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## The digestibility of high protein and high energy feedstuffs by yearling channel catfish

Paul B. Brown

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To the Graduate Council:

I am submitting herewith a thesis written by Paul B. Brown entitled "The digestibility of high protein and high energy feedstuffs by yearling channel catfish." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

R. J. Strange, Major Professor

We have read this thesis and recommend its acceptance:

J. L. Wilson, K. R. Robbins, J. P. Hitchcock

Accepted for the Council:

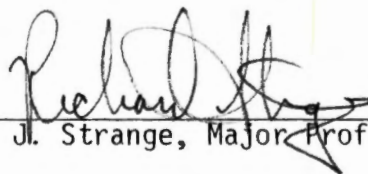
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Vice Provost and Dean of the Graduate School

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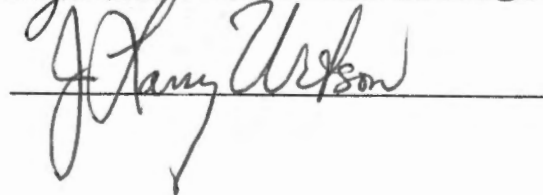
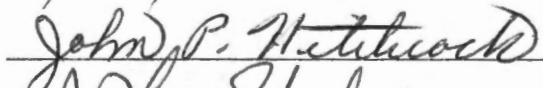
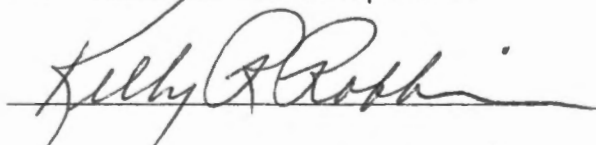
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We have read this thesis  
and recommend its acceptance:



Accepted for the Council:



The Graduate School

THE DIGESTIBILITY OF HIGH PROTEIN AND HIGH ENERGY  
FEEDSTUFFS BY YEARLING CHANNEL CATFISH

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Paul B. Brown

August 1983

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## ABSTRACT

Yearling (1+) channel catfish (Ictalurus punctatus) were held in a regulated environment (28 C and 14 hour light period) and fed high protein and high energy feedstuffs in order to determine apparent digestible crude protein (ADCP) and apparent digestible energy (ADE). ADCP was determined by feeding a semi-purified diet containing 45% carbohydrate and substituting the test feedstuff at an isonitrogenous level. Feedstuffs tested for protein digestibility included corn gluten meal, peanut meal, poultry by-product meal, soybean meal, menhaden fish meal, blood meal, and meat and bone meal. ADE was determined by feeding a practical catfish diet and substituting the test feedstuff at a level of 10% for oil feedstuffs and 20% for meal feedstuffs. The same meal feedstuffs were used for ADE determinations as for ADCP determinations. Oil feedstuffs included safflower oil, corn oil, coconut oil, tallow, lard, margarine, and poultry oil. The fish were offered the experimental diets at a rate equal to 3% of their body weight once daily. Fecal samples were collected 12 hours after the fourth feeding by anal suction. Chromic oxide was used as an external indicator of digestibility. ADCP values ranged from 92% for corn gluten meal to 65% for poultry by-product meal. Mean ADCP from plant sources was higher than the mean ADCP from animal sources. ADE values for meal feedstuffs ranged from 80% for corn gluten meal to 28% for blood meal. Mean ADE values for plant and animal feedstuffs were similar.

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## CHAPTER I

### INTRODUCTION

There is a steadily increasing worldwide demand for protein and, with the exception of eggs and milk, organ meats and fish muscle are the highest quality protein sources available for human consumption. Channel catfish (Ictalurus punctatus) and rainbow trout (Salmo gairdneri) represent the vast majority of freshwater fish production in this country. During the past 10 to 15 years, catfish production has overtaken trout production. Fish are the most efficient producers of muscle protein in terms of protein per unit of ingested energy and are the most efficient in terms of feed to weight gain ratio. These facts are due to the low energy cost of their temperature maintenance, locomotion, reproduction, protein catabolism, and their extensive use of proteins as an energy source (Reid et al. 1980). Despite the increasing demand for protein and the efficiency of protein gain observed in fish, the supply of farm raised fish represents only 4% of the total fish crop reaching the United States public. Feed costs are the major expenditure for fish producers. This expenditure, along with the fact that fish nutrition is one of the least understood aspects of fish culture, makes fish farming an economic gamble. Increased efficiency of protein deposition by fish at a reduced cost is conceivable but implies feeding a carefully formulated diet in which

the availability of all components and any interaction among components is well understood.

All nutrients ingested are not available for use by an animal. These nutrients, or their components, must first be absorbed across the gastrointestinal wall. The basic means of determining availability of nutrients involves analysis of feed and fecal samples. The portion that disappears from ingestion to excretion is the digestible fraction and is expressed either as a percentage (digestion coefficient) or the amount of the nutrient that disappears. The term apparent should be applied to digestibility values, especially energy, crude protein and ether extract digestibilities, since the waste products of intermediary metabolism contribute to the fecal analysis of the aforementioned groups. Apparent digestibility values can be corrected to true digestibilities if endogenous production and eventual fecal excretion can be quantified (Maynard et al. 1979).

The energy component of any animal diet is most important and can be partitioned for a typical endothermic vertebrate as shown in Figure 1. Obviously, some considerations for endothermic vertebrates are of little or no concern for fish energy partitioning or cannot be accurately and economically determined. Cho et al. (1982) proposed a modified scheme for fish (Figure 2). Eructation probably occurs in fish but the energy associated with that gas production is small and not easily quantified. The heat increment ( $H_I$ ) associated with feeding fish is approximately one third that

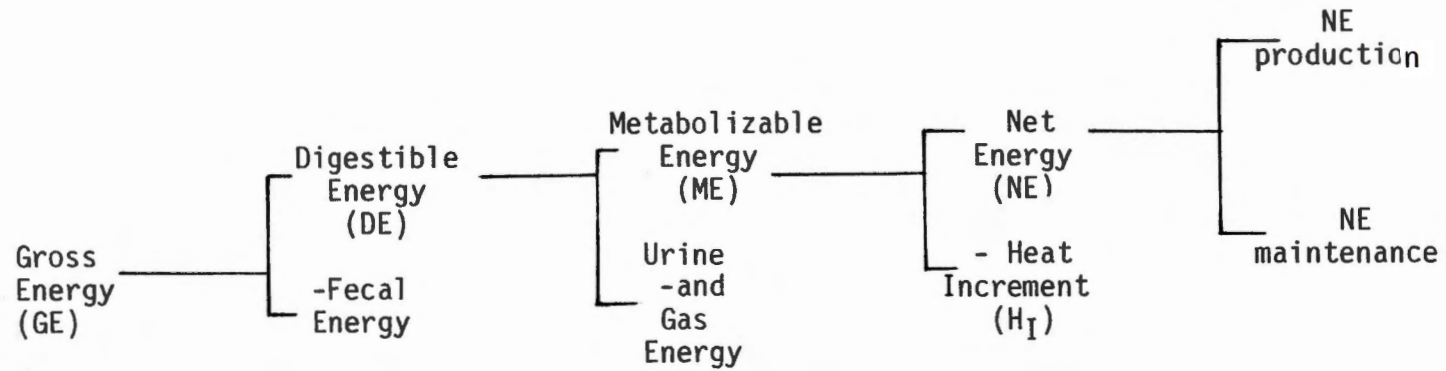


FIGURE 1. Partitioning of the energy component of animal rations.

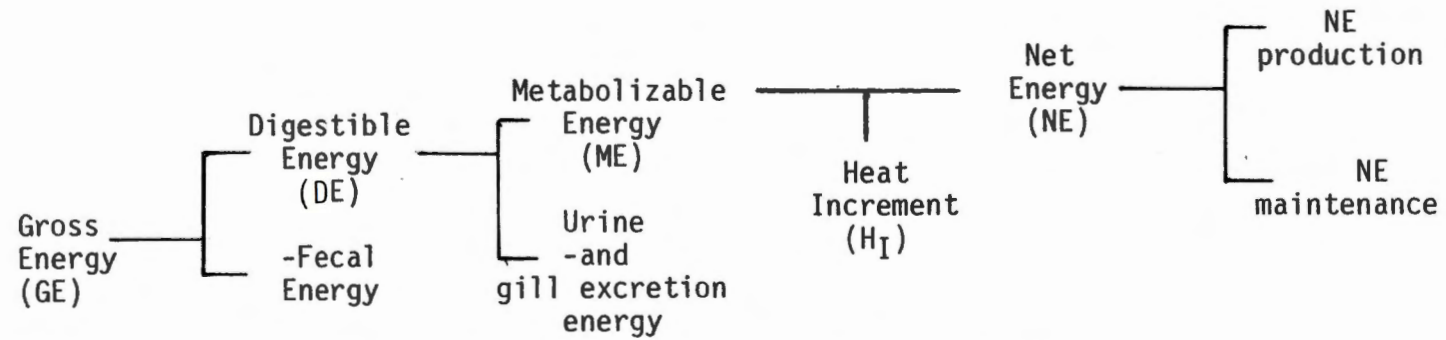


FIGURE 2. Partitioning of the energy component of fish rations.

of endothermic vertebrates (10 versus 30% of gross energy) hence the lesser importance in the overall scheme.

Energy can be supplied in the diet by any of three ways. Most carbohydrates are absorbed as glucose and oxidized by the glycolytic pathway. Fats and oils are absorbed as their component triglycerides. The triglycerides are then hydrolyzed producing free fatty acids and glycerol. These fatty acids are then oxidized forming acetate fractions which can enter the tricarboxylic acid (TCA) cycle. The TCA cycle is the major source of reducing equivalents which can then enter the electron transport chain to produce energy in the form of adenosine triphosphate. Proteins are absorbed as their component amino acids. These absorbed amino acids can enter the TCA cycle at various steps to produce reducing equivalents. Combustible energy values for fats, proteins, and carbohydrates are approximately 9, 5, and 4 kcal/g, respectively. In the production of meat animals, it is desirable to feed a balanced ration in which fats and carbohydrates are oxidized for energy thus sparing the absorbed amino acids for protein formation. This is known as the protein sparing effect (Stryer 1982).

Much of the early nutrition work with any potential meat animal involves comparing growth rates of animals fed different diets. While growth is the desired end-product in animal production, it should be noted from Figure 1 that net energy is divided between maintenance and production. If maintenance requirements are increased, less energy is available for production of edible

tissue. Stress is one factor that can shift the balance of net energy toward increased maintenance conditions. Plasma cortisol increases during stress in both salmonids (Strange et al. 1977) and ictalurids (Strange 1980). Cortisol has profound effects on protein, carbohydrate and mineral metabolism in endothermic animals (Lehninger 1975) and has been shown to effect mineral metabolism in fish (Davis and Simco 1978; Pickering 1981). Stress responses and protein or carbohydrate metabolism are, as yet, not elucidated in fish but this is a potential factor for fish nutrition researchers to consider in the final analysis.

There are two feasible methods for determining in vivo digestibility values for fish--the total fecal collection method and the indicator method. The total fecal collection method becomes the total water collection method when applied to fish. This technique requires a detailed experimental design in order to collect fecal samples quickly and quantitatively (Cho et al. 1982). The indicator method makes use of an undigestible reference component in the experimental diet. External indicators are added to the diet while internal indicators are naturally occurring substances found in the feeds. Examples of external indicators include chromic sesquioxide ( $\text{Cr}_2\text{O}_3$ ), polyethylene and isotopes of Cr, Cs, or P. Internal indicators include chromagens, acid detergent fiber, acid insoluble ash, and hydrolysis resistant organic matter (Buddington 1980). This undigestible component indicates how much of the nutrient disappeared from ingestion to excretion



and allows collection of smaller portions of feces. In fish nutrition work, fecal samples are routinely small and external indicators (mainly  $\text{Cr}_2\text{O}_3$ ) have been used extensively.

The collection of fecal samples from fish poses unique problems. Collection after defecation results in inflated digestibility values due to leaching of nutrients. Sampling anterior to the rectum results in depressed digestibility values due to incomplete absorption (Windell et al. 1978, Smith et al. 1980). Numerous methods of fecal collection have been devised including total water collection (Cho et al. 1982), rotating disc paddle wheels (Choubert et al. 1979), stripping (Austreng 1978), anal suction and rectal dissection (Windell et al. 1978). The paddle wheel method is complex to construct and has not been used to any extent. Stripping of fecal samples results in increased cell sloughing in the alimentary tract and the area sampled can only be approximated. Sampling by anal suction and rectal dissection produced similar protein and energy digestibility values but fish must be sacrificed with the rectal dissection technique (Windell et al. 1978).

Salmonid nutritionists have progressed to the point of routinely determining digestibility values. The Canadian researchers utilize the total water collection method of collecting feces in conjunction with  $\text{Cr}_2\text{O}_3$  indicator (Cho et al. 1982). Fish are allowed to freely feed on a practical reference or control diet. The test feed is then added to the control diet at a level of 30%.



The addition of the test feedstuff dilutes the control diet and the digestibility of the test ingredient can be calculated from overall digestibility values (Cho et al. 1982). American researchers have exclusively used a metabolism chamber (Post et al. 1965, Smith 1971). Fish are restrained and force fed the test feedstuff. Fecal samples were collected by allowing feces to settle in the chamber just under the anus. A comparison of apparent digestible crude protein (ADCP) and apparent digestible energy (ADE) coefficients (Table 1) determined by the two groups of salmonid nutritionists points out the trend toward higher digestion coefficients when determined by the total water collection method. The lone deviants from this trend are poultry-by-product meal and soybean meal. Deviations in digestion coefficients can be expected based on the proximate composition of the feedstuff, individual animal variation, fecal collection method, and overall stress associated with the experiment system, but deviations of this magnitude indicate that leaching of nutrients is occurring. Foltz (1983) observed a linear 1.35% per hour increase in fecal  $\text{Cr}_2\text{O}_3$  concentrations left in water and this could be contributing to the different values generated. Smith et al. (1980) also determined metabolizable energy (ME) coefficients using the metabolism chamber and feeding the same type of experimental diet previously described. There appears to be a consistent relationship between DE and ME (ME = 87% of DE).

TABLE 1. Comparison of Digestion Coefficients for Rainbow Trout Determined by Different Methods

Feedstuff	Investigators			
	Cho et al. (1982)		R. Smith et al. (1980)	
	ADCP	ADE	ADCP	ADE
Alfalfa meal	87	43	22.2	20
Blood meal, spray dried	99	89	65.2	65.8
Fish meal, herring	92	91	86.7	88.8
Meat and bone meal	85	85	70.3	71.7
Poultry by-product meal	68	71	74.7	71.5
Soybean meal	96	75	83.1-86.4	68.9-79.0
Whey, dehy.	96	94	79.7	75.5

Ictalurid nutritionists have concentrated on growth as a means of determining adequacy of experimental diets (Dupree et al. 1970, Stickney and Andrews 1972, Page and Andrews 1973, Andrews and Page 1974; Dupree et al. 1979, Yingst and Stickney 1980). Hastings (1966, as cited by Cruz 1975) conducted the first digestion trials with channel catfish by force feeding the test feedstuff and chromic oxide in a gelatin capsule and then collecting feces by rectal dissection. Smith and Lovell (1973) conducted the first digestion trials using practical catfish diets with a chromic oxide indicator. They calculated whole diet digestibility coefficients and observed higher ADCP coefficients in low fiber diets and increased protein digestibility with increasing crude protein in the diet. They concluded that a close and direct correlation exists between catfish digestibility coefficients and poultry and swine digestibility values. Cruz (1975) determined ADCP and ADE coefficients by feeding the test feedstuff, vitamin and mineral premix, cod liver oil, cellulose, and chromic oxide and collecting feces by either anal suction or rectal dissection. Cruz, as opposed to Hastings, and Smith and Lovell, concluded that there is little correlation between catfish and other nonruminant digestibility coefficients. Andrews et al. (1978) determined ADE of beef tallow by channel catfish by feeding a practical control diet and determining whole diet ADE. They then substituted 5 to 15% tallow at the expense of cellulose and determined whole diet ADE at each level of substitution. This

technique allowed calculation of ADE for the feedstuff. They observed lower digestibility coefficients in cooler water (23 vs 28C) and a decrease in digestibility at substitution levels over 10%. A comparison of digestibility coefficients is presented in Table 2. Coefficients generated by Hastings are generally lower than those of Cruz which could be an indication of the effect of stress and force feeding or an inadequate preliminary period. ADCP values generated by Cruz are not different from Smith's ADCP values for rainbow trout. Digestion coefficients determined by Cruz show a uniform trend toward increased ADE compared to ADCP values. This should be expected since the test feedstuff was the sole source of protein and energy in the diet. ADE values for catfish and trout are similar and no clear trends are apparent.

Determining individual feedstuff digestibility values when fed as the sole protein or energy source is important from the standpoint of selecting potential fish feed ingredients. The data presented in Table 2, however, are not representative of commercially available diets in which mixtures of ingredients are present. It is unknown if these values remain constant when fed in conjunction with other feedstuffs.

The objectives of this study were to determine: (1) the apparent digestible crude protein of high protein meal feedstuffs formulated in a semi-purified diet with a sufficient energy source, and (2) the apparent digestible energy of meal and oil feedstuffs formulated in a practical catfish diet. The meal feedstuffs

TABLE 2. Comparison of ADE and ADCP Coefficients for Channel Catfish

Feedstuff	Investigators			
	Hastings <sup>a</sup>	Cruz (1975)		Andrews et al. (1978)
	ADCP	ADCP	ADE	ADE
Feather meal	63	74.0	83.1	---
Fish meal	73.5-85	87.2-89.9	96.6-97.8	---
Meat and bone meal	40	74.5	77.0	---
Cottonseed meal	76	78.8-83.2	81.2-94	---
Soybean meal	72	76.6-83.7	81.4	---
Raw corn	--	59.5	76.0	---
Cooked corn	--	66.4	96.3	---
Wheat	--	83.8	96.2	---
Wheat bran	--	82.1	94.5	---
Wheat shorts	--	71.5	89.7	---
Alfalfa meal	12	12.5	50.5	---
Blood meal	23	----	----	---
Corn gluten meal	80	----	----	---
Distillers solubles	67	----	----	---
Poultry by-product meal	27	----	----	---
Rice bran	71	----	----	---
Raw soybean	30.2	----	----	---
Fish oil	--	----	97.2	---
Tallow	--	----	----	94
(28C, 10% level of substitution)				

<sup>a</sup>Values quoted from Cruz (1975).

included soybean meal, menhaden fish meal, corn gluten meal, peanut meal, blood meal, meat and bone meal and poultry by-product meal. The oil feedstuffs included corn oil, safflower oil, lard, margarine, coconut oil, tallow, and poultry oil. Evaluation of these feedstuffs includes overlap with existing data and should emphasize component interactions.

## CHAPTER II

### MATERIALS AND METHODS

Yearling (1+) channel catfish were stocked in three 3.0 x 0.6 x 0.6 m fiberglass troughs each divided into three compartments (2121) in The University of Tennessee Fisheries Lab. Mean fish weight per compartment was 228 g (n = 6) for the protein trials and 187.5 (n = 4) for the energy trials. Stocked fish were allowed a minimum of two weeks acclimation prior to initiation of trials. In the event of mortalities during any trial, replacement fish that had been held at the same water temperature were stocked during the fecal collection period and allowed three days acclimation.

Dechlorinated tap water, along with aeration, was supplied to each compartment at a flow rate of 0.72 l/minute (water exchange = 2.96 times/day). Temperatures were maintained at  $28 \pm 1.4$ C. Entire troughs were drained of approximately 25% of their water daily during the digestion trials in order to remove feces and any uneaten food. Additionally, the fish received weekly prophylactic treatment with potassium permanganate (7.5 mg/l for one hour).

Ammonia ( $\text{NH}_3$ ) and dissolved oxygen (DO) values were measured every other day from random compartments within each trough. Ammonia levels were determined as total  $\text{NH}_3$  using a



specific ion probe and DO was measured with a DO meter. The trough water pH was determined weekly with a pH meter.

Fluorescent lighting was regulated daily to 14 hours light and 10 hours dark. All extraneous lighting was eliminated with black plastic. Black plastic was also draped over approximately one third of each compartment in an attempt to reduce stress by providing a darkened refuge area. A regulated environment of 28C and 14 hours light mimics early summer production pond situations. This is a time of active growth in channel catfish.

The basal ADCP diet is given in Table 3. All ingredients except cornstarch and Pel-Aid were mixed in a micro mixer. Experimental diets were formulated by addition of meal feedstuffs at an isonitrogenous level replacing an equal weight of cornstarch. Pel-Aid was added and diets were then hand-mixed.

The basal ADE diet is shown in Table 4. This portion of the diet was mixed in a micro mixer and ground in a Wiley Mill to pass through a 2 mm screen. Chromic oxide (1%) was added and the diet was mixed again in a Hobart mixer. Experimental diets were formulated by substituting the test ingredients for an equal weight of cellulose. Meal feedstuffs were substituted at 20% while oil feedstuffs were substituted at 10%. Control diets contained only the basal diet, cellulose, Cr<sub>2</sub>O<sub>3</sub> and Pel-Aid (Table 5).

Both diets were pelleted with a Parr pellet press and stored frozen. Portions of the experimental diets were heated in an autoclave to 107C and pressure of 7 psi (= 0.49 kg/cm<sup>2</sup>).



TABLE 3. Basal Diet for ADCP Trials

Ingredient	%
Cornstarch	36.21
Dextrose	22.50
Dextrin	22.50
Cellulose	3.00
Cod liver oil	3.00
Mineral Premix <sup>1</sup>	5.37
Vitamin Premix <sup>2</sup>	0.20
Choline chloride	0.20
Ascorbic acid	0.02
Chromic oxide	1.00
Pel-Aid	6.00

(1) Mineral premix composition

(2) Vitamin premix composition

Mineral	Supplied as	mg/kg diet		mg/kg (* = Iu/kg) diet
Ca	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> , CaCO <sub>3</sub>	12,200	Thiamin-HCl	100
P	Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> , K <sub>2</sub> HPO <sub>4</sub>	7,200	Niacin	100
K	K <sub>2</sub> HPO <sub>4</sub> , KI	4,109	Riboflavin	16
Na	NaCl, NaMoO <sub>4</sub> · 2H <sub>2</sub> O, Na <sub>2</sub> SeO <sub>3</sub>	3,509	Ca-pantothenate	20
Cl	NaCl	5,300	Cyanocobalamine	0.02
Mg	MgSO <sub>4</sub> ·H <sub>2</sub> O	590	Pyridoxine-HCl	6
Mn	MnSO <sub>4</sub> ·H <sub>2</sub> O	210	Biotin	0.6
S	MgSO <sub>4</sub> ·H <sub>2</sub> O, MnSO <sub>4</sub> · H <sub>2</sub> O, CuSO <sub>4</sub> ·5H <sub>2</sub> O CoSO <sub>4</sub> ·H <sub>2</sub> O	925	Folate	4
Fe	Ferric citrate	115	Menadione	5
Zn	ZnCO <sub>3</sub>	52	Retinylacetate	10,000*
Cu	CuSO <sub>4</sub> ·5H <sub>2</sub> O	5	Cholicalciferol	600*
Mo	NaMoO <sub>4</sub> ·2H <sub>2</sub> O	40	Tocopherol	10*
I	KI	30		
Co	CoSO <sub>4</sub> ·H <sub>2</sub> O	3		
Se	Na <sub>2</sub> SeO <sub>3</sub>	0.1		

TABLE 4. Basal Diet for ADE Trials<sup>1</sup>

Ingredient	%
Fish meal, menhaden	17.20
Soybean meal	17.20
Corn gluten meal	17.20
Corn	10.00
Mineral premix	5.37
Vitamin premix	0.20
Choline chloride	0.02
Ascorbic acid	0.02
Chromic oxide	1.00
	<u>68.21</u>

<sup>1</sup>Adopted from Andrews et al. 1978.

TABLE 5. Formulation of Experimental Diets for ADE Trials

Ingredient	%
Basal	68.21
Test ingredient	0.00 (Control)
	10.00 (Oils)
	20.00 (Meals)
Cellulose	25.79 (Control)
	15.79 (Oils)
	5.79 (Meals)
Pel-Aid	<u>6.00</u>
	100.00

Approximately 10% water was added to the experimental energy diets to facilitate pelleting. The water released by heating dextrose proved sufficient for the experimental protein diets and no extra water was added. Pellets ranged from 2 to 3 g each and 1/2" (1.27 cm) diameter. They were sectioned into two to three pieces and offered to the fish at a rate equal to 3% of the fishes wet weight as a daily (morning) feeding. Dietary pH of pelleted experimental diets was determined by the method of Nose et al. (1974).

Fecal samples were collected from anesthetized fish (150 mg/l tricaine methanesulfonate, MS-222) using rigid plastic tubing attached to a 20 ml glass syringe. The tubing was inserted rectally 4 to 5 cm and gentle suction was applied to remove feces. Samples were pooled by compartment and dried in a convection oven at 100C overnight prior to analysis (Windell et al. 1978).

Digestion trials were conducted as shown in Figure 3. The preliminary period allowed acclimation of the fish and gut flora to the test diet fed any particular week. A collection period of three to four days is normally conducted with nonruminant animals but the method of fecal collection caused enough stress to decrease voluntary food consumption the following day. As the pelleting process was laborious, it was not practical to continue each trial an additional two or four days. A fecal collection schedule of 12 hours after feeding a single meal assured maximum rectal content.

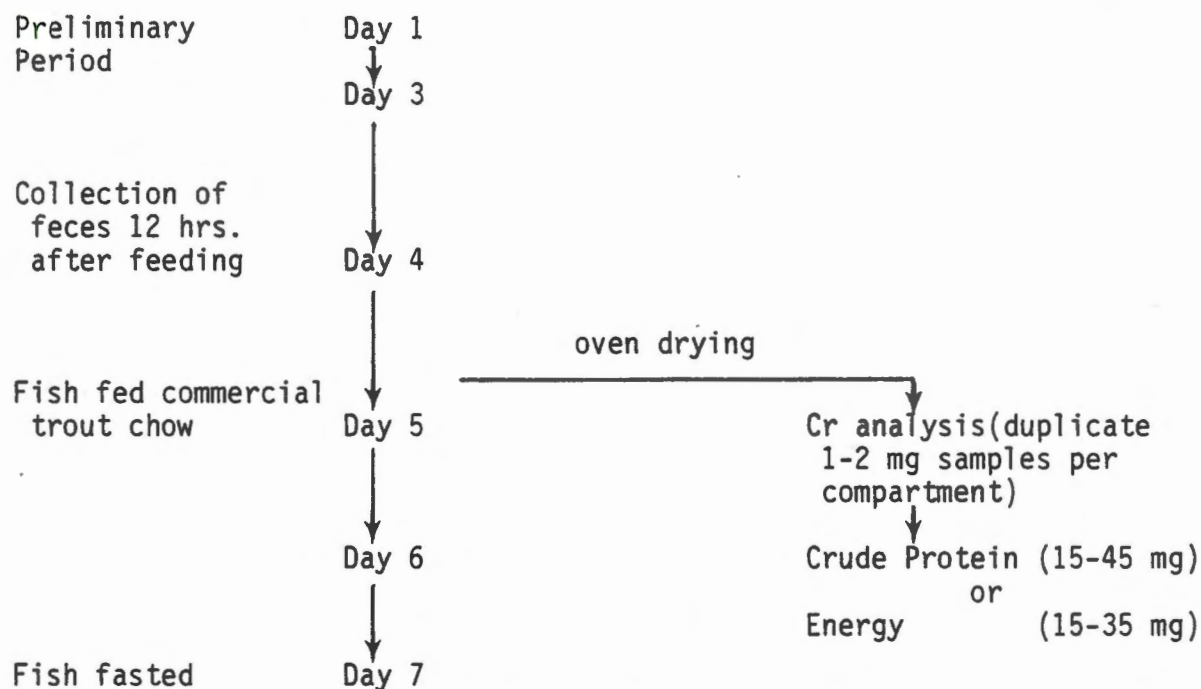


FIGURE 3. Flow chart of digestion trials.

Meal proximate analysis and gross energy were determined by the methods of AOAC (1980) for crude fiber, ether extract, moisture and ash. All crude protein ( $N \times 6.25$ ) analyses were conducted using an automated micro N analyzer. Selenium was used as the digestion catalyst at a temperature of 190.5C (=375F) and steam distillation transferred the volatile  $NH_3$  to the boric acid collection flasks. All energy determinations were analyzed with a Parr 1241 adiabatic oxygen calorimeter (Parr Instrument Co., Moline, IL.) modified with the semi-micro conversion kit.

Chromium content of feed and feces was determined by a modified colorimetric procedure originally described by Saltzman (1952). After wet ashing in concentrated nitric and sulfuric acids,

samples were allowed to go to dryness (Urone and Anders 1950). One to 2 ml of 3% hydrogen peroxide was added and samples were again dried. Ten ml of 0.5N sulfuric acid and 0.5 ml of 0.1N potassium permanganate were added and samples were placed in a hot water bath at 80C for 90 minutes. Samples were removed and decolorized with 5% sodium azide and quantitatively transferred to 50 ml volumetric flasks. Color was developed with s-diphenyl-carbazide reagent and the samples were allowed to stand for 10 minutes. Each flask then received 2.5 ml of 4M sodium dihydrogen phosphate and was taken to volume with distilled water. Absorbance was measured at 540 nm using a Bausch and Lomb Spectronic 70. Standard curves were constructed by plotting absorbance against increasing feed sample weights (1 to 8 mg). Absorbance of fecal samples was determined and compared with the appropriate feed standard curve. This technique allowed calculation of concentration factors (CF) by solving the linear regression equation generated from plotting absorbance against feed sample weight and correcting for weights of fecal samples.

Oil feedstuff fatty acid composition was determined by the procedure described in AOCS Ce2-66 (1975). Methyl esterified samples were injected in a Bendix 2600 gas chromatograph (Bendix Corp., Lewisburg, W.VA) equipped with a flame ionization detector. Samples were separated in a six foot coiled column (1/8" o.d.) packed with 10% Siler 10CP on Chromasorb W. Inlet and detector temperatures were 250C. Samples were run isothermally at 163C

for 14 minutes then increased 3C/minute for the final 7 to 8 minutes. Unknown peaks were identified by injection of appropriate standards.

Apparent digestible energy was calculated for whole diets by the following equation:

$$\text{ADE whole diet} = \text{Energy in diet} - \left( \text{Energy in feces} \times \frac{1}{\text{CF}} \right)$$

The digestibility of individual feedstuffs was then calculated by:

$$\text{ADE feedstuff} = \frac{\text{ADE experimental diet} - \text{ADE control diet}}{\% \text{ substitution}}$$

Apparent digestible crude protein was calculated by the equation:

$$\text{ADCP feedstuff} = 1 - \left( \frac{\text{Fecal Crude Protein/CF}}{\text{Diet Crude Protein}} \right)$$

Each trial was conducted and analyzed statistically as a randomized complete block which allowed three replications of each experimental diet. A control diet was fed each week with the energy trials. This allowed testing of only two feedstuffs per week. Three feedstuffs were tested each week during the protein trials which allowed repetition of two feedstuffs during the final trial. Statistical analyses were conducted with the aid of an IBM 3031 computer equipped with the Statistical Analysis System (SAS) software program and run interactively with the Conversational Monitor System (CMS).

## CHAPTER III

### RESULTS AND DISCUSSION

#### Water Quality, Fish, and Diets

Water quality was good during both sets of digestion trials. DO levels ranged from 7.0 to 7.4 mg/l. Ammonia levels ranged from 0.5 to 1.6 mg/l during the ADCP trials and remained below 1 mg/l during the ADE trials. Trough water pH ranged from 6.7 to 7.1 during both sets of trials. A pH of 7.1 or below dictates that 99% of the ammonia will be in the unionized and less toxic form. Average percent mortality across all three troughs ranged from 9.3 to 5.5% during the three ADCP trials ( $\bar{x}$  = 7.4%) and 13.9 to 0% during the seven ADE trials ( $\bar{x}$  = 5.2%). There was no indication that mortalities were caused by dietary deficiencies or excesses. Since water quality remained acceptable, it is believed that mortalities can be attributed to social interactions within each compartment. Dietary pH ranged from 6.7 to 7.1 for all experimental diets. These should assure efficient utilization of amino acids (Wilson et al. 1977). Results of the proximate analysis and gross energy values are shown in Table 6. These values are in agreement with published standard values (Ensminger and Olentine 1978).

#### Protein Digestibility

Apparent digestible crude protein values are given in Table 7 both on a dry matter and as fed basis. Experimental diet moisture

TABLE 6. Proximate Composition and Gross Energy of Meal Feedstuffs

Feed	Crude Protein(%)	Ether Extract(%)	Crude Fiber(%)	Ash(%)	Moisture (%)	Nitrogen Free Extract(%)	Gross Energy
Dry Matter Basis							
Corn gluten meal	43.41	1.81	1.10	6.73	0	47.58	4460.8
Blood meal	85.09	0.10	0.76	3.25	0	10.80	4818.7
Soybean meal	56.15	1.05	3.84	6.73	0	32.23	4654.1
Fish meal	73.26	7.18	0.25	18.93	0	0.38	4457.1
Peanut meal	56.18	5.60	8.49	4.24	0	25.49	4567.3
Poultry by-product meal	77.33	10.21	0.92	2.88	0	8.66	5507.1
Meat and bone meal	50.10	12.37	2.89	26.36	0	8.28	3714.8
As Fed Basis							
Corn gluten meal	39.79	1.08	1.01	6.17	8.33	43.62	4089.2
Blood meal	83.91	0.01	0.75	3.20	1.38	10.65	4572.2
Soybean meal	51.61	0.96	3.53	6.19	8.08	29.62	4278.0
Fish meal	68.42	6.71	0.23	17.68	6.60	0.35	4162.9
Peanut meal	54.45	5.43	8.23	4.11	3.07	24.71	4427.1
Poultry by-product meal	73.95	9.76	0.88	2.75	4.37	8.28	5266.4
Meat and bone meal	46.64	11.51	2.69	24.54	6.91	7.71	3458.1



TABLE 7. Apparent Digestible Crude Protein (%)\*

Feedstuff	ADCP	
	Dry Matter (DM) Basis	As Fed Basis
Corn gluten meal	92 $\pm$ 1.8 <sup>a</sup>	84
Fish meal 2 <sup>1</sup>	86 $\pm$ 1.0 <sup>a</sup>	80
Peanut meal	86 $\pm$ 2.1 <sup>a</sup>	83
Soybean meal 1 <sup>1</sup>	85 $\pm$ 2.2 <sup>ab</sup>	78
Soybean meal 2 <sup>1</sup>	85 <sup>ab</sup> $\pm$ 2.5 <sup>2</sup>	78
Meat and bone meal	82 $\pm$ 5.6 <sup>ab</sup>	76
Blood meal	74 $\pm$ 2.1 <sup>bc</sup>	73
Fish meal 1 <sup>1</sup>	70 $\pm$ 5.0 <sup>c</sup>	65
Poultry by-product	65 $\pm$ 0.4 <sup>c</sup>	62
Plant sources	87	
Animal sources	75	

\*Associated variability represents the pooled standard error of the mean except where indicated. ADCP values with the same superscript are not significantly different ( $P > .05$ ) as determined by Duncan's multiple range test.

<sup>1</sup>Duplicates.

<sup>2</sup>Simple standard error of the mean.

content ranged from 10.5 to 13.8%. Duplicated trials using the same feedstuffs were conducted with fish meal and soybean meal. Good agreement was observed in the duplication of soybean meal but the duplications of the fish meal are significantly different from one another. It is presumed that the differences observed in the duplication of fish meal are due largely to individual animal variability. This points out the difficulty in determining absolute digestibility values for fish and the wider ranges that are sometimes seen as compared with other nonruminant animals.

The mean ADCP of plant proteins is much higher than the mean ADCP of animal proteins, perhaps due to the heat treatment associated with processing animal-protein feedstuffs. The heat employed must be high to eliminate pathogenic bacteria and this heat induces the Maillard reaction with lysine rendering it nutritionally unavailable.

Comparisons between ADCP values of nonruminant animals as well as the apparent amino acid availability (AAAA) values for catfish (Wilson et al. 1981) are given in Table 8. The comparison between ADCP values determined in this study and by Cruz (1975) for catfish are similar. The differences that are present in ADCP values could be caused by a number of factors including analytical technique, method of feeding, method of fecal collection, experimental holding system, strain of fish or individual animal variability. It is likely that most of the differences shown in Table 8 for catfish are attributable to

TABLE 8. ADCP and Apparent Amino Acid Availability Coefficients for Fish and Other Nonruminant Animals (% DM Basis)

Feedstuff	Channel catfish			Rainbow Trout	Swine
	Present Study	Cruz (1975)	Wilson et al. (AAAA) (1981)	Smith et al. (1980)	Ensminger and Olentine (1978)
Corn gluten meal	92	--	--	82-86	--
Fish meal	70-86	87-90	69-83	85	20
Peanut meal	86	--	88.4	--	54
Soybean meal	85	77-84	81	75	49
Meat and bone meal	82	74	74.3	--	49
Blood meal	74	--	--	69	--
Poultry by-product meal	65	74	--	--	55

individual animal variation. Differences in ADCP as high as 5% have been observed in individual sheep, an animal that has been domesticated for centuries and whose digestive physiology is well understood. In this study, energy was supplied in the diet by dextrose and dextrin (45% of the diet) and the fish did not have to use the test feedstuff as their sole energy source. Since ADCP values of this study and those of Cruz (1975) are similar, it appears that the digestibility of protein is not affected by the presence of 45% carbohydrate in the diet as an energy source. Cruz (1975), in a separate experiment, compared ADCP values in the presence of 30 and 60% carbohydrate in the diet and found depression of the dietary protein digestibility when carbohydrates constituted 60% of the diet. Based on the ADCP values in Table 8, it appears that any interference in protein digestion must occur when carbohydrates comprise greater than 45% of the diet.

Smith and Lovell (1973) observed increased ADCP as the dietary crude protein increased. It is apparent from Table 8 and in direct contrast to Smith and Lovell that the percentage crude protein in the diet does not influence ADCP. The percent crude protein in the experimental diets formulated in this study ranged from 19 to 24% while those formulated by Cruz ranged from 42 to 66% crude protein. The ADCP values generated in this study are similar to the AAAA values of Wilson et al. (1981). It seems that ADCP is a good indicator of AAAA and, since ADCP values are easier to measure, a quick and easy method for estimating AAAA values

might be feasible if further confirmed. Catfish and rainbow trout ADCP values are also similar. It must be remembered that Smith fed only the test feedstuff and it is unknown if trout ADCP is influenced by a carbohydrate or fat energy source in the diet. It is evident, though, that catfish and trout have similar protein digestion capabilities. It is also evident that catfish and trout have much better protein digestion capabilities when compared to swine. This may be due to an evolutionary adaptation on the part of fishes toward a higher percentage protein in their natural diet. Even in a well balanced diet, it has been approximated that fish use up to 50% of the absorbed amino acids for energy production (Brett and Groves 1979). Catfish nutritionists are currently attempting to lower the dietary crude protein and force the fish to use more carbohydrates and fats as energy sources. The inherent trait of substantial energy production from amino acids is one of the major biochemical differences between fish and other nonruminant animals.

Slump et al. (1977) observed good correlation between apparent crude protein digestibilities for chickens, swine, and rats. This relationship became less precise as ADCP values fell below 80%. Figures 4 to 6 are an attempt to relate apparent amino acid availability (AAAA) (Wilson et al. 1981) with the ADCP values generated in this study. Peanut meal ADCP and AAAA are similar (Figure 4). Except for glycine, histidine, and methionine, all AAAA values are the same or higher than the ADCP.

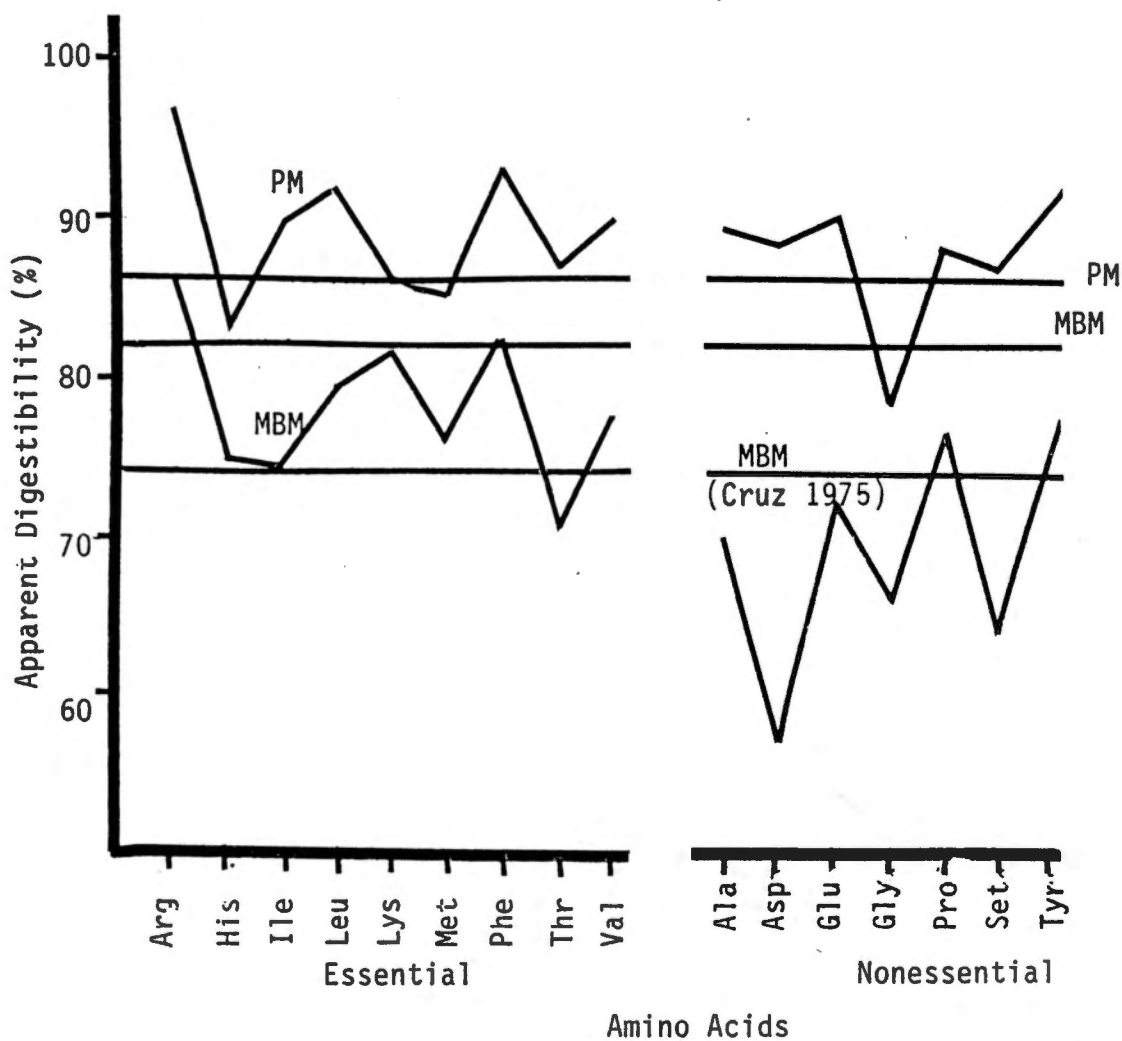


FIGURE 4. Comparison of ADCP and AAAA coefficients for peanut meal (PM) and meat and bone meal (MBM) (DM basis).

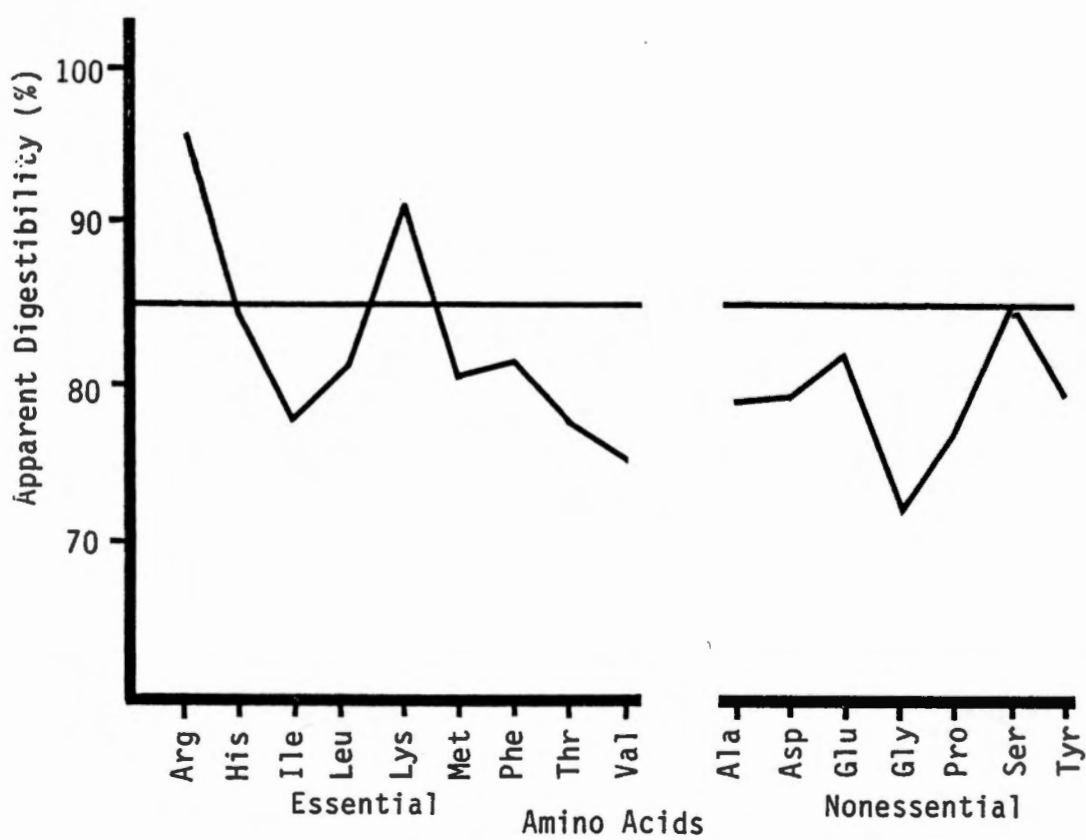


FIGURE 5. Comparison of ADCP and AAAA values for soybean meal (DM basis).

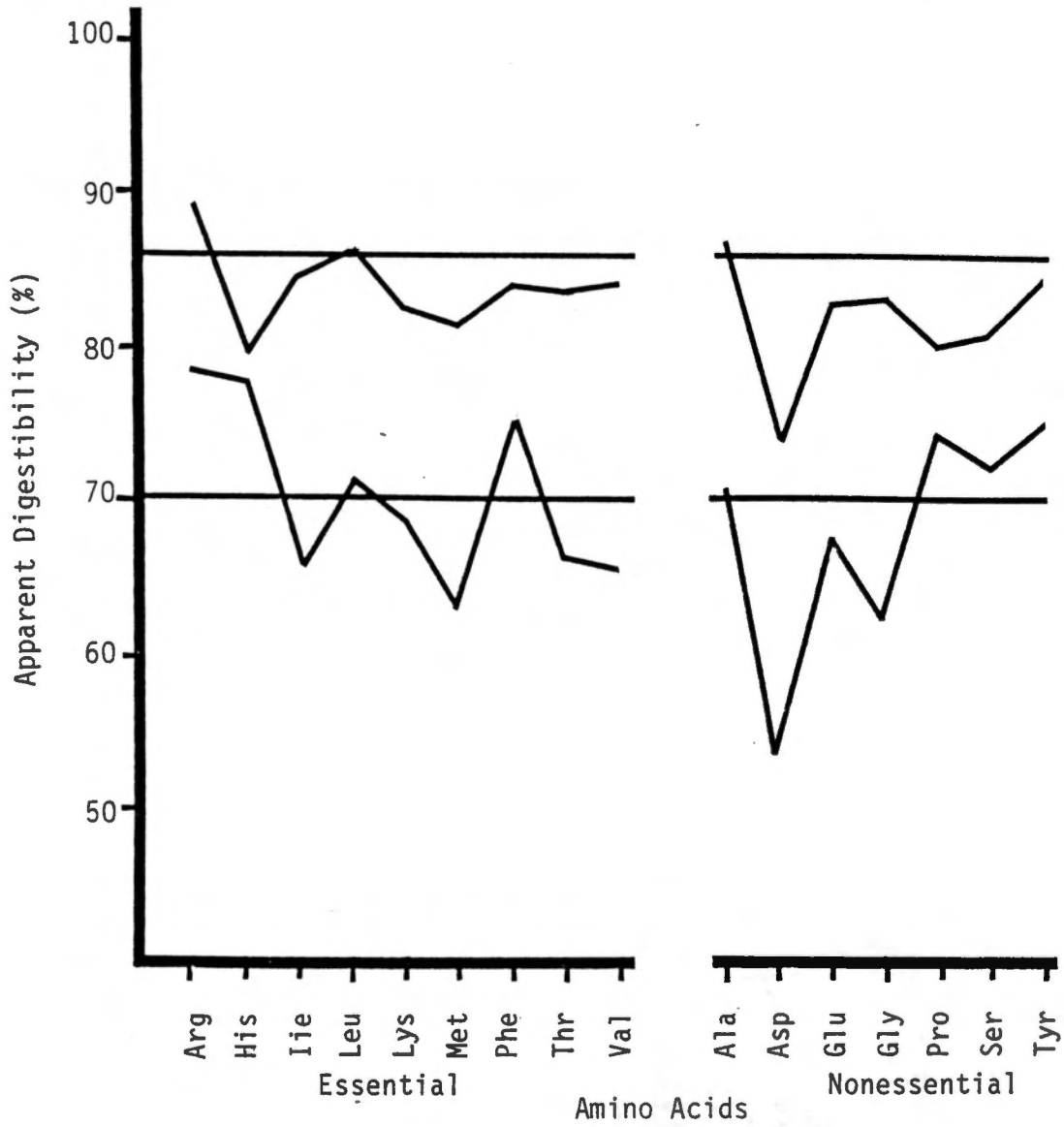


FIGURE 6. Comparison of ADCP and AAAA coefficients for menhaden fish meal (DM basis).



All essential amino acid availabilities are higher than or within 3% of the ADCP. Only phenylalanine and arginine are equal to or higher than the ADCP for meat and bone meal. Proximate composition of feedstuffs varies from one batch to another and the meat scrap meals are probably the least consistent. When the ADCP value of meat and bone meal determined by Cruz (1975) is compared with the AAAA values, all essential amino acids except threonine are equal to or greater than the ADCP. Only arginine, lysine, and serine are equal to or greater than the ADCP of soybean meal (Figure 5). All essential AAAA's are within 9% of the ADCP. Soybean meal is an excellent source of lysine (4.19% of the meal and 90.9% AAAA). Lysine is considered to be the first limiting amino acid in catfish diets, i.e., if the lysine requirement is met, all other essential amino acids are adequate or in excess. The content and availability of lysine makes this meal an important component of catfish diets. Figure 6 is a graph of the ADCP fish meal determinations of this study compared with the AAAA menhaden fish meal values from Wilson et al. (1981). As previously mentioned, ADCP values from this study are duplications with the same batch of fish meal. Wilson et al. used two different lots but lots in which amino acid composition and crude protein were very similar. If low and high values from each study are compared, ADCP appears to be a good prediction of the essential amino acid availability as all AAAA values are greater than or within 7% of the ADCP. Obvious problems are encountered when the high ADCP value is

compared with the low AAAA values and vice versa. Tryptophan is the only essential amino acid not depicted by Wilson et al. as it is usually destroyed on acid hydrolysis.

While the comparisons between ADCP and AAAA values in Figures 4 to 6 represent the use of different lots of the respective feedstuffs and different fish, it appears from these figures and the data in Table 8, page 25, that ADCP can be used as a good prediction of the mean AAAA. More importantly, ADCP values seem to be a reliable predictor of the essential amino acid availabilities. This apparent relationship requires further elucidation with the same batches of feedstuffs.

#### Meal Digestible Energy

An experimental control diet (basal diet + 25.79% cellulose) was fed to the fish each week in conjunction with two experimental diets substituted with the test feedstuffs. It was designed so that experimental test diets could be compared with the control diet fed that particular week. After analysis of all feed and fecal samples, it became apparent that there was enough variability in the ADE values for the control diets that many of the ADE values for the test feedstuffs were calculated as negative numbers. For this reason, the mean ADE for control diets (2661.4 kcal/kg) was used in the formula to calculate ADE of feedstuffs.

Meal ADE values are given in Table 9 both on a dry matter and as fed basis. Experimental meal diet moisture content ranged from 12.7 to 20.1%. Mean ADE for plant feedstuffs is 8% higher than the mean ADE for animal feedstuffs.

Three different components of any feedstuff can supply energy to fish; carbohydrates, protein, and fats. With three dietary components contributing to digestible energy at variable rates, a great deal of variability should be expected and is, in fact, seen in the ADE values. This fact is evident by the analysis of variance of ADE values which indicated that none of the determined values were significantly different from one another.

When ADE values are expressed as a percentage of the organic matter (100-% ash) of the feedstuff, the mean ADE of plant and animal feeds are similar (Table 10). ADE values expressed as a percentage of the potentially digestible portion of the diet (100-(% ash+% crude fiber)) (Table 11) are similar to the values obtained by subtracting only ash.

The soluble carbohydrate portion of the plant feedstuffs (nitrogen free extract) is highest in corn gluten meal, soybean meal, and peanut meal, respectively. This seems to be a good indicator of ADE ( $r = 0.94$ ). As the nitrogen free extract of the three feedstuffs decreases from 47 to 25%, the ADE decreases from 80 to 50% indicating that high (>32%) starch content in feedstuffs is a good source of energy (Figure 7). It was shown

TABLE 9. ADE Values for Meal Feedstuffs\*

Feedstuff	ADE (DM basis)		ADE (As fed basis)	
	%	kcal/kg	%	kcal/kg
Corn gluten meal	80	3558 $\pm$ 1716 <sup>1</sup>	73	3262
Meat and bone meal	75	2792 $\pm$ 210	70	2599
Soybean meal	71	3305 $\pm$ 497 <sup>1</sup>	65	3038
Poultry by-product meal	70	3847 $\pm$ 865	67	3679
Menhaden fish meal	59	2626 $\pm$ 380	55	2453
Peanut meal	50	2282 $\pm$ 594 <sup>1</sup>	48	2212
Blood meal	28	1370 $\pm$ 700 <sup>1</sup>	28	1351
Plant source	67			
Animal source	59			

\*Variability represents the pooled standard error of the mean except where indicated. There were no significant differences as determined by analysis of variance ( $P > .05$ ).

<sup>1</sup>Simple standard error of the mean.

TABLE 10. ADE Values for Meal Feedstuffs as a Percentage of the Organic Matter (ADE/100- % Ash)

Feedstuff	ADE (DM basis)	
	%	kcal/kg
Corn gluten meal	85	3815
Meat and bone meal	102	3791
Soybean meal	76	3543
Poultry by-product meal	72	3961
Menhaden fish meal	73	3239
Peanut meal	52	2382
Blood meal	29	1416
Plant source	71	
Animal source	69	

TABLE 11. ADE Values as a Percentage of the Potentially Digestible Material (ADE/(100- % Ash + % Crude Fiber))

<u>Feedstuff</u>	<u>ADE (DM basis)</u> <u>%</u>
Corn gluten meal	87
Meat and bone meal	106
Soybean meal	79
Poultry by-product meal	73
Fish meal	73
Peanut meal	57
Blood meal	29
Plant source	74
Animal source	70

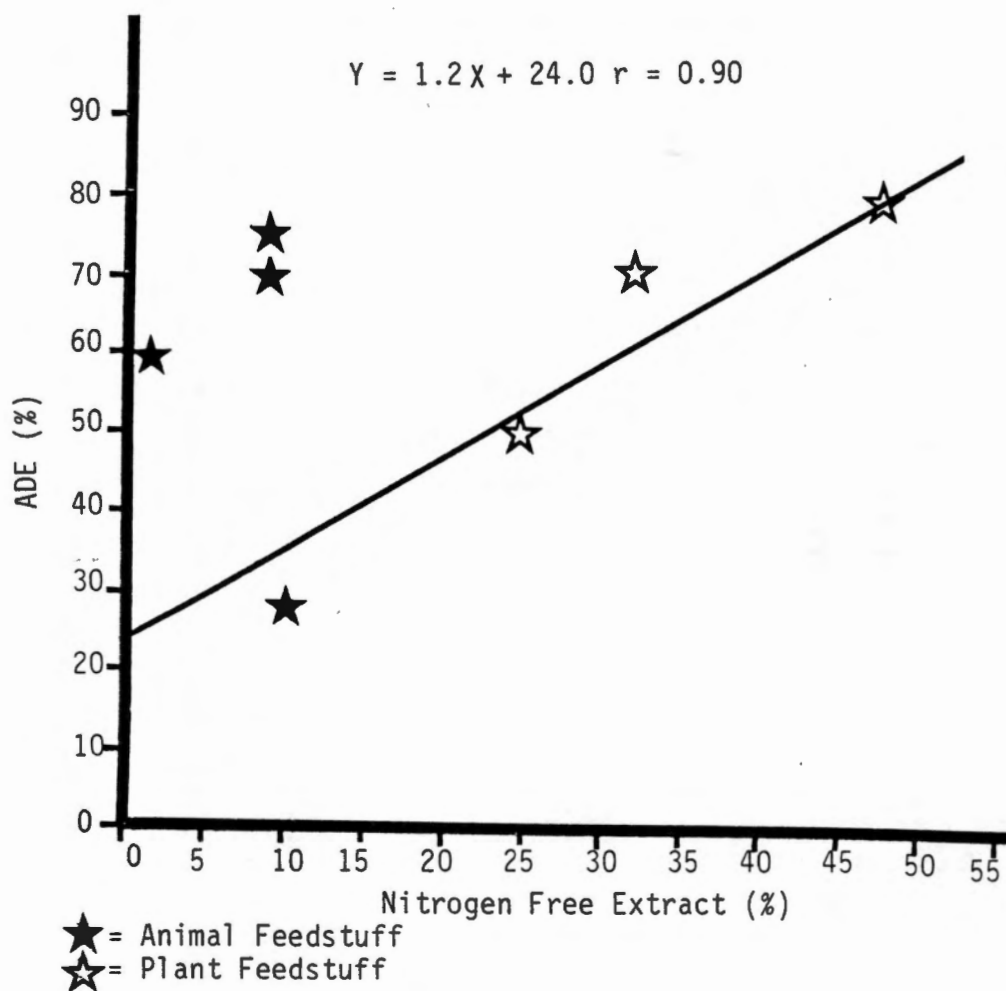


FIGURE 7. Relationship between nitrogen free extract and ADE (DM basis).

by Cruz (1975) that cooked starch is much better digested than raw starch (26 vs 58% in corn). If the experimental diets used in this study had been heat extruded forming a floating pellet, higher ADE values in the feedstuffs with high starch content would probably have been observed due to the heat employed in the extrusion process.

There is a good relationship ( $r = 0.99$ ) between the percentage ether extract (crude fat) and ADE between the animal feedstuffs. As the ether extract percentage decreases from 12 to 0.1% in meat and bone meal, poultry by-product meal, fish meal, and blood meal, respectively, the ADE decreases from 75 to 28% (Figure 8). While these relationships concerning nitrogen free extract and ether extract appear to be good indications of ADE, the protein component of feedstuffs also contributes to the digestible energy. There does not appear to be any clear relationship between ADCP and ADE (Figure 9). Crude protein in the feedstuff appears to be negatively correlated with the ADE values in Table 9 (Figure 10). The starch and lipid components appear to be the major factors determining ADE of meal feedstuffs.

Comparisons among ADE values for fish and other nonruminant animals are shown in Table 12. Comparisons between ADE values determined in this study and those determined by Cruz (1975) are generally not similar. Again, as with ADCP, many factors could be contributing to these differences with individual animal variability being a major contributing factor as well as the other



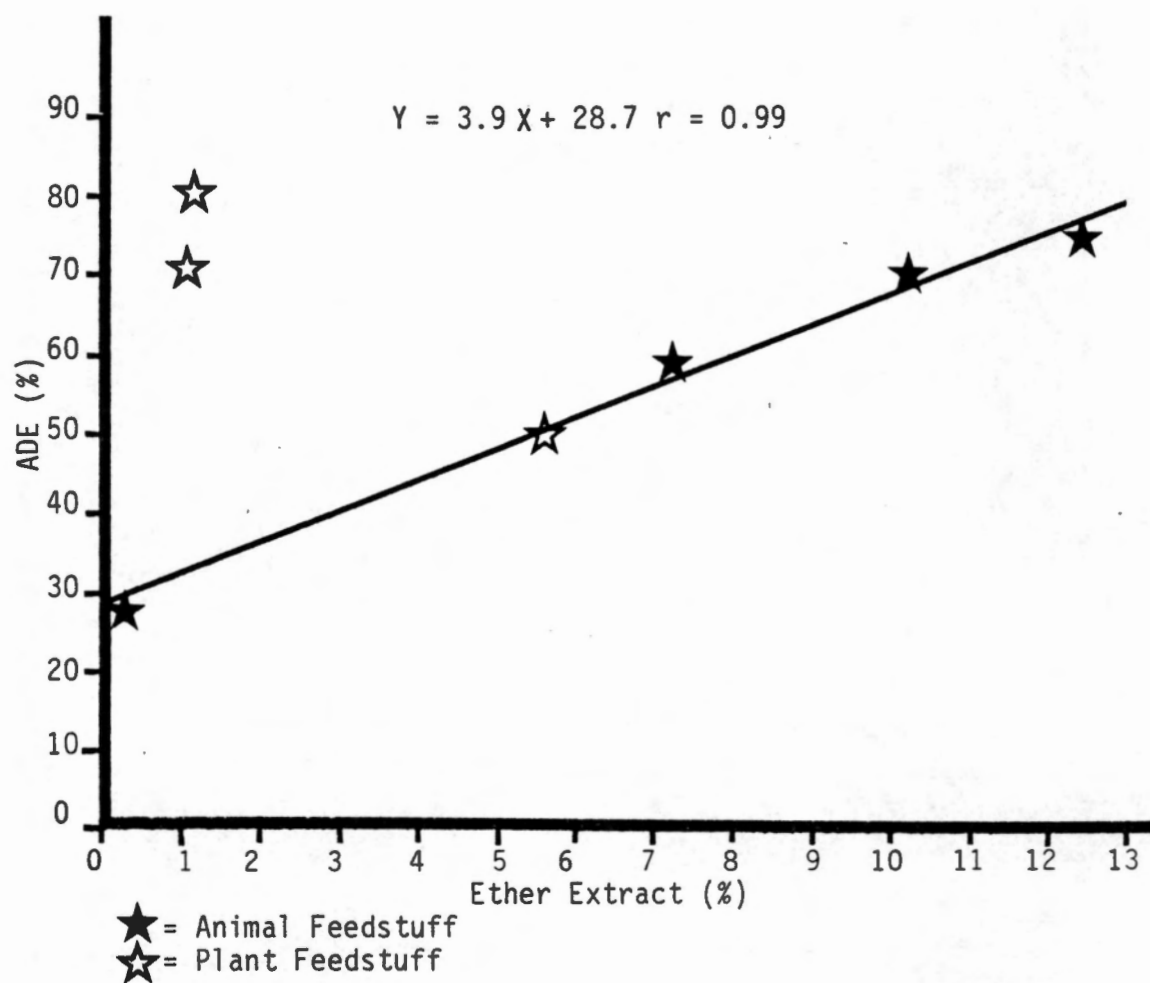


FIGURE 8. Relationship between ether extract and ADE (DM basis).

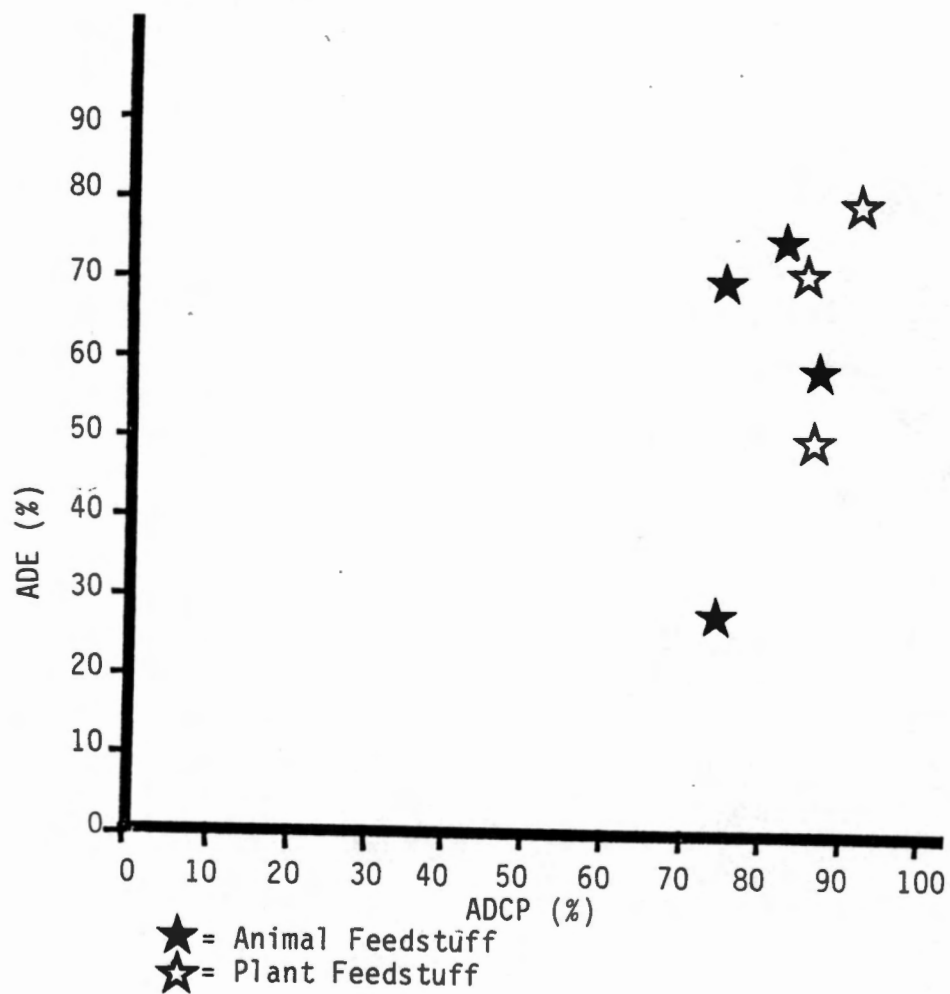


FIGURE 9. Relationship between ADCP and ADE (DM basis).

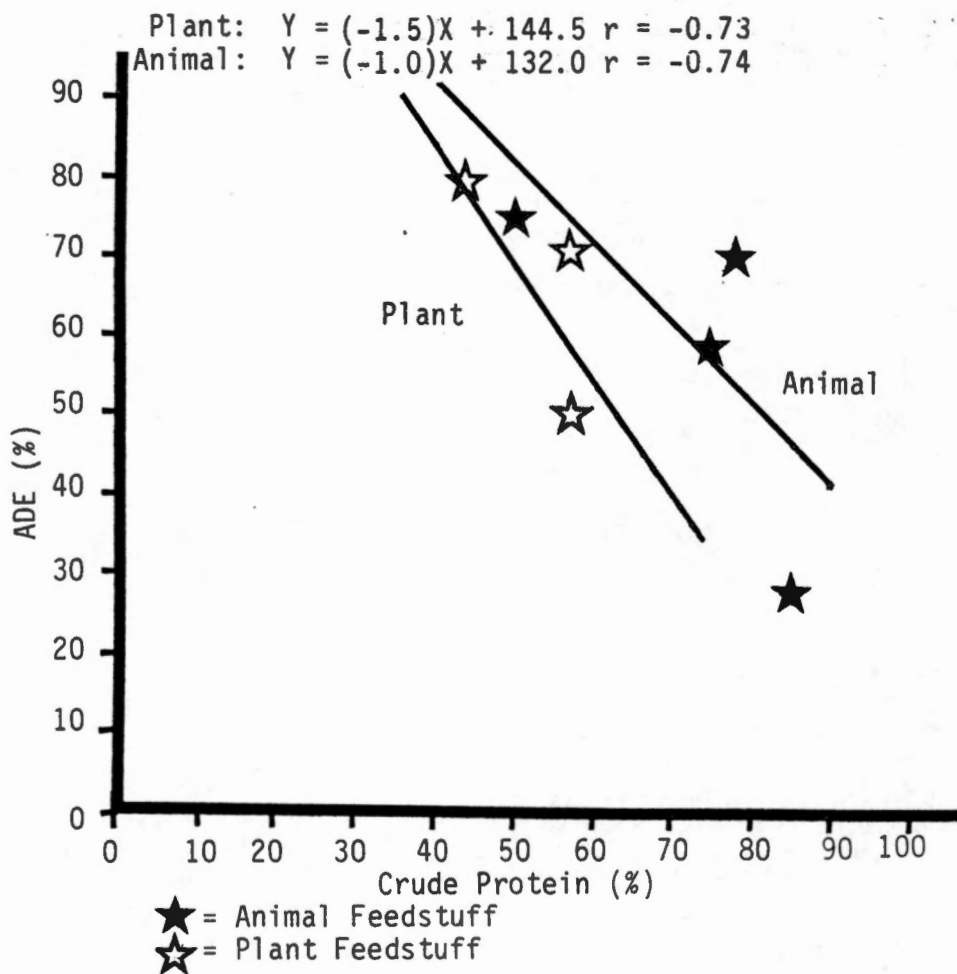


FIGURE 10. Relationship between ADE and crude protein (DM basis).

TABLE 12. ADE Values for Fish and Other Nonruminant Animals (kcal/kg DM basis)

Feedstuff	Channel catfish		Rainbow Trout	Swine	Poultry (ME <sub>n</sub> )
	Present Study	Cruz (1975)	Smith et al. (1980)	Ensminger and Olentine (1978)	NRC (1977)
Corn gluten meal	3558	--	3712-4683	3562	3231
Meat and bone meal	2792	3469	--	2114	2304
Soybean meal	3305	2560-2521	2981-3539	4368	2725
Poultry by-produce	3847	3414	--	3305	3033
Menhaden fish meal	2626	4079-3732	--	3561	3091
Peanut meal	2282	--	--	3888	2928
Blood meal	1370	--	3855-4612	--	2998

feedstuffs in the diet. Gross energy of the feedstuff must be considered as well as technique of determining ADE. Gross energy determinations are similar for the respective feedstuffs. Cruz fed a semi-purified diet in which the test feedstuff was the sole energy source. The experimental diets formulated in this study were practical but energy deficient diets substituted at 20% with the test feedstuff. Catfish producers do not feed a single feedstuff but use a diet containing numerous feedstuffs and, consequently, numerous sources of energy. For this reason, it is believed that the values determined in this study are closer to the average ADE for typical catfish feed formulations.

Comparisons between ADE values determined in this study for catfish and ADE values for rainbow trout are similar for soybean meal and corn gluten meal but not for blood meal. Smith et al. (1980), like Cruz, fed semi-purified diets with only one energy source and the values tend to be somewhat higher than the ADE values determined in this study. It is unknown if these values change when fed in a practical diet. The blood meal value determined by Smith is unusually high when compared with the blood meal ADE value determined in this study. It is unclear as to whether the value determined for trout fed a semi-purified diet was artificially high or whether the value determined for catfish fed a practical diet was artificially low.

There are no clear similarities between the ADE values of this study, ADE values of swine and  $ME_n$  values for chickens

( $ME_n$  = metabolizable energy corrected for nitrogen excretion). In general, catfish ADE values determined in this study are more similar to chicken  $ME_n$  values than swine ADE values but they are not similar enough to warrant general use of chicken  $ME_n$  values in balancing catfish diets.

### Oil Digestible Energy

Oil ADE values are shown in Table 13 on a dry matter basis. Experimental diet moisture content ranged from 12.4 to 15.9%. Values represented were determined by using a mean control diet ADE value as with the meal feedstuffs (2661.4 kcal/kg). The percentages were determined using the approximate energy value of 9000 kcal/kg for fats and oils except where gross energy values were available from the manufacturers or from NRC (1969).

These values appear unlikely to be correct since three of the values were calculated over 100%. Also, corn oil and safflower oil have very similar fatty acid composition but the ADE of safflower oil is less than one half of the corn oil ADE.

The concentration factors determined for the oil fecal samples appeared to be over estimates as most ranged from 2.7 to 3.7 as compared to 1.6 to 2.6 for the meal ADE CF's. It was postulated that the oils in the diet were depressing their respective chromium feed standard curves. A separate experiment was conducted to determine if the substitution of oils depressed the analysis of chromium in the diet. Coconut oil was substituted

TABLE 13. Digestibility Values for Oil Feedstuffs (DM basis).

Feedstuff	ADE		Swine	Chickens
	%	kcal/kg	ADE Ensminger and Olentine (1978)	(ME <sub>n</sub> ) NRC (1977)
Corn oil	133	11416	7620	8820
Lard	128	11557	7620	8820
Poultry oil	120	10831	--	8170
Margarine	117	8352	--	--
Coconut oil	83	7503	--	--
Safflower oil	64	5485	--	8800
Tallow	63	5555	--	6568

at 2.5, 5.0, 10.0, and 20% for an equal weight of cellulose. The observed slope increased from 0.042 to 0.066 as the substituted oil increased from 2.5 to 10%. At the 20% level of substitution, the slope fell to .039. Correlation coefficients were calculated and found to be 0.93 or higher indicating linearity of the data points. It is apparent from this data that oil level in the diet does not negatively influence the standard curve.

The original description for substituting test feedstuffs for cellulose in catfish diets (Andrews et al. 1978) points out further discrepancies. They calculated both an apparent lipid absorbability (ALA) and ADE value for beef tallow substituted at 10%. While the ALA value was determined to be 94%, the ADE value determined was 7000 kcal/kg. Using an approximated value of 9000 kcal/kg combustible energy for oils, 7000 kcal/kg of digestible energy represents only 78% ADE. The reasons for this difference are unknown as are the reasons for the wide ranges between similar oil feedstuffs and the values over 100% determined in this study. Leaching of the oil is unlikely since most experimental diets were eaten quickly. Analytical technique contributes variability with Cr analysis contributing the most. However, Cr values posed no apparent problems in determining ADCP or meal ADE values and it is believed that the Cr values determined for the oil ADE values are, at least, close to correct. Component interaction remains as a possible cause and, since this study represents only the second attempt at determining oil digestibility values when fed to fish, further speculation is not appropriate.



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## LITERATURE CITED

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APPENDIX

TABLE 14. Relative Percentages of Fatty Acid Groups in Oil Feedstuffs

Fatty Acid (chain length: number of double bonds)	Feedstuff						
	Corn Oil	Safflower Oil	Tallow	Margerine	Poultry Oil	Lard	Coconut Oil
6	----	----	----	----	----	----	0.2
8	----	----	----	tr	----	----	5.6
10	----	----	tr	----	----	tr	4.6
12	----	----	tr	tr	tr	tr	58.3
14	----	----	2.1	----	0.1	0.9	17.6
14:1	----	----	1.4	----	0.6	----	----
16	10.1	5.7	26.0	10.5	0.2	26.2	6.5
16.1	----	----	3.6	----	27.8	2.3	----
17	----	----	1.9	----	----	----	----
17:1	----	----	1.3	----	----	0.2	----
18	1.2	1.6	0.4	0.6	6.9	0.2	tr
18:1	23.0	10.2	59.6	50.0	44.6	0.9	1.5
18:2	64.6	82.5	2.3	38.1	18.5	43.8	----
18:3	1.0	----	tr	0.5	1.0	7.9	4.1
20	----	----	----	0.3	0.1	16.1	----

tr = trace.

## VITA

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