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# DIGESTIBILITY OF NUTRIENTS IN SEMI-PURIFIED RATIONS BY CHANNEL CATFISH IN STAINLESS STEEL TROUGHS [1]

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## ABSTRACT

The digestibility of nutrients in six semi-purified rations containing variable levels of protein, cellulose and starch by channel catfish in troughs was evaluated by using chromium oxide as an inert reference in the feeds. Excreta were collected using an indirect trough collection method and a direct intestinal collection method.

Digestibility coefficients determined on excreta collected in troughs were higher than coefficients determined from excreta collected from the intestine. There was no significant difference in protein or fat digestibility from the six rations when the trough collection of excreta method was used.

In the intestinal collection method, excreta were collected, separately, from four areas of the digestive tract, namely, the stomach, upper intestine, lower intestine and rectum. The absorption of protein in two-year old channel catfish occurred the length of the intestine up to and possibly including the rectum.

There was a significant difference in protein digestibility among the six rations for collection of excreta from the rectal area. A higher percentage of protein was digested in rations containing 40% protein than in those containing 20% protein. Starch did not seem to affect protein digestibility but cellulose did. Protein digestibility coefficients ranged from 72 to 93%.

## INTRODUCTION

Although the nutrient composition of most conventional feedstuffs used in catfish feeds is known, the nutrient availability to catfish has been scarcely explored. It has been assumed that availability is similar to that of domestic mammals, but information supporting this assumption is limited. Hastings (1966) determined the apparent digestibility of protein in several natural feedstuffs with channel catfish by employing an indicator technique and collecting undigested food material from the lower one-third of the gut of sacrificed fish. His data, the only published source of digestibility coefficients for channel catfish, showed reasonable similarity to those determined with monogastric farm animals. The influence of diet composition upon the relative absorption of the major nutrients has not been investigated in catfish. Smith (1971) reported that the apparent digestibility of protein by rainbow trout was not affected by level of protein or the presence of starch, glucose or dextrin, but that a high level of cellulose did depress protein digestibility.

Methods for the determination of alimentary absorption of nutrients have typically involved either a direct or an indirect quantitative measurement of the nutrient ingested and the nutrient excreted. The direct method involves collection and measurements of total quantities of waste excreted by the fish in the aquatic environment (Tunnison et al., 1942). The indirect method employs the use of an inert indicator, such as chromium oxide, in the feed which negates the need for total collection of excreta (Hastings, 1966; Nose, 1966). Samples of undigested feed have been taken directly from the intestine of sacrificed fish or from the bottoms of the aquariums.

This study involved the feeding of semi-purified diets of six protein, cellulose, starch ratios to channel catfish in steel troughs and measuring

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apparent nutrient digestibility by using two fecal collection methods. Specific objectives were as follows:

1. Determine digestion coefficients for protein, starch, fat, and cellulose when the ratio of protein to cellulose to starch was varied.
2. Measure absorption of protein in four areas of the digestive tract of two-year-old channel catfish.

## MATERIALS AND METHODS

### *Facilities*

Stainless steel troughs, each 30 cm x 25 cm x 213 cm, were used in the experiment. Each trough contained an individual air and water supply and a standpipe drain at the end opposite the water supply. Water from the city of Auburn's domestic water supply was passed through an activated charcoal filter and then into the troughs. The flow rate was 2.5 liters per minute. The water temperature was regulated at 25 C during the experiment by a thermostatic mixing valve.

Fingerlings and two-year-old channel catfish, *Ictalurus punctatus*, from the Auburn University Fisheries Research Unit were used in this experiment. The fingerlings were 10 to 13 cm total length with an average weight of 12 g. The two-year fish averaged 425 g. The fish were brought indoors and subjected to artificial conditions for several months prior to the collection of samples for digestibility determinations.

### *Experimental Rations*

Six semi-purified diets containing casein, uncooked corn starch and purified cellulose as the major ingredients were formulated for the digestibility studies. One percent chomium oxide was added to each as an inert reference material for calculating digestibility. The ingredient composition is given in Table 1 and the per cent nutrient composition is as follows:

| | Ration | | | | | |
|---|---|---|---|---|---|---|
| Nutrient | 1 | 2 | 3 | 4 | 5 | 6 |
| Protein | 20 | 20 | 20 | 40 | 40 | 40 |
| Cellulose | 5 | 20 | 35 | 5 | 20 | 35 |
| Starch | 60 | 45 | 30 | 40 | 25 | 10 |
| Fat | 7 | 7 | 7 | 7 | 7 | 7 |

TABLE 1. Ingredients in Experimental Rations (g/kg).

| | Ration | | | | | |
|---|---|---|---|---|---|---|
| Ingredient | 1 | 2 | 3 | 4 | 5 | 6 |
| Casein [1] | 235 | 235 | 235 | 471 | 471 | 471 |
| Cellulose | 565 | 465 | 265 | 329 | 179 | 29 |
| Corn Starch (raw) | 50 | 200 | 350 | 50 | 200 | 350 |
| Corn Oil | 50 | 50 | 50 | 50 | 50 | 50 |
| Cod Liver Oil | 20 | 20 | 20 | 20 | 20 | 20 |
| Chromium Oxide | 10 | 10 | 10 | 10 | 10 | 10 |
| Mineral Mix [2] | 40 | 40 | 40 | 40 | 40 | 40 |
| Vitamin Mix [3] | 30 | 30 | 30 | 30 | 30 | 30 |

1 Casein was 84.95% protein on air dry basis.
2 U.S.P. XIV Salt Mixture.
3 A mixture of the following vitamins triturated in dextrose (g/kg): Vitamin A, 4.5; Vitamin D, 0.25; Alpha Tocopherol, 5.0; Ascorbic Acid, 45.0; Inositol, 5.0; Choline Chloride, 75.0; Menadione, 2.25; p Aminobenzoic Acid, 5.0; Niacin, 4.5; Riboflavin, 1.0; Pyridoxine Hydrochloride, 1.0; Thiamine Hydrochloride, 1.0; Calcium Pantothenate, 3.0; Biotin, 0.09; Folic Acid, 0.02; Vitamin B-12, 0.001.

### *Digestibility Trials*

*Trough collection of excreta.* The six experimental rations, replicated twice, were randomly assigned to 12 troughs, each containing 2 kg of

12-g fingerling channel catfish. Each ration was fed daily in dry-pellet form at 3% of the weight of the fish.

Separate troughs were maintained for feces deposition and feeding. Forty-five minutes after feeding, the fish in each of the 12 troughs were transferred to clean troughs for feces collection. Feces were collected just prior to feeding for five consecutive days following a nine-day adjustment period. Fecal particles were collected from the trough bottom by siphoning with ⅜-inch diameter rubber tube. The feces were concentrated by centrifugation and dried and stored for chemical analysis.

*Intestinal collection of excreta*

The six rations, replicated twice, were randomly assigned to 12 troughs of the two-year-old channel catfish. Each trough contained five fish averaging 425 g each. The fish in each trough were fed the semi-purified diets at 2% of their body weight daily. After two weeks the fish were consuming less than one-half of their allowance. Daily fecal deposition was too slight to warrant collection when the fish were allowed to feed voluntarily, consequently, force-feeding became necessary.

Fish were anesthetized prior to force-feeding by placing them in a 30-ppm quinaldine solution for one minute. They were given approximately one per cent of body weight of the experimental diet by use of a trocar for seven successive days. Eighteen hours after the last feeding the fish were anesthetized and the gastro-intestinal tract was exposed. Unabsorbed ingesta was removed from four areas of the tract: the rectum or the area between the anus and a sphincter-like structure approximately 4 cm proximal to the anus; the posterior half of the remainder of the intestine; the anterior half of the remainder of the intestine extending up to the pylorus; and the stomach. For each area of the tract the residue from the five fish receiving similar diets was composited, dried and stored for chemical analysis.

*Chemical Analyses and calculation of digestibility*

The same methods of analysis were used for the feed and the dried fecal samples. Where possible duplicate chemical analyses were made on all samples. Samples were stored in dehydrated form in a desiccator until after all collections were completed.

Nitrogen analysis was determined with a Coleman Model 29A Nitrogen Analyzer II. Protein for casein was calculated as nitrogen X 6.38. Since casein was the only protein source, the factor of 6.38 was used instead of the conventional factor of 6.25.

Starch was determined by digesting a 0.25 to 1.0 −g sample with 1.0 N hydrochloric acid for 4.5 hours at 90 C to hydrolyze the starch to reducing monosaccharides. Reducing sugars were then determined by ferricyanide reduction as described by Friedemann et al. (1967).

Cellulose was determined by placing a 1 −g fat-free sample in 20 ml of 80% acetic acid with 1 ml of concentrated nitric acid and refluxing for 20 minutes. After digestion the sample was extracted with hot 95% ethanol and then washed with hot benzene followed by hot ethanol and ether. The sample was dried at 100 C for two hours to determine a base weight and then ashed at 500 C for four hours. The base weight minus the ash weight represented the weight of cellulose.

Fat was determined by ether extraction on a Goldfisch extractor. One-gram samples were placed in 22 x 80 mm extraction thimbles and extracted with ethyl ether for four hours. The ether extract was collected in a tared beaker and dried and weighed.

Chromium oxide values were determined by wet ashing a 50 to 100-mg sample with perchloric acid and following the photometric procedure described by Furukawa and Tsukahara (1962). A standard curve was prepared, expressed by the equation $Y = -2.2355 + 1.0091\ X$ where Y is the optical density at 350 mu and X is chromium oxide content of the test samples.

Data on nutrient and chromium oxide contents of the feeds and feces were used to calculate per cent digestibility for each nutrient in the experimental rations using the following formula:

$$\text{Digestibility (\%)} = 100 \times \frac{\text{\% Chromium Oxide in Feed}}{\text{\% Chromium Oxide in Feces}} \times \frac{\text{\% Nutrient in Feces}}{\text{\% Nutrient in Feed}}$$

Analysis of variance (Snedecor and Cochran, 1967) employing an F-test was used to test for difference among treatment means. Duncan's multiple range test was used to compare treatment means where significant differences existed.

## RESULTS

*Trough Collection of Excreta*

The mean per cent apparent-digestable protein in the six semi-purified rations fed to the fingerling channel catfish is given in Table 2. There was no significant difference in protein digestibility among the diets at the 0.05 level of probability. The per cent digestible protein was high at both the 20 and 40 per cent dietary levels, ranging from 94 to 99% digestible. These data indicated that the catfish utilized protein (casein) at dietary levels of 20 and 40% equally well, and the amount of starch or cellulose in the ration had little effect upon protein digestibiity.

TABLE 2. Average Per Cent Apparent Digestibiity of Protein, Starch, Fat and Cellulose in Rations Determined from Excreta Collected from Troughs of Fingerling Channel Catfish

| Ration Composition (Per Cent) | | | Apparent Digestibility (Per Cent) | | | |
|---|---|---|---|---|---|---|
| 20 | 5 | 60 | Protein | Starch | Fat | Cellulose |
| 20 | 20 | 45 | 97.26 | 69.65 | 99.54 | 24.86 |
| 20 | 35 | 30 | 96.76 | 88.58 | 95.39 | 13.48 |
| Protein | Cellulose | Starch | 95.96 | 81.44 | 96.52 | 13.26 |
| 40 | 5 | 40 | 98.45 | 72.67 | 97.54 | 1.47 |
| 40 | 20 | 25 | 98.18 | 87.56 | 99.02 | .0 |
| 40 | 35 | 10 | 98.25 | 84.59 | 96.02 | 1.11 |

There was a signfiicant difference at the 0.01 level among treatment means for starch digestibility. Protein level of the diet apparently had little influence on starch digestibility; however, ratio of starch to cellulose did. High levels of starch or high starch to cellulose ratios resulted in the lowest starch digestibility coefficients. Twenty per cent cellulose in the diet provided for highest starch digestibility. Dupree and Sneed (1966) found 20% cellulose to be the optimum level in purified diets for catfish fingerling growth.

The data in Table 2 show that the calculated disappearance of cellulose in the digestive tract was 10.37 to 31.67 per cent for rations 1, 2, 3, but only 0 to 1.47 per cent for rations 4, 5, 6. The difference in the apparent losses of cellulose from the low protein rations and the high protein rations cannot be explained. Smith (1971) reported an apparent digestion coefficient for cellulose in trout of 13.7 per cent.

The digestibility coefficients for fat were high, ranging from 93.24 to 99.72, as shown in Table 2. There was no significant difference at the 0.05 level among treatment means.

Data obtained with the trough-collection technique indicated that the alimentary absorption of protein by the fish was not affected by the level of protein in the diet or by the levels of starch or cellulose; that uncooked starch was relatively highly digestible; and that the amount of cellulose, but not protein, in the diet affected starch digestion.

*Intestinal Collection of Excreta*

The quantity of material recovered from the rectum, or distal area of the intestine of the two-year-old catfish was not sufficient to permit analyses other than for protein and chromium oxide. Ingesta residue collected from all four sections of the digestive tract was analyzed. Per cent protein of the dry material is given in Table 3. The progressive decrease in protein as the ration passed through the digestive tract is evident in most cases. The per cent protein of the stomach contents 18 hours after feeding was approximately 50% of the dietary protein level. This indicates that the casein protein went into solution at a faster rate than some other constituents of the diet. Other sources of protein, particularly plant proteins, may not have hydrolyzed so rapidly.

The protein digestibility coefficients for the six rations at different areas of the digestive tract are shown in Table 4. The values in the table represent averages of two determinations. There were significant differences at the 0.01 level among treatments, among parts of the digestive tract, and in the interaction of the two effects.

Values from the rectum represent the final digestibility of the rations. Data from this area of the digestive tract were analyzed using a one-way analysis of variance. There was a significant difference at the 0.01 level among treatment means. Means were compared using Duncan's multiple range test. The three low-protein rations were significantly different at the 0.05 level from each other and from the three high-protein rations. The means for rations 4 and 6 and 5 and 6 were not significantly different.

Protein digestibility was higher in the 40% protein rations than in the 20% protein rations. Within each protein level, the highest digestibility values occurred at the lowest (5%) cellulose level. Starch did not seem to hinder protein digestion since at each protein level the highest digestibilities were at the highest starch levels.

The apparent digestibility of protein was significantly different within each part of the digestive tract. At the end of 18 hours an average of 61% of the dietary protein had disappeared from the stomach. The determination of a "digestibility of coefficient," as such, from the stomach contents is misleading. Although the protein was probably broken down to lower molecular weight peptides, it is unlikely that it was absorbed through the stomach wall; rather, the hydrolyzed protein moved out of the stomach faster than the starch or cellulose.

The digestibility coefficients in Table 4 represent the amount of protein absorbed at the end of 18 hours. As the samples were taken nearer the rectum the apparent protein digestibility progressively increased. This indicates that absorption occurred the length of the intestine up to or possibly even including the rectum.

The degree to which absorption occurs throughout the length of the intestine is an important consideration in the removal of fecal material for digestibility studies. If significant absorption occurs in the area from which the feces are removed, then the digestibility coefficients may show a lower value than actually exists.

## DISCUSSION

The protein digestion coefficients determined by collecting the excrement from the troughs are markedly greater than those obtained with the intestinal collection method. It is probable that all of the digestibility values calculated from the trough-collection data are higher than actually exist. This is due to the exposure of the feces to slowly moving water for periods up to 23 hours which presents the possibility that significant quantities of nutrients leached out. No estimate was made of the amount of leaching which might have occurred.

The relatively high apparent digestibility of starch may be due in part to partially hydrolyzed starch in the excreta going into solution in the trough and not appearing in the fecal analysis. Even assuming that these starch absorption values have been magnified by the excreta collection method, they are still two to there times larger than the

TABLE 3. Average Per Cent Protein in Unabsorbed Feed Taken from Four Areas of the Digestive Tract of Two-Year-Old Channel Catfish 18 Hours After Feeding.

| Ration | Ration Composition (Per Cent) | | | Area of Digestive Tract | | | |
|---|---|---|---|---|---|---|---|
| | Protein | Cellulose | Starch | Stomach | Upper Intestine | Lower Intestine | Rectum |
| 1 | 20 | 5 | 60 | 12.97 | 14.67 | 11.88 | 5.64 |
| 2 | 20 | 20 | 45 | 7.79 | 10.14 | 8.96 | 9.41 |
| 3 | 20 | 35 | 30 | 7.75 | 6.41 | 5.71 | 5.48 |
| 4 | 40 | 5 | 40 | 11.96 | 14.65 | 5.61 | 4.49 |
| 5 | 40 | 20 | 25 | 21.35 | 13.63 | 11.87 | 10.42 |
| 6 | 40 | 35 | 10 | 25.64 | 17.00 | 12.59 | 5.84 |



TABLE 4. Average Per Cent Apparent Digestibility of Protein in Semi-Purified Rations Determined from Excreta Collected from Four Areas of the Digestive Tract of Two-Year-Old Channel Catfish

| Ration | Ration Composition (Per Cent) | | | Area of Digestive Tract | | | |
|---|---|---|---|---|---|---|---|
| | Protein | Cellulose | Starch | Stomach | Upper Intestine | Lower Intestine | Rectum |
| 1 | 20 | 5 | 60 | 51.69 | 56.99 | 71.15 | 85.74 [1]* |
| 2 | 20 | 20 | 45 | 64.94 | 64.84 | 74.62 | 72.85 [2] |
| 3 | 20 | 35 | 30 | 68.05 | 74.38 | 78.88 | 81.05 [3] |
| 4 | 40 | 5 | 40 | 73.37 | 67.02 | 91.85 | 93.07 [4] |
| 5 | 40 | 20 | 25 | 64.13 | 83.06 | 88.09 | 90.49 [5] |
| 6 | 40 | 35 | 10 | 46.53 | 66.61 | 79.55 | 91.23 [4, 5] |

* Means with the same superscript are not significantly different at the 0.05 level. Means with different superscripts are significantly different.

values determined for trout (Phillips, 1948; Smith, 1971). These data indicate that catfish may be more adaptable to digesting unprocessed starches than trout. High levels of starch did not appear to hinder protein absorption in either experiment.

High levels of cellulose in the diet depressed starch digestion but did not show a consistent effect upon protein digestion. Smith (1971) fed a higher level, 50%, in diets to trout and got a profound reduction in protein digestion.

The fish from which the feces were taken from the intestines were under stress due to the unnatural feeding which could have affected digestion (Windell, 1966). Special measures were taken to minimize stress such as acclimatizing the fish prior to the experiment, anesthetizing the fish prior to handling, and forcing the trocar into the esophagus only once each day. The low plane of feeding, 1 per cent of body weight, undobutedly influenced the digestion coefficients in an upward direction. Nonetheless, the intestinal collection method is considered the superior of the two techniques used in this study. Because there was considerable difference in the protein digestion coefficients obtained in the two experiments, the trough collection method is not considered satisfactory for deriving this type of information.

Although the removal of fecal material from a lower section of the gut, either by stripping or sacrificing the fish, precludes the loss of undigested nutrients into the water, it presents another problem: Was the absorption of nutrients completed before the removal of the material from the tract? The fact that average protein digestion coefficients for several of the treatments were above 90 per cent is considered evidence that this fecal collection method was satisfactory. Hastings (1966) determined his digestion coefficients by removing all of the food residue from the lower one-third of the intestine, which included a markedly larger area than that sampled in this experiment. Data in table 3 show that considerable protein absorption occurred in the lower part of the intestine.

The higher apparent digestibility for protein in the high-protein diets may possibly be explained on the basis of level of endogenous nitrogen being removed from the gut along with the food residue. In determining apparent digestibility endogenous nitrogen, or nitrogen from the animal, is not distinguished from exogenous nitrogen, or that from the food. A significant amount of endogenous nitrogen in the fecal samples would lower digestion coefficients more for a low-protein diet than it would for a high protein diet.

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