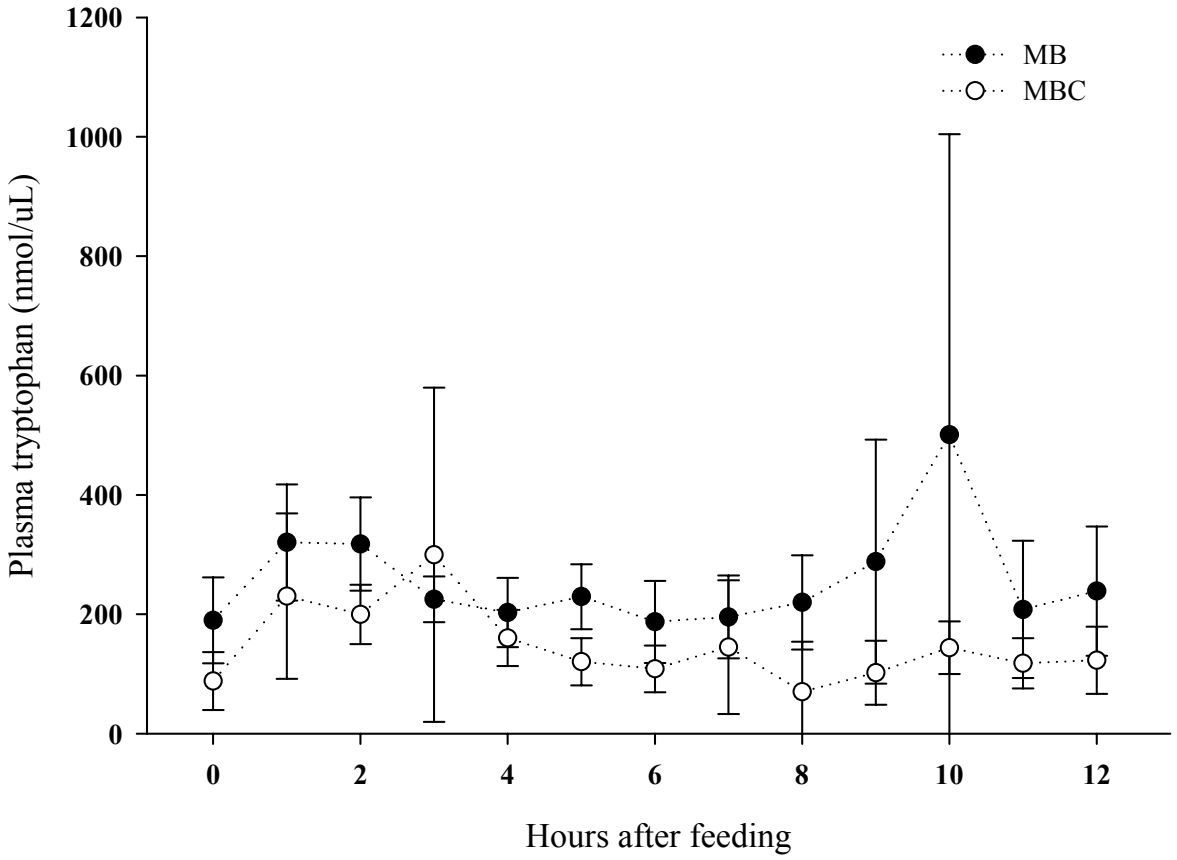

FIGURE 36. Concentrations of tryptophan in the HPV blood of channel catfish during the 12-h period after force-feeding of MB or MBC. There were no significant differences between or within treatments.

6) Basic series histidine– (Fig. 43) and lysine (Fig. 44): No difference between SB and SBC at any time during the 12-h sampling period.

7) Heterocyclic series– tryptophan (Fig. 45): No difference between SB and SBC at any time during the 12-h sampling period.

8) Sulfur-containing series– methionine: Not detected at any time, in either treatment group, during the 12-h sampling period.

One hour after feeding, concentrations of the acidic amino acids, aspartic acid and glutamic acid, and the aliphatic amino acids, glycine and serine, were significantly higher in the HPV blood of fish fed the purified amino acid mixture (SBC) than in the blood of fish fed intact protein (SB). Concentrations of the aliphatic amino acids, leucine, threonine and valine, and the basic amino acid, arginine, were significantly higher in the HPV blood of fish fed SBC, 3 h after feeding, than in the blood of fish fed SB. Blood levels of these amino acids did not differ significantly between treatments at any of the other sampling times. Concentrations of seven amino acids in HPV blood did not differ significantly between treatments at any time during the 12-h postprandial sampling period, and one amino acid was not detected in any of the samples.

Fish fed purified amino acids mixtures had significantly high concentrations of alanine and glycine in the HPV blood of catfish, 1 h, 2 h and 3 h after feeding. Aspartic acid and glutamic acid concentrations in the HPV blood of catfish fed SBC were significantly higher at 1 h after feeding than it was before feeding (0 h). Otherwise there were no differences in concentrations of aspartic acid and glutamic acid of blood samples atken 1-12 h after feeding SBC. Concentration of serine in HPV blood of catfish fed SBC was significantly high at 2 h. The tyrosine content of HPV blood of catfish fed SBC was slightly but higher, 3 h after force-feeding than it was before feeding (0 h). Glutamic acid was the only amino acid that showed

significantly high concentrations at 10 h after feeding the SB treatment. Arginine (Fig. 42), leucine (Fig. 38), phenylalanine (Fig. 41), threonine (Fig. 39) and valine (Fig. 40) had significantly high concentrations in the HPV blood of catfish fed SBC at 3 h. Isoleucine concentrations in the HPV blood of catfish fed SBC were significant high at 7 h.

3.2.6 Wheat Middlings (WM and WMC)

Statistically significant differences between treatments, at one hour intervals, for 12 h after feeding, were as follows:

1) Acidic series– aspartic acid (Table 27) and glutamic acid (Table 28): No difference between WM and WMC at any time during the 12-h sampling period.
2) Aliphatic series– alanine (Table 27) glycine (Table. 28), isoleucine (Fig. 46), leucine (Fig. 47), serine (Table 29), threonine (Fig. 48) and valine (Fig. 49): No difference between WM and WMC at any time during the 12-h sampling period.
3) Aromatic series– phenylalanine (Fig. 50) and tyrosine (Table 29): No difference between WM and WMC at any time during the 12-h sampling period.
4) Basic series– arginine (Fig. 51), histidine (Fig. 52) and lysine (Fig. 53): No difference between WM and WMC at any time during the 12-h sampling period.
5) Sulfur-containing series– methionine: Not detected at any time, in either treatment group, during the 12-h sampling period.
6) Heterocyclic series– tryptophan (Fig. 54): No difference between WM and WMC at any time during the 12-h sampling period.

Concentrations of 15 amino acids in HPV blood did not differ significantly between treatments at any time during the 12-h postprandial sampling period, and one amino acid was not detected in any of the samples.

TABLE 24. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids alanine and aspartic acid, in the HPV blood of channel catfish during a 12-h period after force-feeding of SB or SBC. Within ingredients, means in the same row with different letters are significantly different ($P \leq 0.05$).

Time in h	Alanine		Aspartic acid	
	SB	SBC	SB	SBC
	nmol/µL	nmol/µL	nmol/µL	nmol/µL
0	617.3 ± 0.0	302.5 ± 34.5	129.0 ± 78.1	39.1 ± 10.0
1	444.2 ± 68.7	2013.3 ± 1394.6	127.5 ± 95.0[b]	3074.1 ± 692.2[a]
2	829.1 ± 481.4	1636.1 ± 217.0	116.6 ± 35.6	145.8 ± 104.7
3	836.1 ± 565.6	1869.9 ± 203.6	120.6 ± 58.4	266.5 ± 237.9
4	433.9 ± 168.3	431.2 ± 198.9	111.7 ± 58.4	188.6 ± 236.2
5	646.9 ± 148.3	520.4 ± 123.0	165.8 ± 99.9	103.7 ± 49.2
6	506.6 ± 0.0	402.6 ± 97.7	410.2 ± 172.2	99.0 ± 31.0
7	432.2 ± 70.0	612.0 ± 300.7	144.9 ± 109.4	116.3 ± 44.1
8	392.1 ± 0.0	492.3 ± 281.2	231.4 ± 25.3	61.0 ± 48.7
9	452.4 ± 11.7	435.8 ± 251.3	202.1 ± 130.4	27.2 ± 5.4
10	834.8 ± 28.0	382.7 ± 82.9	206.8 ± 185.7	39.2 ± 11.4
11	361.2 ± 35.1	242.6 ± 75.6	153.2 ± 119.2	31.3 ± 18.3
12	457.9 ± 0.0	390.8 ± 143.4	169.8 ± 59.6	28.9 ± 4.1

TABLE 25. Concentrations (mean + SD, n=3) of the dietary non-essential amino acids, glutamic acid and glycine, in the HPV blood of channel catfish during a 12-h period after force-feeding of SB or SBC. Within ingredients, means in the same row with different letters are significantly different ($P \leq 0.05$).

Time in h	Glutamic Acid		Glycine	
	SB	SBC	SB	SBC
	nmol/μL	nmol/μL	nmol/μL	nmol/μL
0	102.6 ± 68.1	65.3 ± 29.1	322.2 ± 216.6	254.0 ± 4.8
1	94.1 ± 27.3[b]	3262.0 ± 958.0[a]	285.4 ± 44.6[b]	2321.4 ± 1695.6[a]
2	321.4 ± 72.5	209.9 ± 195.3	322.8 ± 149.4[b]	1620.7 ± 761.9[a]
3	132.7 ± 80.4	278.6 ± 220.8	422.7 ± 92.8	1139.1 ± 131.1
4	145.5 ± 68.7	279.0 ± 343.1	371.3 ± 49.6	391.6 ± 116.9
5	142.2 ± 24.3	185.7 ± 165.2	305.7 ± 81.6	490.0 ± 203.2
6	150.4 ± 27.6	155.3 ± 72.9	450.9 ± 288.5	381.1 ± 79.9
7	101.6 ± 5.6	208.9 ± 132.7	234.0 ± 31.7	536.8 ± 173.5
8	126.6 ± 2.5	60.6 ± 38.4	353.8 ± 167.2	338.1 ± 125.4
9	142.8 ± 116.3	66.4 ± 53.4	306.6 ± 97.3	323.5 ± 50.3
10	403.7 ± 9.1	74.3 ± 26.4	351.5 ± 49.4	439.8 ± 152.8
11	104.5 ± 45.6	74.9 ± 80.0	284.1 ± 47.3	255.3 ± 23.6
12	103.9 ± 13.5	59.5 ± 38.7	259.7 ± 149.9	244.7 ± 77.2

TABLE 26. Concentrations (mean + SD, n=3) of the dietary non-essential amino acids, serine and tyrosine, in the HPV blood of channel catfish during a 12-h period after force-feeding of SB or SBC. Within ingredients, means in the same row with different letters are significantly different ($P \leq 0.05$).

Time in h	Serine		Tyrosine	
	SB	SBC	SB	SBC
	nmol/μL	nmol/μL	nmol/μL	nmol/μL
0	483.4 ± 402.4	256.3 ± 19.1	129.0 ± 78.1	122.3 ± 29.6
1	331.1 ± 77.9[b]	641.1 ± 90.9[a]	164.9 ± 42.1	297.4 ± 63.8
2	220.8 ± 10.4	1079.6 ± 290.6	235.6 ± 173.5	296.0 ± 74.4
3	429.9 ± 176.6	859.6 ± 106.0	236.0 ± 143.8	526.5 ± 216.9
4	384.9 ± 120.3	390.9 ± 78.1	163.4 ± 40.1	171.2 ± 14.8
5	353.8 ± 39.5	376.8 ± 15.7	219.2 ± 34.6	216.1 ± 74.3
6	630.4 ± 459.6	376.8 ± 88.8	410.2 ± 172.2	245.0 ± 72.6
7	319.1 ± 66.5	530.1 ± 93.7	245.4 ± 32.6	424.7 ± 137.0
8	500.8 ± 141.1	343.7 ± 141.2	231.4 ± 25.3	213.1 ± 101.8
9	406.9 ± 55.1	328.3 ± 36.0	249.9 ± 71.3	208.1 ± 17.2
10	557.2 ± 138.9	470.8 ± 132.8	307.7 ± 42.9	170.5 ± 65.6
11	374.3 ± 137.6	249.1 ± 6.9	214.8 ± 51.9	164.3 ± 82.6
12	393.4 ± 170.7	287.8 ± 93.6	169.8 ± 59.6	161.5 ± 42.6

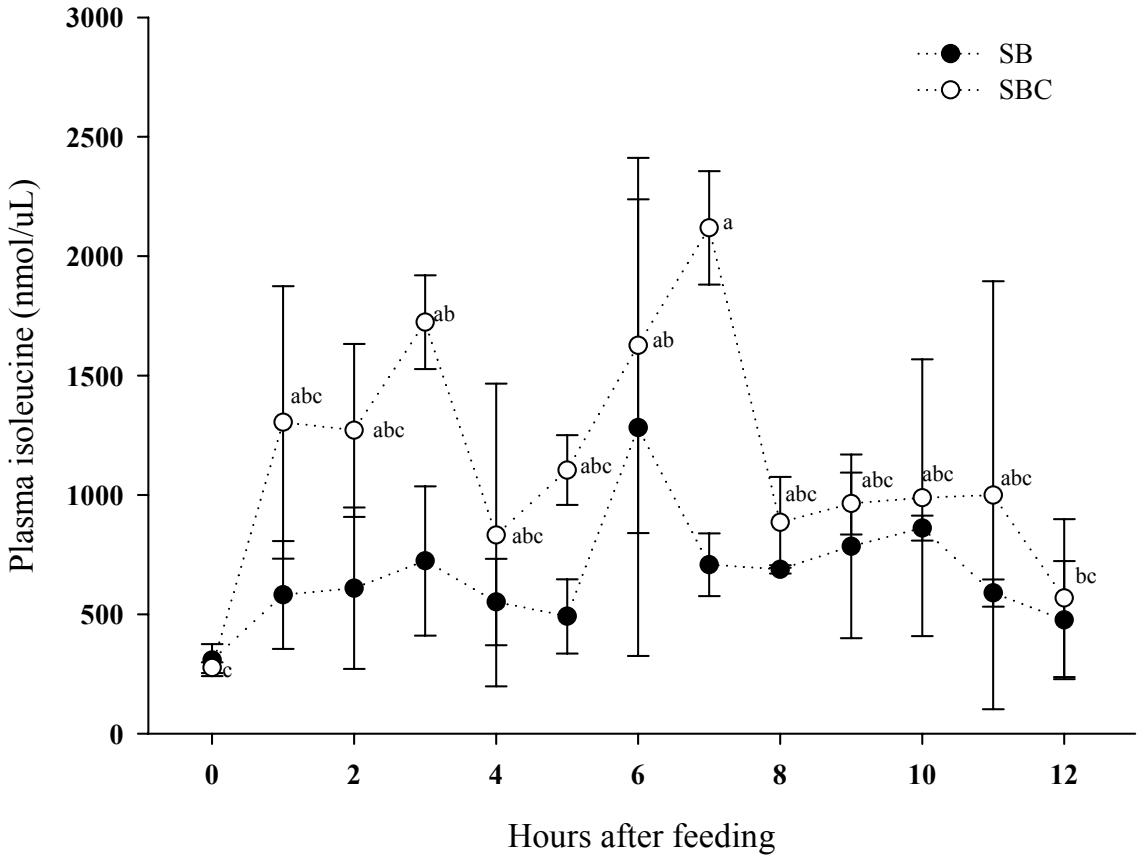

FIGURE 37. Concentrations of isoleucine in the HPV blood of channel catfish during the 12-h period after force-feeding of SB or SBC. There were no significant differences between or within treatments. Points on the SBC line with different letters are significant different. There were no significant differences within SB treatment.

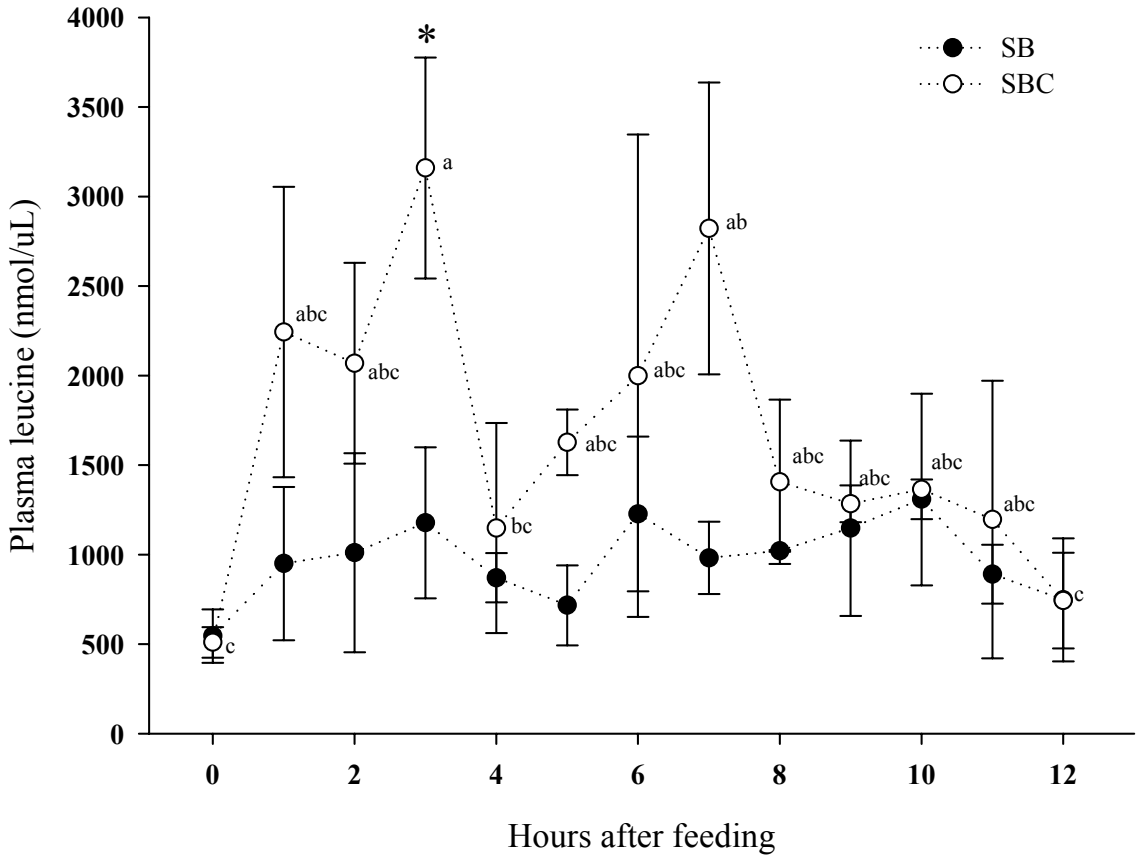

FIGURE 38. Concentrations of leucine in the HPV blood of channel catfish during the 12-h period after force-feeding of SB or SBC. An asterisk indicates a significant difference between treatments. Points on the SBC line with different letters are significantly different. There were no significant differences within SB treatment.

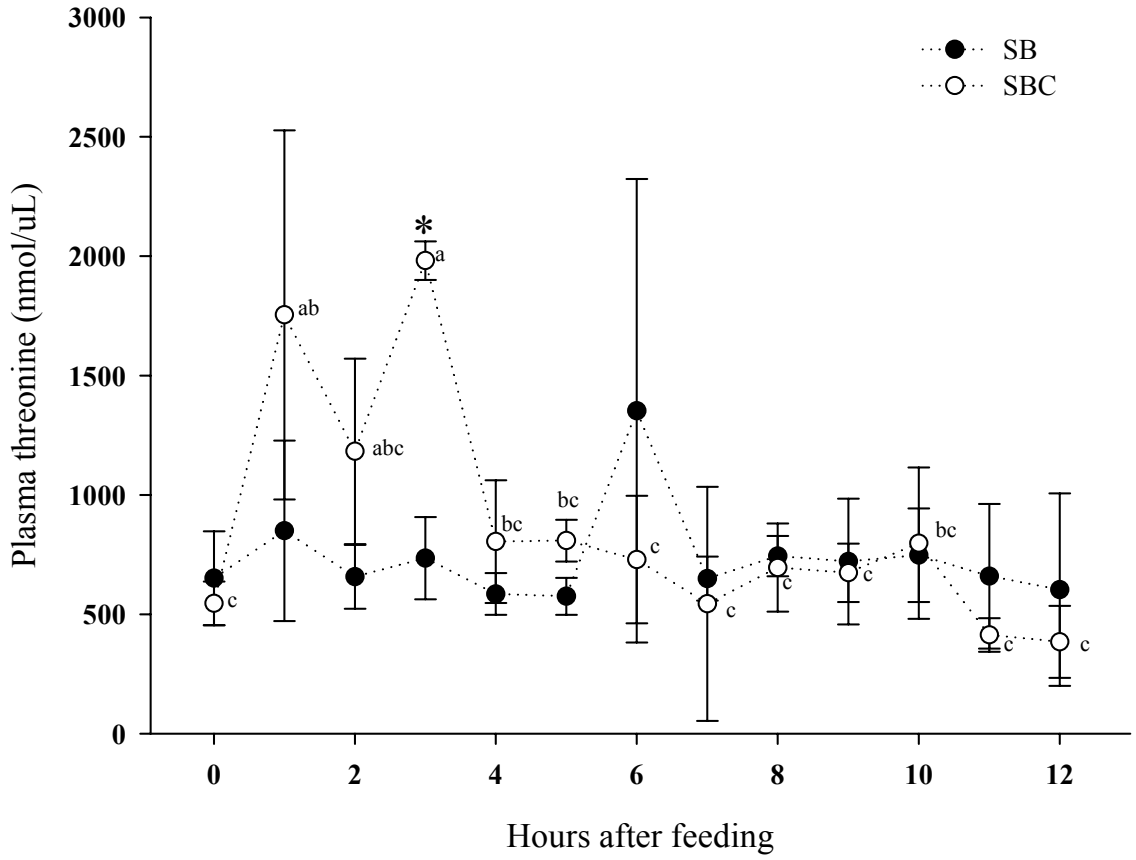

FIGURE 39. Concentrations of threonine in the HPV blood of channel catfish during the 12-h period after force-feeding of SB or SBC. An asterisk indicates a significant difference between treatments. Points on the SBC line with different letters are significantly different. There were no significant differences within SB treatment.

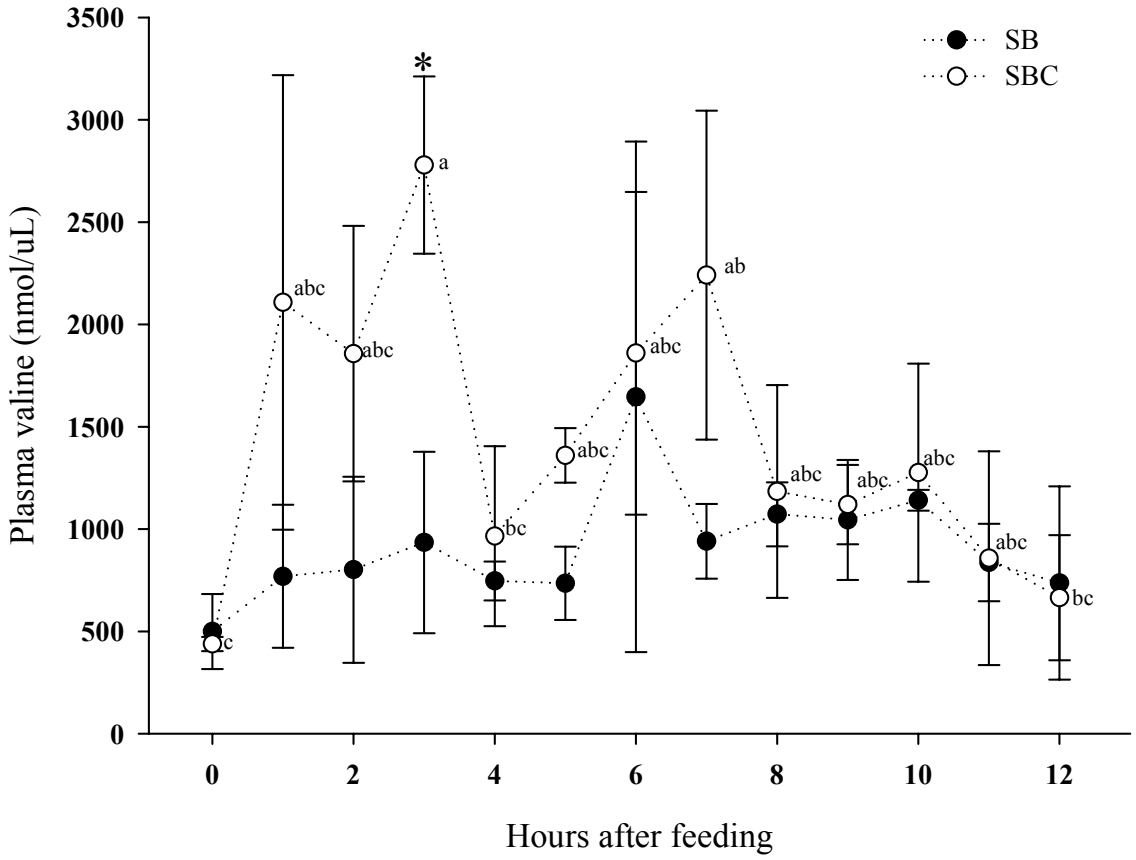

FIGURE 40. Concentrations of valine in the HPV blood of channel catfish during the 12-h period after force-feeding of SB or SBC. An asterisk indicates a significant difference between treatments. Points on the SBC line with different letters are significant different. There were no significant differences within SB treatment.

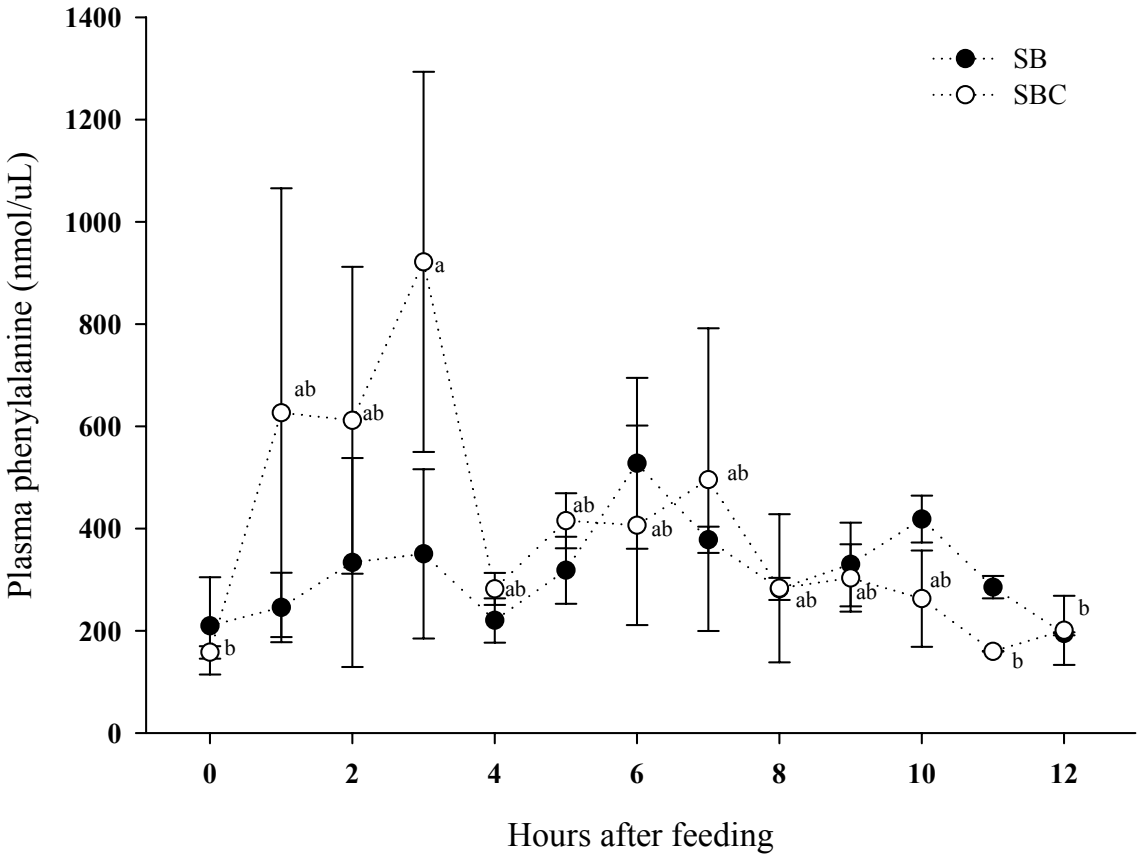

FIGURE 41. Concentrations of phenylalanine in the HPV blood of channel catfish during the 12-h period after force-feeding of SB or SBC. There were no significant differences between or within treatments. Points on the SBC line with different letters are significantly different. There were no significant differences within SB treatment.

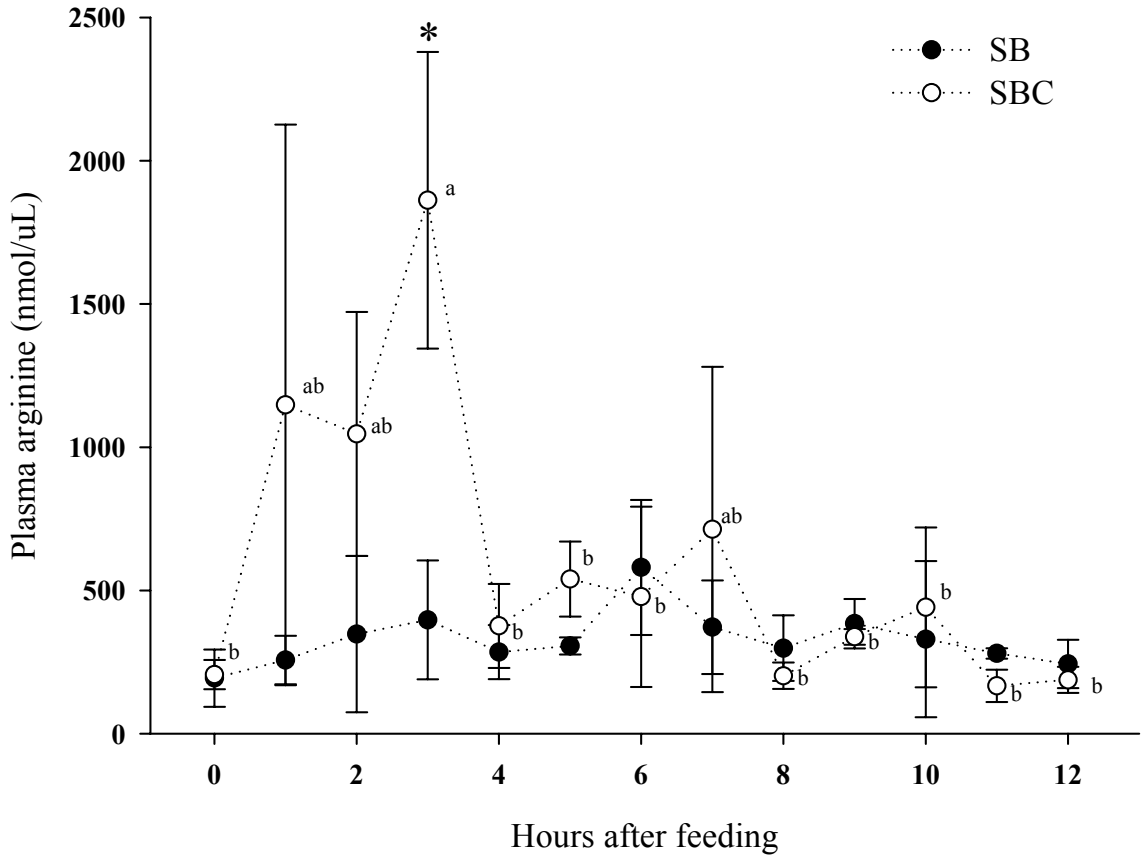

FIGURE 42. Concentrations of arginine in the HPV blood of channel catfish during the 12-h period after force-feeding of SB or SBC. An asterisk indicates a significant difference between treatments. Points on the SBC line with different letters are significantly different. There were no significant differences within SB treatment.

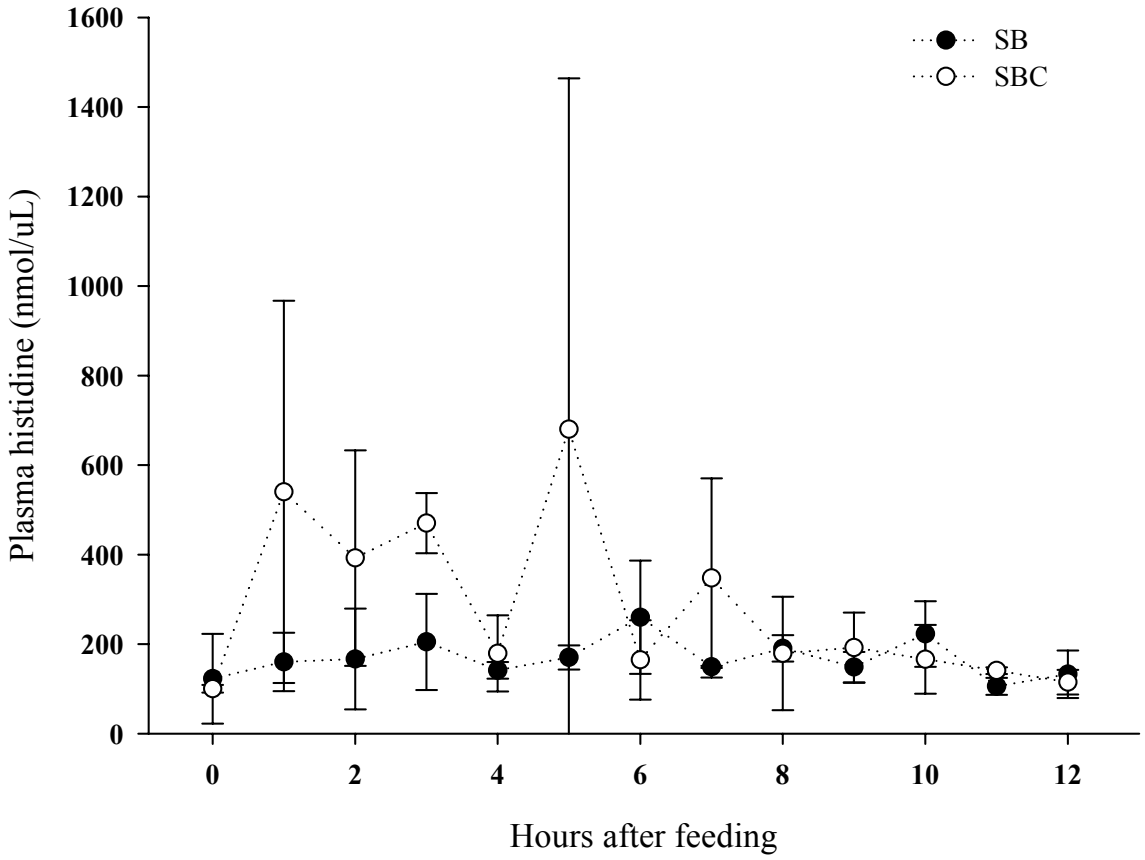

FIGURE 43. Concentrations of histidine in the HPV blood of channel catfish during the 12-h period after force-feeding of SB or SBC. There were no significant differences between or within treatments.

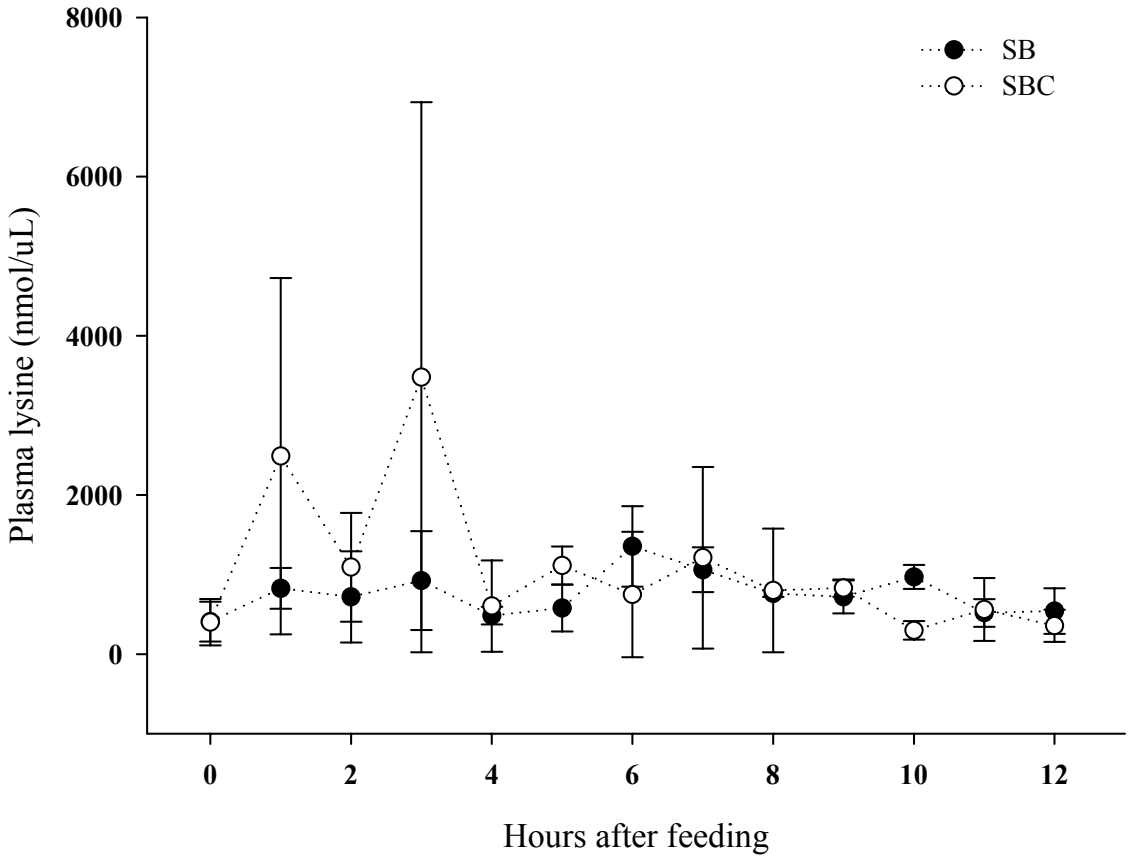

FIGURE 44. Concentrations of lysine in the HPV blood of channel catfish during the 12-h period after force-feeding of SB or SBC. There were no significant differences between or within treatments.

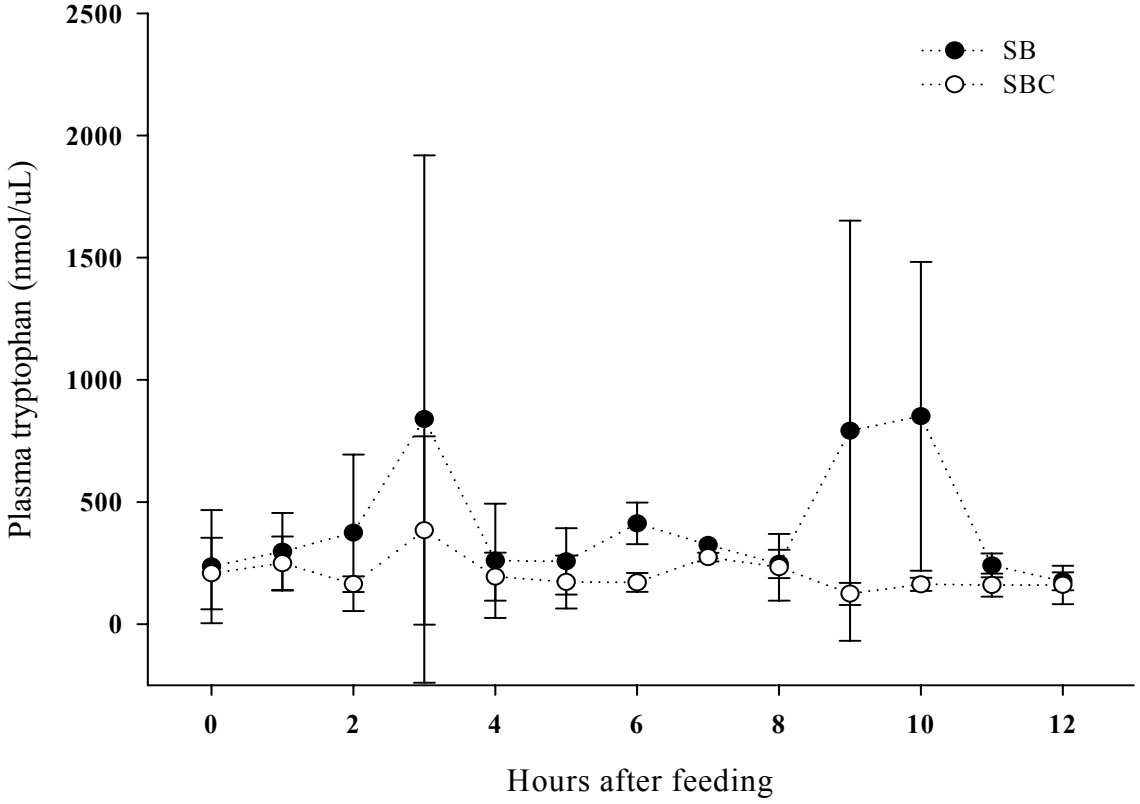

FIGURE 45. Concentrations of tryptophan in the HPV blood of channel catfish during the 12-h period after force-feeding of SB or SBC. There were no significant differences between or within treatments.

TABLE 27. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids alanine and aspartic acid, in the HPV blood of channel catfish during a 12-h period after force-feeding of SB or SBC. Within ingredients, means in the same column with different letters are significantly different ($P \leq 0.05$).

Time in h	Alanine		Aspartic acid	
	SB	SBC	SB	SBC
	nmol/µL	nmol/µL	nmol/µL	nmol/µL
0	617.3 ± 0.0	302.5 ± 34.5^{c}	129.0 ± 78.1	39.1 ± 10.0^{b}
1	444.2 ± 68.7	2013.3 ± 1394.6^{a}	127.5 ± 95.0	3074.1 ± 692.2^{a}
2	829.1 ± 481.4	1636.1 ± 217.0^{ab}	116.6 ± 35.6	145.8 ± 104.7^{ab}
3	836.1 ± 565.6	1869.9 ± 203.6^{a}	120.6 ± 58.4	266.5 ± 237.9^{ab}
4	433.9 ± 168.3	431.2 ± 198.9^{c}	111.7 ± 58.4	188.6 ± 236.2^{b}
5	646.9 ± 148.3	520.4 ± 123.0b^{c}	165.8 ± 99.9	103.7 ± 49.2^{ab}
6	506.6 ± 0.0	402.6 ± 97.7^{c}	410.2 ± 172.2	99.0 ± 31.0^{ab}
7	432.2 ± 70.0	612.0 ± 300.7^{bc}	144.9 ± 109.4	116.3 ± 44.1^{b}
8	392.1 ± 0.0	492.3 ± 281.2^{bc}	231.4 ± 25.3	61.0 ± 48.7^{b}
9	452.4 ± 11.7	435.8 ± 251.3^{c}	202.1 ± 130.4	27.2 ± 5.4^{b}
10	834.8 ± 28.0	382.7 ± 82.9^{c}	206.8 ± 185.7	39.2 ± 11.4^{b}
11	361.2 ± 35.1	242.6 ± 75.6^{c}	153.2 ± 119.2	31.3 ± 18.3^{b}
12	457.9 ± 0.0	390.8 ± 143.4^{c}	169.8 ± 59.6	28.9 ± 4.1^{b}

TABLE 28. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, glutamic acid and glycine, in the HPV blood of channel catfish during a 12-h period after force-feeding of SB or SBC. Within ingredients, means in the same column with different letters are significantly different ($P \leq 0.05$).

Time in h	Glutamic Acid		Glycine	
	SB	SBC	SB	SBC
	nmol/μL	nmol/μL	nmol/μL	nmol/μL
0	102.6 ± 68.1^{c}	65.3 ± 29.1^{b}	322.2 ± 216.6	254.0 ± 4.8^{c}
1	94.1 ± 27.3^{c}	3262.0 ± 958.0^{a}	285.4 ± 44.6	2321.4 ± 1695.6^{a}
2	321.4 ± 72.5^{ab}	209.9 ± 195.3^{b}	322.8 ± 149.4	1620.7 ± 761.9^{ab}
3	132.7 ± 80.4^{bc}	278.6 ± 220.8^{b}	422.7 ± 92.8	1139.1 ± 131.1^{abc}
4	145.5 ± 68.7^{bc}	279.0 ± 343.1^{b}	371.3 ± 49.6	391.6 ± 116.9^{c}
5	142.2 ± 24.3^{c}	185.7 ± 165.2^{b}	305.7 ± 81.6	490.0 ± 203.2^{bc}
6	150.4 ± 27.6^{bc}	155.3 ± 72.9^{b}	450.9 ± 288.5	81.1 ± 79.9^{c}
7	101.6 ± 5.6^{bc}	208.9 ± 132.7^{b}	234.0 ± 31.7	536.8 ± 173.5^{bc}
8	126.6 ± 2.5^{bc}	60.6 ± 38.4^{b}	353.8 ± 167.2	338.1 ± 125.4^{c}
9	142.8 ± 116.3^{c}	66.4 ± 53.4^{b}	306.6 ± 97.3	323.5 ± 50.3^{c}
10	403.7 ± 9.1^{a}	74.3 ± 26.4^{b}	351.5 ± 49.4	439.8 ± 152.8^{c}
11	104.5 ± 45.6^{c}	74.9 ± 80.0^{b}	284.1 ± 47.3	255.3 ± 23.6^{c}
12	103.9 ± 13.5^{c}	59.5 ± 38.7^{b}	259.7 ± 149.9	244.7 ± 77.2^{c}

TABLE 29. Concentrations (mean + SD, n=3) of the dietary non-essential amino acids, serine and tyrosine, in the HPV blood of channel catfish during a 12-h period after force-feeding of SB or SBC. Within ingredients, means in the same column with different letters are significantly different ($P \leq 0.05$).

Time in h	Serine		Tyrosine	
	SB	SBC	SB	SBC
	nmol/μL	nmol/μL	nmol/μL	nmol/μL
0	483.4 ± 402.4	256.3 ± 19.1^{e}	129.0 ± 78.1	122.3 ± 29.6^{c}
1	331.1 ± 77.9	641.1 ± 90.9^{bc}	164.9 ± 42.1	297.4 ± 63.8^{abc}
2	220.8 ± 10.4	1079.6 ± 290.6^{a}	235.6 ± 173.5	296.0 ± 74.4^{abc}
3	429.9 ± 176.6	859.6 ± 106.0^{b}	236.0 ± 143.8	526.5 ± 216.9^{a}
4	384.9 ± 120.3	390.9 ± 78.1^{bcde}	163.4 ± 40.1	171.2 ± 14.8^{bc}
5	353.8 ± 39.5	376.8 ± 15.7^{cde}	219.2 ± 34.6	216.1 ± 74.3^{bc}
6	630.4 ± 459.6	376.8 ± 88.8^{cde}	410.2 ± 172.2	245.0 ± 72.6^{bc}
7	319.1 ± 66.5	530.1 ± 93.7^{bcd}	245.4 ± 32.6	424.7 ± 137.0^{ab}
8	500.8 ± 141.1	343.7 ± 141.2^{de}	231.4 ± 25.3	213.1 ± 101.8^{bc}
9	406.9 ± 55.1	328.3 ± 36.0^{de}	249.9 ± 71.3	208.1 ± 17.2^{bc}
10	557.2 ± 138.9	470.8 ± 132.8^{bcde}	307.7 ± 42.9	170.5 ± 65.6^{bc}
11	374.3 ± 137.6	249.1 ± 6.9^{de}	214.8 ± 51.9	164.3 ± 82.6^{bc}
12	393.4 ± 170.7	287.8 ± 93.6^{e}	169.8 ± 59.6	161.5 ± 42.6^{bc}

TABLE 30. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, alanine and aspartic acid, in the HPV blood of channel catfish during a 12-h period after force-feeding of WM or WMC. No significant differences were observed between treatments.

Time in h	Alanine		Aspartic acid	
	WM	WMC	WM	WMC
	nmol/µl	nmol/µl	nmol/µl	nmol/µl
0	177.6 ± 43.0	224.5 ± 33.1	45.6 ± 24.3	34.2 ± 8.4
1	801.2 ± 270.9	502.5 ± 208.7	52.4 ± 5.0	47.5 ± 33.5
2	1028.9 ± 432.5	637.8 ± 466.7	101.9 ± 68.4	42.4 ± 14.1
3	514.2 ± 215.1	405.0 ± 85.6	52.8 ± 40.3	32.6 ± 17.0
4	546.2 ± 100.4	474.2 ± 46.3	52.8 ± 31.2	33.9 ± 6.1
5	536.6 ± 98.3	525.0 ± 136.4	32.7 ± 14.6	33.3 ± 4.8
6	406.7 ± 27.4	303.5 ± 19.4	52.6 ± 24.3	30.8 ± 11.1
7	807.8 ± 148.5	489.1 ± 238.9	58.3 ± 9.8	41.1 ±11.6
8	461.0 ± 87.1	338.7 ± 51.3	35.4 ± 11.4	35.8 ± 9.6
9	765.0 ± 539.3	435.8 ± 357.4	56.8 ± 24.5	25.4 ± 7.7
10	378.4 ± 224.9	385.6 ± 157.2	44.7 ± 30.6	28.7 ± 5.9
11	399.0 ± 0.7	445.4 ± 145.2	28.3 ± 9.5	27.8 ± 2.2
12	400.9 ± 44.0	518.2 ± 45.7	36.3 ± 26.6	28.3 ± 7.1

TABLE 31. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, glutamic acid and glycine, in the HPV blood of channel catfish during a 12-h period after force-feeding of WM or WMC. No significant differences were observed between treatments.

Time in h	Glutamic acid		Glycine	
	WM	WMC	WM	WMC
	nmol/µl	nmol/µl	nmol/µl	nmol/µl
0	72.3 ± 3.6	69.1 ± 17.9	261.0 ± 99.4	284.3 ± 6.1
1	229.5 ± 96.4	162.0 ± 124.7	501.4 ± 127.9	351.4 ± 169.1
2	251.0 ± 45.8	128.2 ± 15.3	491.5 ± 86.9	482.1 ± 215.6
3	117.8 ± 21.0	98.7 ± 33.7	512.5 ± 357.2	615.9 ± 458.0
4	178.5 ± 135.0	93.8 ± 49.9	386.6 ± 110.9	314.9 ± 63.6
5	93.6 ± 34.0	102.4 ± 28.7	356.7 ± 123.7	427.0 ± 178.0
6	87.3 ± 18.4	75.9 ± 10.3	375.7 ± 155.1	314.4 ± 138.3
7	176.9 ± 119.7	143.0 ± 84.6	570.1 ± 256.2	395.1 ± 71.3
8	151.0 ± 124.6	146.2 ± 48.1	348.4 ± 149.6	340.9 ± 176.1
9	177.8 ± 121.5	73.8 ± 31.0	608.4 ± 307.1	263.8 ± 108.7
10	77.6 ± 31.3	93.4 ± 39.5	313.3 ± 162.4	279.9 ± 56.1
11	85.2 ± 21.5	125.7 ± 40.0	318.8 ± 86.4	261.2 ± 56.0
12	93.9 ± 47.4	93.5 ± 50.2	310.0 ± 59.1	339.8 ± 68.1

TABLE 32. Concentrations (mean ± SD, n=3) of the dietary non-essential amino acids, serine and tyrosine, in the HPV blood of channel catfish during a 12-h period after force-feeding of WM or WMC. No significant differences were observed between treatments.

Time in h	Serine		Tyrosine	
	WM	WMC	WM	WMC
	nmol/μL	nmol/μL	nmol/μL	nmol/μL
0	261.0 ± 99.4	284.3 ± 6.1	76.5 ± 33.0	122.8 ± 18.8
1	501.4 ± 127.9	351.4 ± 169.1	284.3 ± 66.8	183.5 ± 66.9
2	491.5 ± 86.9	482.1 ± 215.6	256.2 ± 78.0	185.8 ± 65.8
3	512.5 ± 357.2	615.9 ± 458.0	196.5 ± 64.8	153.4 ± 83.0
4	386.6 ± 110.9	314.9 ± 63.6	202.4 ± 24.8	143.0 ± 75.5
5	356.7 ± 123.7	427.0 ± 178.0	163.2 ± 17.0	206.1 ± 69.5
6	375.7 ± 155.1	314.4 ± 138.3	260.8 ± 77.1	198.1 ± 18.3
7	570.1 ± 256.2	395.1 ± 71.3	326.7 ± 238.8	184.7 ± 42.1
8	348.4 ± 149.6	340.9 ± 176.1	187.8 ± 53.3	142.7 ± 39.9
9	608.4 ± 307.1	263.8 ± 108.7	292.8 ± 239.0	107.1 ± 42.1
10	313.3 ± 162.4	279.9 ± 56.1	131.3 ± 59.9	120.1 ± 19.4
11	318.8 ± 86.4	261.2 ± 56.0	182.4 ± 34.1	112.4 ± 35.0
12	310.0 ± 59.1	339.8 ± 68.1	180.5 ± 46.3	133.6 ± 74.4

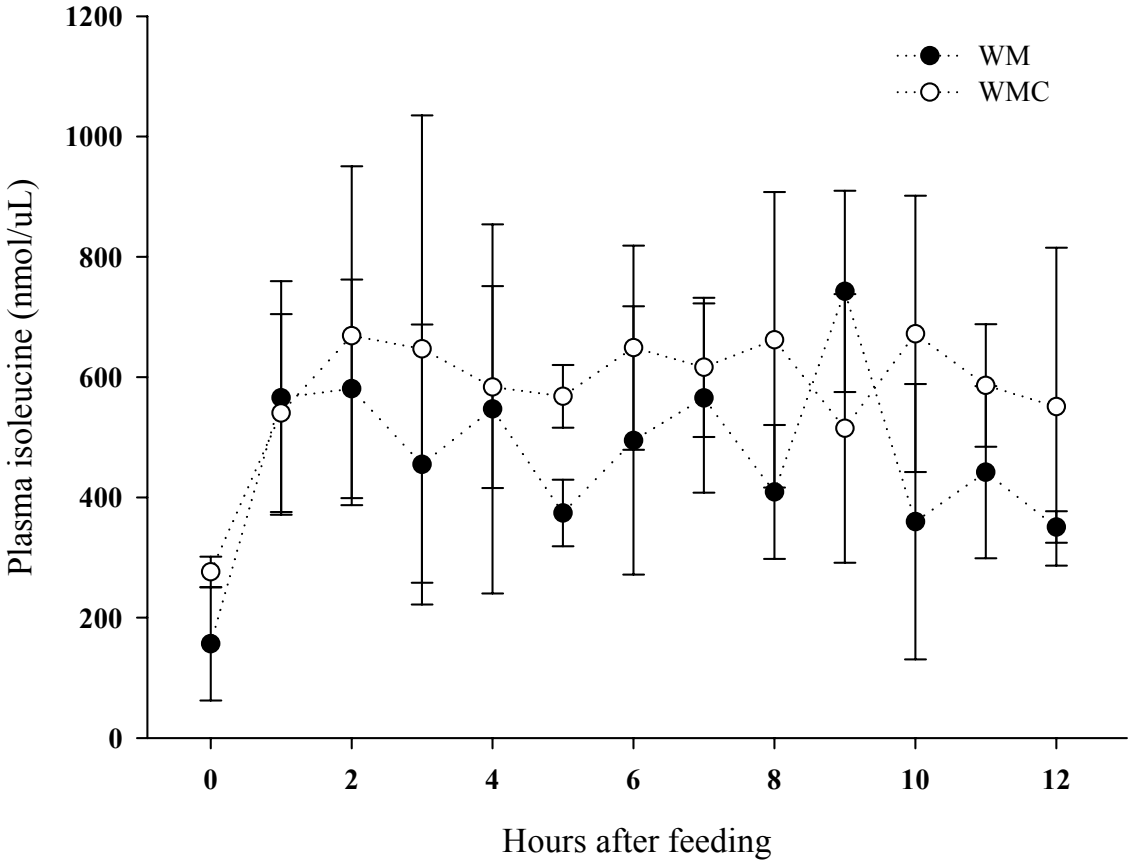

FIGURE 46. Concentrations of isoleucine in the HPV blood of channel catfish during the 12-h period after force-feeding of WM or WMC. There were no significant differences between or within treatments.

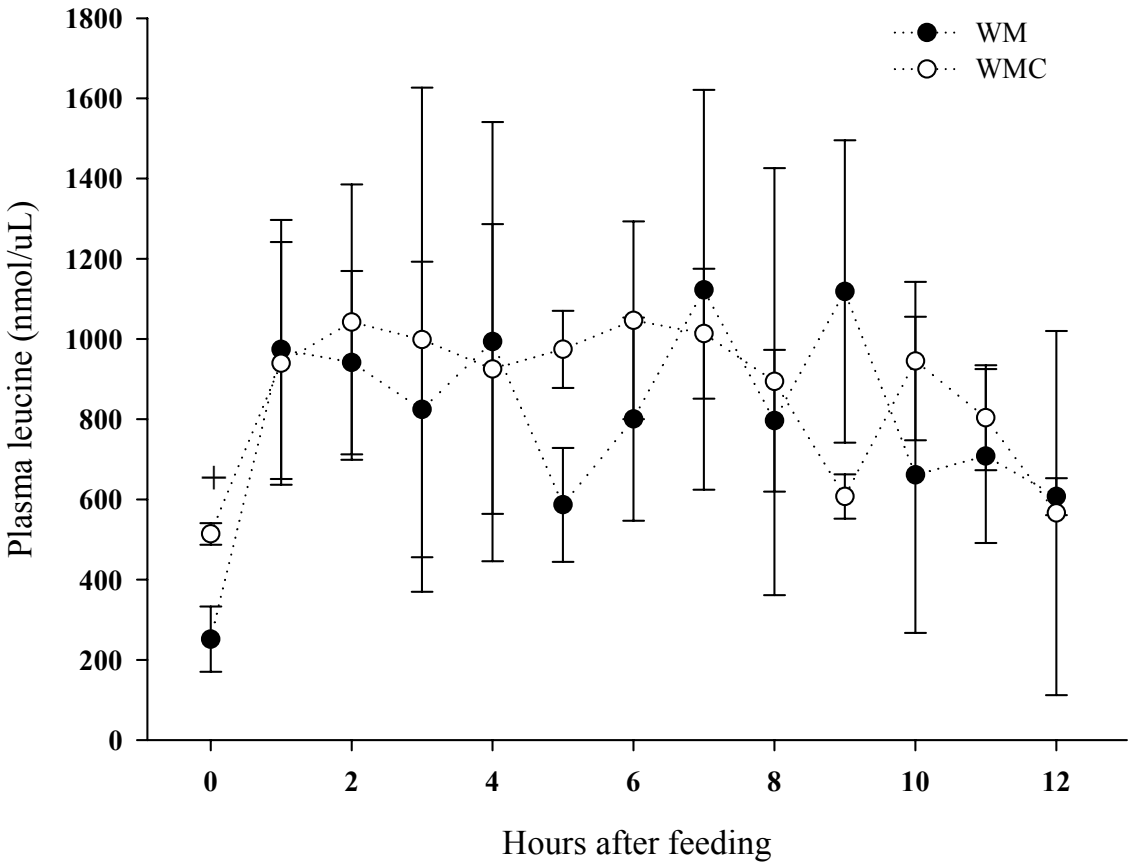

FIGURE 47. Concentrations of leucine in the HPV blood of channel catfish during the 12-h period after force-feeding of WM or WMC. There were no significant differences between or within treatments.

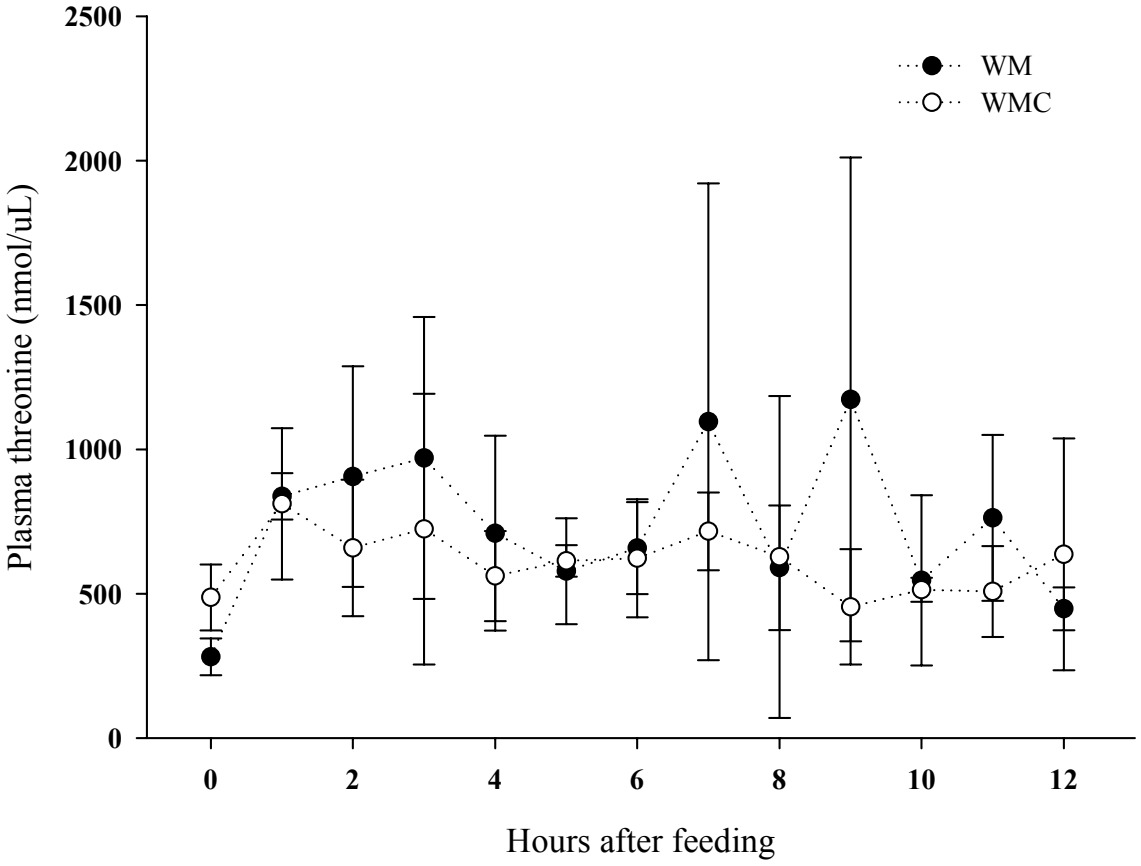

FIGURE 48. Concentrations of threonine in the HPV blood of channel catfish during the 12-h period after force-feeding of WM or WMC. There were no significant differences between or within treatments.

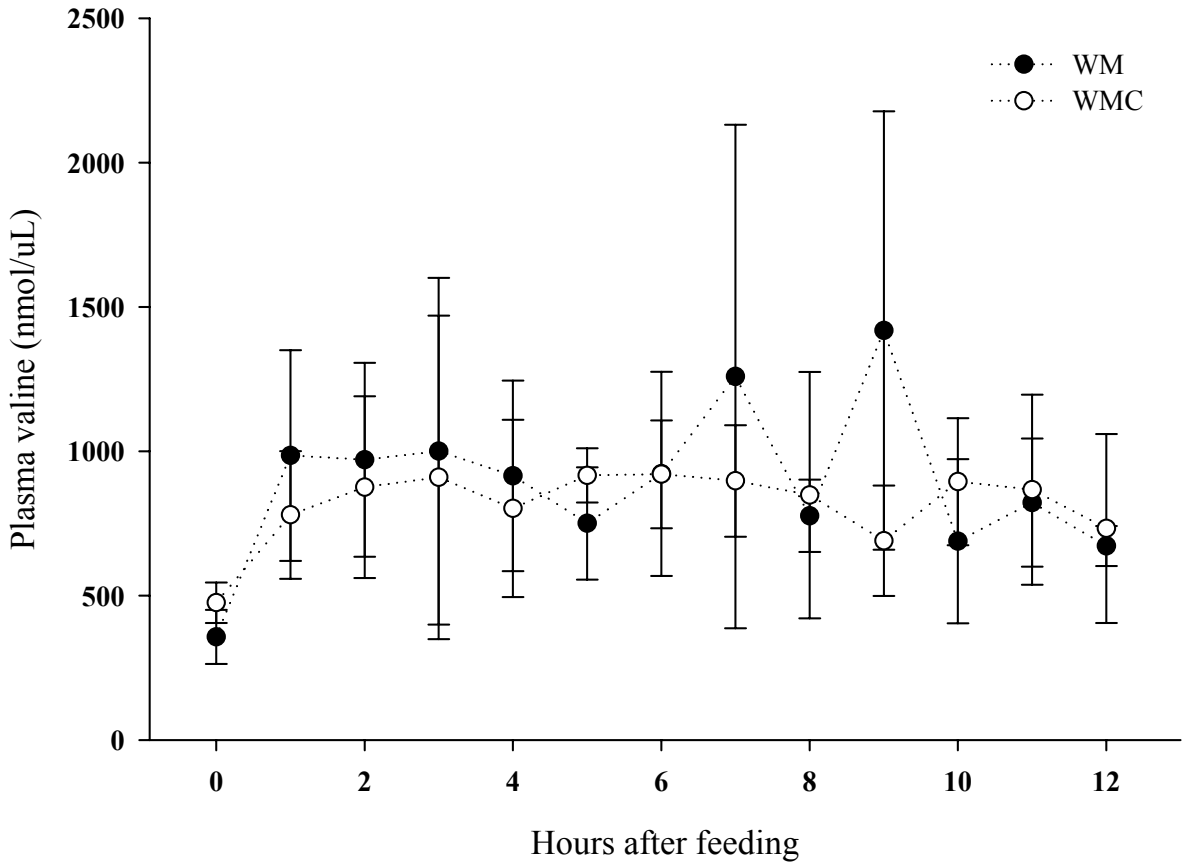

FIGURE 49. Concentrations of valine in the HPV blood of channel catfish during the 12-h period after force-feeding of WM or WMC. There were no significant differences between treatments.

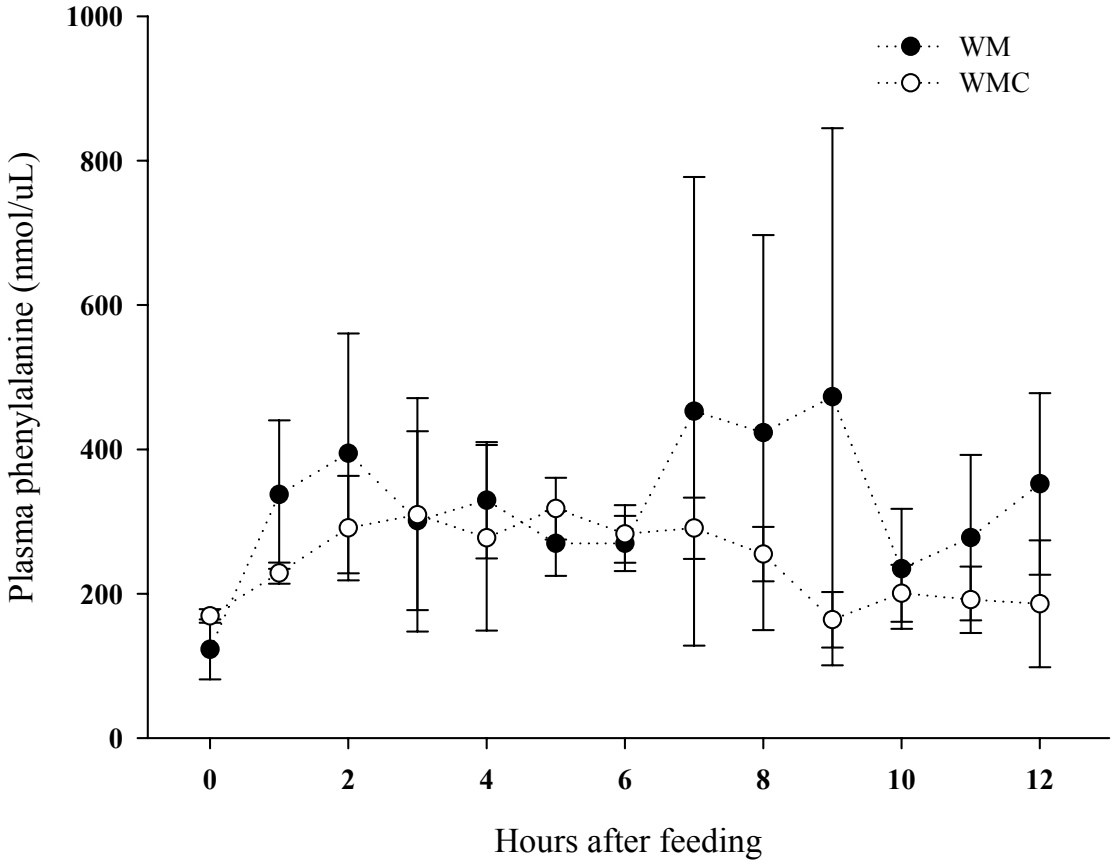

FIGURE 50. Concentrations of phenylalanine in the HPV blood of channel catfish during the 12-h period after force-feeding of WM or WMC. There were no significant differences between or within treatments.

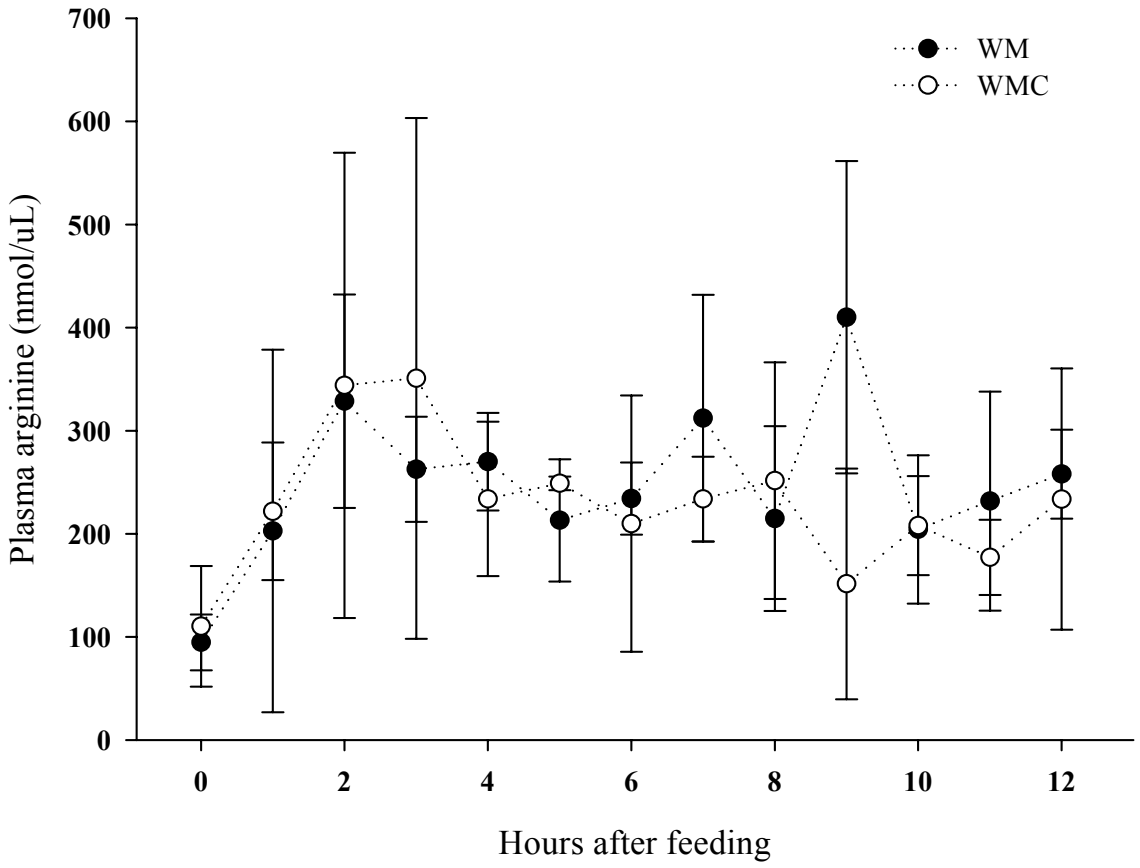

FIGURE 51. Concentrations of arginine in the HPV blood of channel catfish during the 12-h period after force-feeding of WM or WMC. There were no significant differences between or within treatments.

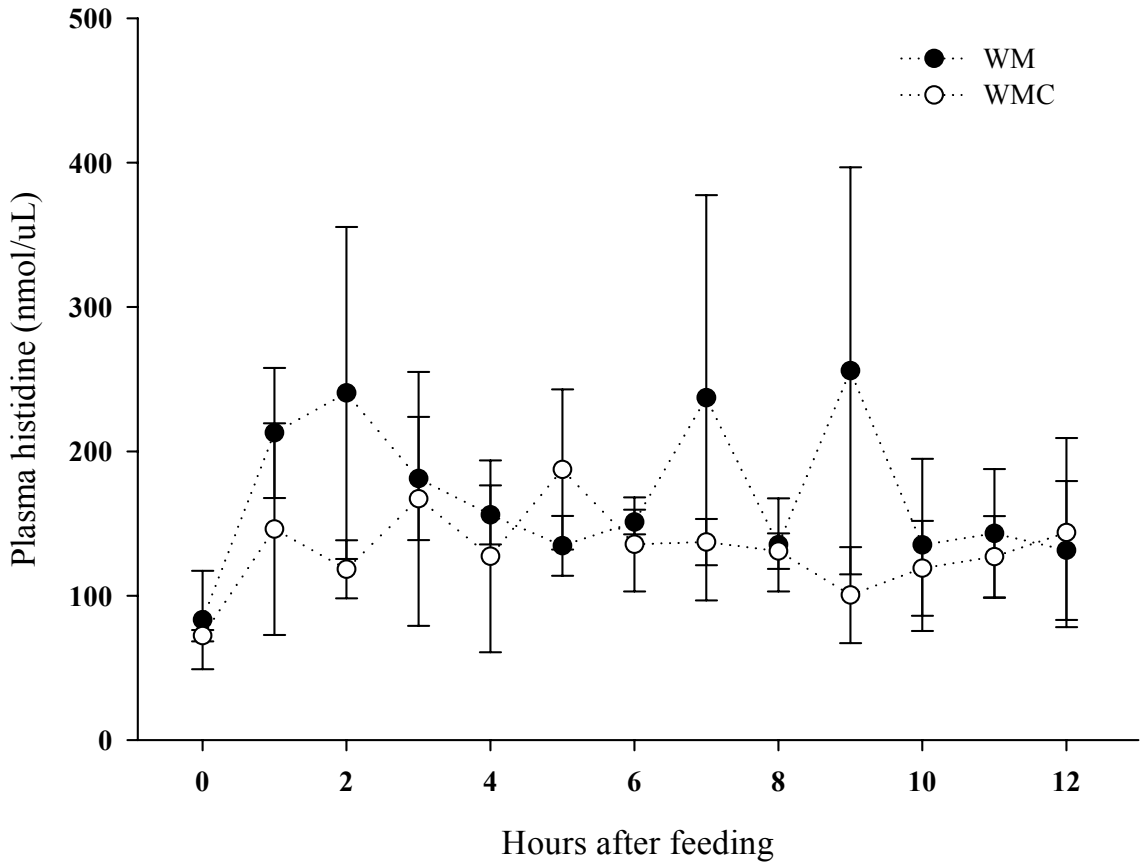

FIGURE 52. Concentrations of histidine in the HPV blood of channel catfish during the 12-h period after force-feeding of WM or WMC. There were no significant differences between or within treatments.

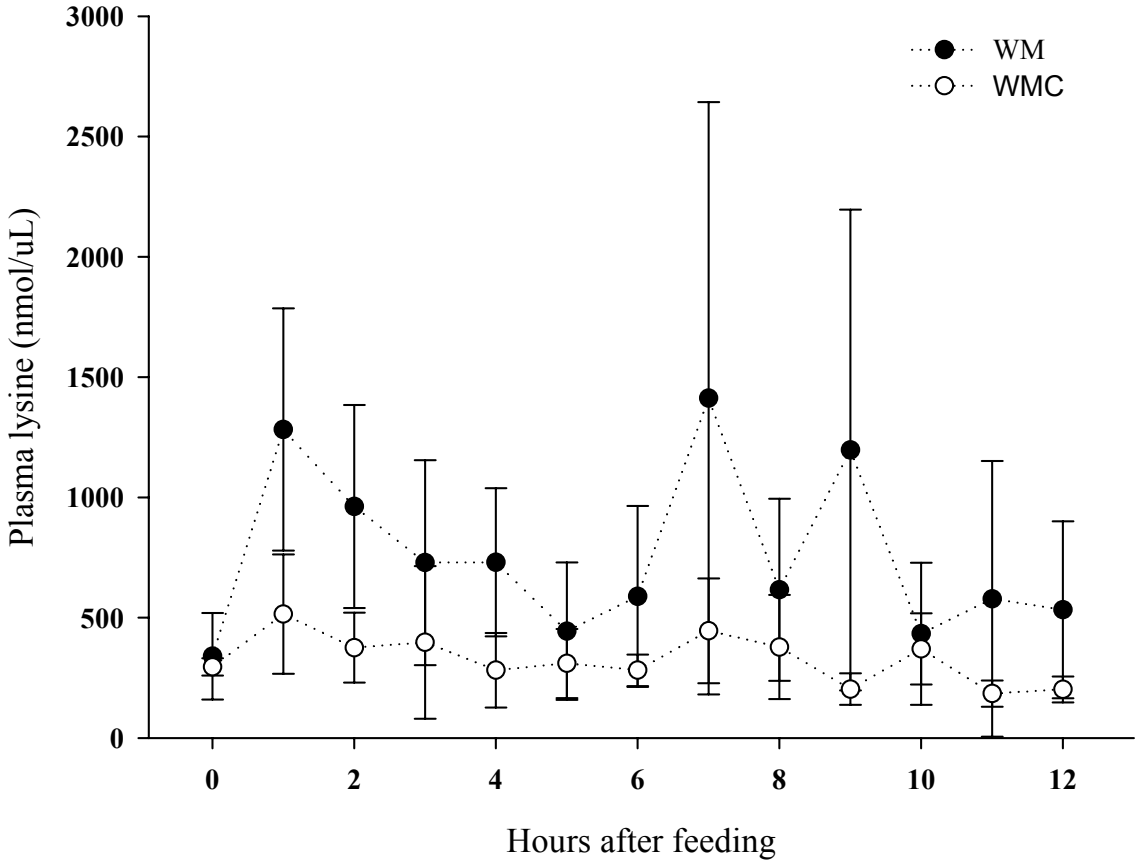

FIGURE 53. Concentrations of lysine in the HPV blood of channel catfish during the 12-h period after force-feeding of WM or WMC. There were no significant differences between or within treatments.

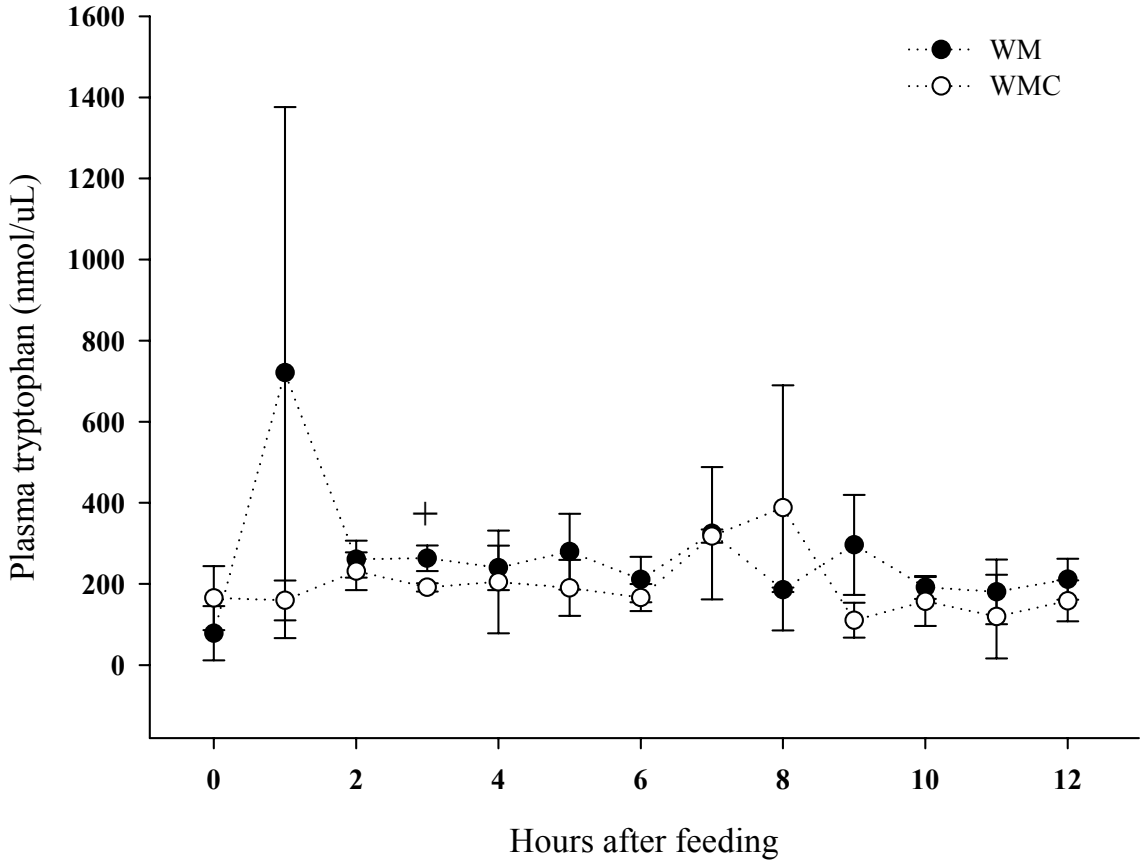

FIGURE 54. Concentrations of tryptophan in the HPV blood of channel catfish during the 12-h period after force-feeding of WM or WMC. There were no significant differences between or within treatments.

CHAPTER 4

DISCUSSIONS

Feed cost is a major component of the cost of production in many intensive fish farming operations. Increased competition in the feed manufacturing and fish farming industries, and the rising cost of premium protein supplements, has led to increased use of less expensive ingredients in fish feeds whenever possible. As larger quantities of amino-acid deficient feedstuffs are used in prepared diets, the need to supplement these diets with purified amino acids will increase. However, the efficacy of supplementing fish diets with purified amino acids is unclear.

In an effort to measure the effectiveness of amino acid supplementation of catfish diets, the research presented here identified differences in the digestibility and rates of absorption (i.e., temporal concentrations in the HPV blood) of amino acids obtained from intact proteins or mixtures of purified (crystalline) amino acids designed to match the amino acid composition of intact protein in a selection of feed ingredients with diverse chemical compositions. The ADCs of 15 amino acids in six common feed ingredients — blood meal, corn meal, menhaden fish meal, meat and bone meal, soybean meal, and wheat middlings — were determined, as well as the ADCs of those same amino acids in mixtures of purified amino acids that duplicated the composition of each practical ingredient in proximate composition as well as amino acid content. Concentrations of amino acids in the HPV blood of channel catfish fed intact protein or a mixture of purified amino acids also were measured up to 12 h after feeding.

4.1 Digestibility

The ingredients selected for testing were chosen because of their chemical compositions and the manner in which they are used in channel catfish diets. Blood meal (80% crude protein,

hereafter referred to as CP; Dale and Batal 2004), menhaden fish meal (62% CP; Dale and Batal 2004), meat and bone meal (50% CP; Dale and Batal 2004), and soybean meal (48% CP; Dale and Batal 2004) are the primary protein supplements in catfish feeds. Soybean meal usually composes about half of the diet by weight. Fish meal, which used to be the primary animal protein supplement in catfish diets (4-10% by weight), has been replaced in recent years with a mixture of meat and bone meal and blood meal that provides an amino acid profile similar to fish meal. Fish meal, however, is still used in large quantity in diets for carnivorous fishes (e.g., Atlantic salmon). The eventual replacement of fish meal in diets for these species with plant protein supplements is a primary research objective of nutritionists working with carnivorous fishes.

Corn (8% CP; Dale and Batal 2004) is added to catfish diets as a nutritive binder, usually at a level of about one-quarter to one-third of diet weight. Its high starch content helps bind the diet's ingredients and contributes to the excellent water stability of the extruded (floating) pellets fed to catfish. Corn also provides a small amount of protein and some energy to the fish. Like corn, wheat middlings (15% CP; Dale and Batal 2004) is added to catfish diets (10-15% by weight) primarily for its binding properties. It also adds a small amount of protein and energy to a typical catfish diet.

Proteins in most commonly used feedstuffs are highly digestible to fish (National Research Council 1993). Indeed, results of this study indicated that intact proteins from the animal and plant products tested were well digested by channel catfish, even though these products composed 99% of the diet. There were few statistically significant differences between the ADCs of individual amino acids regardless of the form (i.e., intact protein or purified mixture) in which they were provided (Table 30). In most cases, the digestibility of amino acids

in the practical feed ingredient was not significantly different from the digestibility of amino acids provided in a mixture of purified compounds. This general lack of difference in amino acid ADCs could have been related to the well-established ability of fishes to effectively digest protein from diverse sources, many of which are not natural fish foods. The efficiency with which channel catfish digest dietary protein ranges from a low of about 12% for alfalfa meal up to 97% for corn grain and soybean meal (Tucker and Robinson 1990, Wilson 1991). The digestibility of protein in most of the dozen or so ingredients used in catfish diets typically ranges from 60% to well over 90%, and the availability of the amino acids in these feedstuffs is often in excess of 80% for channel catfish (Tucker and Robinson 1990, Wilson 1991).

Because the proteins in the selected ingredients were highly digestible (i.e., published ADCs are: blood meal, 74%; corn meal, 97%; menhaden fish meal, 85%; meat and bone meal, 82%; soybean meal, 97%; and wheat middlings, 92%; Tucker and Robinson 1990, Wilson 1991), it is possible that the release of amino acids from these ingredients by normal digestive processes during the 6-h period between force-feeding and fecal collection could have produced digestibility coefficients that were not significantly different from the coefficients produced by the artificial ingredients that contained the purified amino acid mixtures. However, when differences between ADCs did occur for individual amino acids, higher ADCs usually were associated with the purified amino acid mixtures. Also, in most (four of six) cases the average ADC of all the amino acids in an ingredient was significantly higher when the amino acids were provided in a purified mixture rather than in intact proteins. Ingredients with the highest protein content — blood meal (86% CP) and fish meal (61% CP) — had the most significant differences in ADCs between treatments, with all of the higher ADCs occurring among fish fed purified amino acids (Table 30).

Table 33. Summary of the differences in amino acid ADCs between intact protein (I) in six practical feed ingredients and purified amino acid mixtures (P) designed to duplicate the amino acid compositions of the intact ingredients. Within each column, the presence of I or P denotes a significantly ($P \leq 0.05$) higher ADC for the amino acid indicated.

Amino acid	Blood	Corn	Fish	Meat/ bone	Soybean	Wheat middlings
Acidic Series						
Aspartic acid	–	–	–	–	–	–
Glutamic acid	–	–	–	–	–	–
Aliphatic series						
Alanine	P	–	–	–	–	–
Glycine	–	–	P	–	–	–
Isoleucine	–	–	–	–	–	–
Leucine	–	–	–	–	–	–
Serine	P	I	P	–	–	–
Threonine	–	I	–	–	–	–
Valine	–	–	–	–	–	–
Aromatic series						
Phenylalanine	–	–	–	–	–	–
Tyrosine	–	–	–	–	–	–
Basic series						
Arginine	–	–	–	–	–	–
Histidine	–	–	–	–	–	–
Lysine	–	–	–	–	–	–
Sulfur-containing series						
Methionine	P	–	–	–	–	–
Mean ADC[1]	P	I	P	P	P	–

[1]Average of all amino acid ADCs.

Meat and bone meal (56% CP) and soybean meal (48% CP) had significant differences only between the mean ADCs of fish fed intact protein and those fed the purified amino acid mixtures, and mean ADCs were always higher among fish fed purified amino acids. No significant differences were observed among individual amino acid ADCs or mean ADCs of fish fed wheat middlings (15% CP), a relatively low-protein feedstuff. Corn meal, the ingredient with the lowest protein level (7% CP), was the only ingredient in which all significantly higher ADCs occurred among fish fed intact proteins (Table 30).

These results suggest that the protein content of an ingredient influenced the likelihood that a significant difference would occur between the mean ADC of amino acids in intact protein and the mean ADC of amino acids provided in purified form. It is possible that digestion of the large amount of protein in some ingredients, and absorption of the amino acids released, took longer than digestion of the small amount of protein in other ingredients. If so, the efficiency of digestion of intact protein, as measured by the difference between the amino acid content of the diet and the amino acid content of the feces after a 6-h period of digestion, could be lower for fish fed intact protein than for fish fed the same mixture of amino acids in purified (i.e., predigested) form. Thus, the disappearance of amino acids from the digesta might tend to be more rapid among fish fed high concentrations of purified amino acids than among fish fed high concentrations of intact protein. The fact that the test ingredient composed 99% of the weight of each "diet" no doubt had some effect on the efficiency of digestion of dietary protein and the efficiency of absorption of amino acids. Whether this same effect would be seen in a practical diet in which the same high protein supplements are likely to be used at levels from one-twentieth to one-half the levels used in this experiment is not known. Nonetheless, the results demonstrate that intact dietary protein, regardless of source, was well digested by channel catfish

and in many cases intact proteins appeared to be digested and absorbed as effectively as mixtures of purified amino acids that were readily available from the diet without additional processing.

Results of the digestibility trials did not demonstrate obvious negative effects associated with the use of either intact protein or purified amino acids in channel catfish diets. However, digestibility coefficients could not provide accurate information on the relative rates of uptake of amino acids from the gut contents, which could provide useful information on the rates at which amino acids from intact proteins and purified mixtures were being provided to the bloodstream. To measure relative rates of amino acid absorption with time, concentrations of amino acids in blood plasma were measured during a 12-h period after force-feeding the same diets that were used in the digestibility trials.

4.2 Plasma Free Amino Acids

The caudal vein, which is relatively easy to access, is often the preferred site for extracting blood from fishes. However, because blood from the caudal vein has passed through the tissues of the body, changes in the amino acid concentrations of caudal vein blood often do not accurately reflect the amino acid composition of the diet. Amino acids absorbed from the digesta are transported through the hepatic portal vein from the intestine to the liver, where they are rapidly metabolized. Therefore, concentrations of free amino acids in blood taken from the HPV are likely to reflect the rates at which amino acids are being absorbed in the intestine more accurately than blood collected elsewhere in the circulation. In this study, blood was taken from the HPVs of groups of fish, at 1-h intervals after feeding, so that concentrations of free amino acids could be measured before the amino acids entered the liver.

Concentrations of plasma free amino acids in HPV blood were measured for a 12-h period after force-feeding each of the practical ingredients and purified amino acid mixtures.

Results indicated that significant differences, when they occurred, were usually caused by higher concentrations of plasma free amino acids in fish fed the purified amino acid mixtures. In 22 of 23 cases in which significant differences between treatments were identified, fish fed purified amino acids had higher concentrations of amino acids in the HPV blood than fish fed intact protein (Table 31). Most of these differences (nine each) occurred among catfish that received the fish meal and soybean meal treatments, and in nearly all cases significant increases in amino acid concentrations in the blood occurred 1 h to 3 h after feeding (Table 31).

Murai et al. (1987) sampled blood from the HPV of rainbow trout 3, 6, 12, 24, 48, and 72 h after force-feeding casein or a mixture of amino acids similar in composition to casein, and reported that plasma concentrations of most amino acids rose sharply within 3 h after force-feeding either diet. Free amino acid concentrations in that study peaked by 12 h after feeding and returned to pre-treatment levels by 24 h after feeding. Moreover, the absorption pattern of dietary essential amino acids was highly correlated with the essential amino acid profile of the diet, while the absorption pattern of non-essential amino acids was not correlated with dietary concentrations. Schuhmacher et al. (1997), in another study with rainbow trout in which blood samples were obtained by heart puncture, reported a similar rate of increase and decrease in plasma free amino acids following the force-feeding of mixtures of amino acids or practical feedstuffs. In the present study a similar response was seen in channel catfish, although the time required to reach peak levels of amino acids in the blood was much shorter in most cases than has been reported for rainbow trout. The length of time required to achieve complete digestion in coldwater species (e.g., trout) is usually much longer than in warmwater species (e.g., catfish). Hence, it is reasonable to expect that the average time required for a similar physiological response to occur would be less in channel catfish than in rainbow trout.

Table 34. Summary of the differences, between treatments, in the concentrations of amino acids in the HPV blood of channel catfish fed intact protein (I) in six practical feed ingredients or purified amino acid mixtures (P) designed to duplicate the amino acid compositions of the intact ingredients, during a 12-h period after force-feeding. Within each column, the presence of I or P denotes a significantly ($P \leq 0.05$) higher concentration for amino acid indicated. Numbers following a letter indicate the sampling times (h) at which significant differences occurred.

Amino acid	Blood	Corn	Fish	Meat/ bone	Soybean	Wheat middlings
Acidic Series						
Aspartic acid	–	–	P1	–	P1	–
Glutamic acid	–	–	P1	–	P1	–
Aliphatic series						
Alanine	–	–	P1	–	–	–
Glycine	–	–	P1	–	P1,2	–
Isoleucine	–	–	–	–	–	–
Leucine	P3	–	P4,8	–	P3	–
Serine	–	–	–	–	P1	–
Threonine	–	–	–	–	P3	–
Valine	–	–	–	–	P3	–
Aromatic series						
Phenylalanine	–	–	–	–	–	–
Tyrosine	–	–	–	–	–	–
Basic series						
Arginine	P3	–	P1	P4	P3	–
Histidine	–	–	P1	–	–	–
Lysine	–	–	P1	P4	–	–
Heterocyclic series						
Tryptophan	–	I8	–	–	–	–
Sulfur-containing series						
Methionine	nd[1]	nd	nd	nd	nd	nd

[1]Not detected.

Some researchers have reported a lag of 24-36 h in rainbow trout between the time of force-feeding and the appearance of peak levels of amino acids in the blood (Yamada et. al 1981). Yamada (1981), and Cowey and Walton (1988) suggested that this lag could be related to the starvation periods that were imposed in these studies prior to feeding and to the stress of the force-feeding itself. However, Murai et al. (1987) and Schuhmacher et al. (1997) observed no lag in rainbow trout that had been fasted and force-fed, and in the present study no lag appeared to occur among channel catfish that were fasted and force-fed prior to blood sampling. Thus a 24-h fast, followed by force-feeding, appears to be a suitable method for evaluating uptake of dietary amino acids in channel catfish.

The fact that soybean protein appeared to be digested, and its constituent amino acids absorbed, at a significantly lower rate than purified amino acids is of interest when one considers that soybean meal composes almost half of a typical catfish diet. This suggests that purified amino acids, which might be added to a diet to eliminate deficiencies caused by the presence of lower quality protein supplements, are likely to be absorbed more quickly than amino acids released from the digestion of soybean protein, the primary dietary protein source. This difference in uptake rate could have negative effects on the efficiency of nitrogen retention, especially if the supplemented amino acid is one of the amino acids limiting the rate of protein synthesis. Nonetheless, most of the purified dietary essential amino acids that exhibited significantly higher apparent rates of uptake in one or more ingredients (i.e., leucine, threonine, valine, and arginine; Table 31) are not supplemented in practical catfish diets at the present time because the levels of these amino acids are usually adequate when the diet is formulated to meet the lysine requirement of channel catfish. It is possible that as new ingredients are used in catfish diets in the future the need to supplement some of these amino acids may develop. In that

case, knowledge of the differences in uptake rates that might exist between amino acids supplemented in purified form and those provided in intact protein could be useful.

In most cases, lysine, which is often the most limiting/most commonly supplemented amino acid in catfish feeds, appeared to be digested and absorbed equally well whether it was provided as a constituent of intact protein or as a purified dietary supplement (Tables 30 and 31). Fish meal and meat and bone meal were the only treatments in which the apparent rate of lysine absorption differed significantly between fish fed intact protein and those fed purified amino acids (Table 31). In both cases, a significant increase in blood lysine concentration occurred among fish fed the purified amino acid mixtures within 1 h (fish meal) to 4 h (meat and bone meal) after feeding. Temporal differences in the apparent rate of lysine absorption (Table 32) did not occur among fish in the meat and bone meal treatment, but significant temporal differences in lysine uptake did occur among catfish in the fish meal treatment (Table 32). The mean lysine concentration in the blood of catfish fed purified amino acids rose significantly 1 h after feeding, from a few hundred nmol/μL (initial) to more than 4,500 nmol/μL, relative to fish fed intact protein (Fig. 26). The mean lysine concentration in the blood of catfish fed fish meal did not change significantly during the same period, nor did it change significantly from one sampling to the next during the subsequent 12-h sampling period. Two hours after feeding, the mean lysine concentration in the plasma of catfish fed purified amino acids had returned to a level not significantly different from that of catfish fed fish meal, and plasma lysine concentrations between fish in the two treatment groups remained similar ($P > 0.05$) during the remaining 10 h of sampling (Fig. 26).

Table 35. Summary of the differences, within treatments, in the concentrations of dietary essential amino acids in the HPV blood of channel catfish fed intact protein (I) in six practical feed ingredients or purified amino acid mixtures (P) designed to duplicate the amino acid compositions of the intact ingredients, during a 12-h period after force-feeding. Within each column, the presence of I or P denotes a significantly ($P \leq 0.05$) higher concentration of amino acid indicated. Numbers following a letter indicate the sampling times (h) at which significant differences occurred.

Amino acid	Blood	Corn	Fish	Meat/ bone	Soybean	Wheat middlings
Aliphatic series						
Isoleucine	–	–	–	–	P7 > P0,12	–
Leucine	–	–	–	I10 > I0	P3 > P0,4,12	–
Threonine	–	–	–	–	P3 > P0,4-12	–
Valine	–	–	–	–	P3 > P0,4,12	–
Aromatic series						
Phenylalanine	–	–	–	–	P3 > P0,11-12	–
Basic series						
Arginine	–	–	–	–	P3 > P0,4-6,8-12	–
Histidine	–	–	–	–	–	–
Lysine	–	–	P1 > P0,2-12	–	–	–
Heterocyclic series						
Tryptophan	–	–	–	–	–	–
Sulfur-containing series						
Methionine	nd[1]	nd	nd	nd	nd	nd

[1]Not detected.

The large number of significant differences associated with soybean meal, both between and within treatments (Tables 31 and 32, respectively), suggest that the protein in soybean meal may be more difficult to digest than the protein in some of the other ingredients tested. This is noteworthy because soybean meal constitutes a large part (nearly 50%) of the diet of farm-raised catfish. Similar results were obtained for menhaden fish meal, but fish meal is a small component of modern catfish diets. Indeed, fish meal has been eliminated from many catfish feeds in recent years and replaced with a 60/40 mixture of meat and bone meal/blood meal. High-temperature processing is known to reduce the digestibility of protein and the availability of amino acids in fish meals. Processing effects could have been a factor affecting the reduced uptake of amino acids from fish-meal protein, relative to amino acids absorbed from purified amino acid mixtures, but this could not be determined conclusively under the conditions of this study.

If, as the results of this study indicate, amino acids provided in purified form tend to be more rapidly absorbed by catfish than amino acids obtained from protein in the diet, what benefits might accrue from adding supplemental, crystalline amino acids to catfish feeds? When crystalline amino acids are added to the diet, peak levels in the blood tend to be reached sooner after feeding than peak levels of amino acids that are obtained from digested protein. The presence of purified amino acids in the diet tends to favor rapid uptake of these "predigested" compounds, which can lead to increased catabolism of amino acids that are absorbed in excess of metabolic needs. Thus, selective catabolism of the very amino acids that were added to a diet to compensate for deficiencies in the amino acid composition of its intact proteins could occur as a result of providing the amino acids in purified form. Amino acids released by the digestion of protein tend to be absorbed in smaller quantities, at a more constant rate, over a longer period of

time than purified amino acids. This difference in the timing of amino acid uptake could result in more efficient use of the amino acids obtained from protein and perhaps more efficient protein synthesis (Tantikitti and March 1995).

A cell's quantitative requirements for the amino acids used in protein synthesis and other metabolic activities are met by transport of amino acids into the cell and synthesis of amino acids within the cell. Active transport systems move amino acids across cell membranes and the regulation of amino acid transport affects amino acid metabolism. Amino acid transport systems can be affected by factors such as the concentration of ions in the cell, the affinity of specific substrates for a particular amino acid, intracellular amino acid concentrations, pH, and the nutritional status of the organism (Collarini and Oxender 1987). Intracellular amino acid pools are not determined by the passive movement of amino acids, but are regulated by active trans-membrane transport mechanisms (Lyndon et al. 1993). Inconsistencies in the absorption of free amino acids from digested protein or purified amino acids could be related to the effects mentioned above.

Some reports (Kangsen et al. 1988, Williams et al. 2001) suggest that feeding frequency can affect the efficiency of utilization of purified amino acids. Multiple feedings per day could provide more opportunities for supplemented, purified amino acids to complement the profile of amino acids obtained from digested dietary protein. This could reduce the amount of supplemented amino acids that are shunted to non-productive uses and improve fish growth. The poor growth reported for some species fed diets containing purified amino acids is due, in part, to the fact that supplemented amino acids are not absorbed simultaneously with the amino acids released from dietary protein, which can impede the rate of protein synthesis. It is also possible that the role of enzymes in amino acid transport could be a factor affecting absorption.

Dabrowski et al. (2003) compared growth of rainbow trout fed diets containing free amino acids, dipeptides, or proteins and reported that a diet containing purified dipeptides produced better growth than a diet containing free amino acids. They suggested that the rate of transport and hydrolysis of dipeptides in the intestine was sufficient to support protein synthesis and growth. If so, supplementation of diets with dipeptides rather than free amino acids might be useful for fish that do not respond favorably to diets supplemented with combinations of individual, purified amino acids. However, it was also noted that the growth of fish fed dipeptides was inferior to that of fish fed proteins (Dabrowski et al. 2003).

If the results obtained in this study with channel catfish also apply to fishes with similar digestive processes, it is possible that the practical value of adding purified amino acids to fish feeds will vary with the composition of the diet. Fish fed diets that contain ingredients with easily digested proteins may benefit more from amino acid supplementation than fish fed diets containing proteins that are slowly or inefficiently digested. In the latter case, amino acid supplements are likely to be absorbed too soon to significantly improve the efficiency of protein synthesis. The faster dietary protein is digested, the more effective purified amino acids are likely to be. Thus, effective use of amino acid supplements in fish feeds may require that the ingredient composition of the diet be considered. In some cases it may be best to formulate a diet with a mixture of intact proteins only, despite the increased cost that is likely to be incurred. In other cases a mixture of intact proteins and purified amino acids might be suitable, if the diet's ingredient composition promotes the efficient utilization of amino acids from both sources. Someday, advancements in encapsulation technology might provide the opportunity to supplement diets with amino acids that have been protected with digestion-resistant coatings that

match their rates of absorption in the fish intestine to the rates of absorption of amino acids released from digested proteins.

4.3 Conclusions

1) Intact proteins were well digested in all of the ingredients tested, although in some ingredients (fish meal and soybean meal) the rates of digestion appeared to be slower than in others.
2) The mean digestibility (availability) of purified amino acids was higher in most cases than the mean digestibility of amino acids in intact proteins.
3) Uptake of purified amino acids tended to be higher within the first four hours after feeding than uptake of amino acids in intact proteins.
4) Uptake rates of purified amino acids did not appear to be affected by the type of amino acid (acidic, aliphatic, aromatic, basic, or heterocyclic) involved.
5) Different rates of absorption between purified amino acids and the amino acids obtained from intact proteins in some ingredients could cause amino acid supplementation to be more useful in some dietary formulations than in others. The effectiveness of supplemental amino acids may depend on the speed with which intact protein is digested.
6) The use of purified amino acids that have been modified (e.g., encapsulated) to reduce their rates of absorption to match those of amino acids derived from intact proteins could improve the efficiency of amino acid supplementation.

4.4 Future Research

Future studies should take into consideration the different digestive and absorptive capacities of individual fish. Ok et al. (2001) reported that patterns of amino acid concentrations in the blood of rainbow trout with and without dorsal aorta cannulation were similar. Perhaps repeated collections of blood samples from the same fish, by HPV cannulation, could be used

effectively to measure postprandial amino acid concentrations in the blood. This approach might provide more accurate and consistent results by eliminating the effect of individual variation among fish.

A different approach, perhaps with greater practical application, is research aimed at the development of micro-encapsulation techniques that can delay the uptake of purified amino acids into the blood stream. Improving the effectiveness of amino acid supplementation could allow many poorer quality feedstuffs that are now avoided in diet formulations to be used in the fish feeds of the future.

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