Nutritional Value of Freshwater Crayfish Waste Meal

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Dried waste material, consisting of shell, muscle, and viscera, from freshwater crayfish processing plants was subjected to inorganic and organic analyses using an atomic absorption spectrometer and conventionally accepted methods of chemical analysis of feedstuffs. Three methods were compared for chitin determination. True digestibility of protein in crayfish waste meal was compared with that of methionine-supplemented soy protein. Ten collections of crayfish waste had the following mean composition values: calcium, 18.1%; phosphorus, 1.2%; manganese, 157 p.p.m.; magnesium, 2656 p.p.m.; potassium, 1400 p.p.m.; iodine, 1313 p.p.m.; iron, 8.8 p.p.m.; chitin-free protein, 32.2%; ether extract, 4.9%; ash, 29.0%; and crude fiber, 14.2%. The most accurate method of chitin determination (slow, 24 hours at room temperature, decalcification with 5% hydrochloric acid followed by reflux over steam with 5% sodium hydroxide for 1 hour) showed crayfish meal to contain 14.1% chitin; 19.8% of the total nitrogen was in the form of chitin. Digestibility coefficients for chitin-free protein in crayfish waste and soy protein were 87.54 and 86.61%, respectively.

Freshwater crayfish are a commercially important food product in Louisiana and in adjoining areas along the Gulf Coast. The Bureau of Commercial Fisheries (1966) reported a commercial catch of 8.6 million pounds of crayfish in Louisiana, and Spath (1966) listed 34 licensed crayfish processing plants in the state. Presently, the waste material, which approximates 85% of the live weight of freshwater crayfish, from the processing plants has no commercial value and its disposal is an expense to the industry.

Although no research had been conducted with waste from the processing of freshwater crayfish, data have been published concerning chemical composition and nutritional value of other crustacean wastes and their use as animal feeds. Shrimp meal (Brown, 1959; Goodwin and Srisuckh, 1949) and crab meal (Lubitz *et al.*, 1943; Parkhurst *et al.*, 1943) have been processed commercially in this country and fed to fish and poultry, and salt-water crayfish meal has been manufactured for poultry feed in South Africa (Black and Schwartz, 1950). An analogous presumption would be that crayfish meal would have nutritional qualities comparable with those of other crustacean meals. Since no information is available from which estimates of the nutritional value of crayfish waste meal can be made, a study was conducted to determine chemical and biological properties of the material which would be useful in estimating its feeding value.

All feedstuffs which contain crustacean shells contain chitin, an acetylated glucosamine polymer, which is indigestible and has no protein value for monogastric animals. The chitin concentration in crustacean meals, which may vary from 10 to 20% (Lubitz *et al.*, 1943), must be quantitatively accounted for to predict accurately the protein value of the meal. Several procedures have been used to analyze for chitin in crustacean shells (Black and Schwartz, 1950; Brown, 1959; Giles *et al.*, 1958; Lubitz, *et al.*, 1943), although none has been universally accepted. Enzymatic methods of removing the proteinaceous or the chitinous nitrogen have been reported (Giles *et al.*, 1958); however, these were less satisfactory than methods involving acid decalcification and alkaline hydrolysis of the nonchitinous organic matter. Three methods for measuring chitin in the crayfish meal were compared in this study.

EXPERIMENTAL METHODS

Ten collections of waste material were obtained from two processing plants representing crayfish of varying sizes, from several production areas and harvested at different periods during the season. The waste was dried in a convection oven at 85° C., ground first in a Wiley mill through a 2-mm. screen, and then powdered with a ball mill.

Mineral Analyses. Duplicate samples from 10 batches of meal were ashed at 550° C. for 12 hours and analyzed for several inorganic materials. Calcium, magnesium, manganese, iron, and potassium were analyzed with a Perkin-Elmer atomic absorption spectrometer (Perkin-Elmer, 1964). Phosphorus and iodine were analyzed according to methods of the Association of Official Agricultural Chemists (1965).

Crude Protein. Crude protein was determined by multiplying the nonchitinous nitrogen content of the meal by 6.25. Nonchitinous nitrogen represented the difference between total nitrogen and chitinous nitrogen as determined by the procedures described below. Nitrogen was determined by the Kjeldahl method. Nonchitinous nitrogen is assumed to be essentially in the form of protein.

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Chitin Determination. FORMIC ACID METHOD. This procedure is similar to that used by Brown (1959) with shrimp. One hundred milliliters of 90% formic acid and 10 grams of fat-free crayfish meal were agitated at room temperature for 24 hours in a 250-ml. centrifuge tube. The tubes were centrifuged at 2000 r.p.m. for 20 minutes, and the acid and solubilized calcium were decanted. The residue was washed and recentrifuged with 100 and 70% acetone, refluxed for 90 minutes with 5% sodium hydroxide, and filtered and washed with water over ashless 11-cm. filter paper. The residue was dried, weighed, ashed at 550° C. for 24 hours, and reweighed. The organic material in the residue was assumed to be intact chitin.

HOT HYDROCHLORIC ACID. The formic acid method was followed except decalcification was brought about by refluxing the samples with 1*N* hydrochloric acid, instead of formic, on a steam bath for 1 hour, as described by Black and Schwartz (1950) with salt-water crayfish meal.

COLD HYDROCHLORIC ACID. The formic acid procedure was followed except 5% hydrochloric acid was used for decalcification instead of formic acid.

Comparison of the Three Chitin Determination Procedures. Three criteria were used to compare the effectiveness of the three chitin analysis methods. These were the acetic acid solubility, nitrogen content, and ash content of the chitinous residue following extraction by one of the three procedures. Four samples of each of the 10 collections of meal were carried through the three extraction processes and the chitinous residues recovered, dried, and weighed. Two of the residue samples from each extraction procedure were refluxed for 1 hour on a steam bath in 5% acetic acid, filtered, and reweighed. Assuming that any deacetylated chitin would be solubilized by the acetic acid, a loss in weight would indicate deacetylation by the chitin determination procedure. The other samples of the chitinous residue were analyzed for nitrogen by the Kjeldahl procedure. Nitrogen values above the theoretical nitrogen content of chitin would indicate insufficient removal of protein; values below the theoretical percentage would indicate loss of the acetamido group, or that the chitin molecule did not remain intact.

The ash content of the residue is the difference in weight before and after ashing as described above. Incomplete decalcification would result in a high percentage of ash in the residue.

Proximate Composition of Crayfish Meal. The 10 collections of crayfish meal were analyzed in duplicate for proximate composition using Association of Official Agricultural Chemists methods (1965).

Digestibility of Nitrogen in Crayfish Meal. The availability of total nitrogen and chitin-free nitrogen in crayfish meal was compared with the nitrogen availability in methionine-supplemented soy protein (90%, Protamine-D, Central Soya) by feeding 100-gram rats the semipurified rations shown in Table I. Purified soy protein is a conveniently reproducible reference protein which, when supplemented with methionine, provides a satisfactory amino acid ratio for the rat (National Academy of Sciences–National Research Council, 1962). Four groups of eight rats each were randomly assigned to experimental ration 1, 2, 3, or 4. The rations contained either crayfish meal or soy protein as the only nitrogen source with empirical allowances of 4 and 8% of uncorrected crude protein. The rats received the four experimental rations according to a feeding regime which consisted of a 4-day adjustment period followed by a 7-day collection period in which feed consumption was measured and feces were collected. The rats were then placed on one of the nitrogen-free rations in which the crayfish meal and soy protein were replaced with Alphacel and sand, for a 3-day adjustment period and a 5-day collection period. The nitrogen-free rations were fed to determine endogenous nitrogen excretion. Nitrogen contents of the feed and feces were determined by the Kjeldahl method, and true digestibility was calculated according to the method described in Hawk *et al.* (1954).

Table I. Composition of Experimental Rations

Ingredient	Ration, %[a] 1	2	3	4	N-free 1, 3	N-free 2, 4
Corn starch	76.5	62.5	76.4	65.0	76.5	62.5
Vegetable oil	3.0	7.0	3.0	7.0	3.0	7.0
Sucrose	10.0	10.0	10.0	10.0	10.0	10.0
Crayfish meal	10.0	20.0	...	...	...	...
Soy protein	...	...	4.4	8.8	...	...
Methionine	...	...	0.12	0.24	...	...
Alphacel	...	...	...	...	4.4	8.8
Mineral mix[b]	3.0	3.0	3.0	3.0	3.0	3.0
$CaCO_3$	...	...	4.5	9.0	4.5	9.0
NaH_2PO_4	0.7	1.4	0.7	1.4	0.7	1.4
Vitamin mix[c]	2.2	2.2	2.2	2.2	2.2	2.2
Sand	...	...	1.1	2.2	1.1	2.2

[a] Ration 1. 4% uncorrected crude protein from crayfish meal. Ration 2. 8% uncorrected crude protein from crayfish meal. Ration 3. 4% uncorrected crude protein from soy protein. Ration 4. 8% uncorrected crude protein from soy protein. [b] Nutritional Biochemicals Co. Ca-free mineral mix. [c] Nutritional Biochemicals Co. complete vitamin fortification mix.

RESULTS AND DISCUSSION

Chemical analyses which are indicative of the nutritional quality of crayfish waste meal are summarized in Table II.

Table II. Average Composition of Fresh-Water Crayfish Waste Meal[a]

Component	Quantity Present in Meal	S.D.
Minerals		
Magnesium, p.p.m.	2656.0	196.21
Manganese, p.p.m.	157.0	9.73
Potassium, p.p.m.	1400.0	83.45
Iodine, p.p.m.	1313.0	253.22
Iron, p.p.m.	8.8	2.89
Calcium, %	18.1	1.08
Phosphorus, %	1.2	0.39
Corrected crude protein, %	32.2	2.44
Ether extract, %	4.9	0.27
Chitin, %	14.1	0.98
Crude fiber, %	14.2	1.06
Ash, %	29.0	1.70

[a] Each determination was the average of 10 samples.

Minerals. Crayfish meal contains a high concentration of calcium (as calcium carbonate) and should be a good supplemental source of this element. It is not as good a source of supplemental phosphorus as meat or bone meal; however, it is relatively well fortified in the other elements investigated. The ash percentage of crayfish meal is not an authentic estimate of the nutritionally useful inorganic elements in the material. This value overemphasizes the mineral value of crayfish meal in that approximately 30% of the ash is carbonate, since calcium carbonate is not completely pyrolyzed at 550° C. By subjecting reagent grade calcium carbonate to these ashing conditions, there was only 10.8% weight loss.

Corrected Crude Protein. The average uncorrected crude protein content of the meal as determined by proximate analysis was 40.1%. However, the extracted chitin contained 19.8% of the total nitrogen of the meal leaving 80.2% of the total nitrogen presumably in the form of protein. Hence, the nonchitinous nitrogen multiplied by 6.25 gives 32.2% corrected crude protein. This compares very closely with the corrected crude protein value for crab meal which was 32.7% as reported by Lubitz *et al.* (1943).

Chitin. The average chitin percentages of the crayfish meal for each determination procedure are presented in Table III. The criteria for evaluating the effectiveness of each procedure are compared in Table IV. The per cent ash in the residue indicates that the two hydrochloric acid decalcification procedures removed more of the inorganic material from the samples than did formic acid. The per cent nitrogen in the residue from the cold hydrochloric acid procedure was nearest to the theoretical percentage of nitrogen for chitin which is 6.89. The per cent acetic acid-soluble material was least in the cold hydrochloric acid-treated samples. These three criteria indicate that the cold hydrochloric procedure is the preferred method of extracting chitin from crayfish waste meal. Formic acid, apparently, was not as effective for decalcification and caused loss of acetamido groups. The hot hydrochloric acid and the formic acid seemingly caused more deacetylation of the chitin than did the cold hydrochloric acid. Hence, the value of 14.06 as determined by the cold hydrochloric acid procedure is considered to be the most accurate measurement of the chitin percentage of the crayfish meal samples. This value compares with 12.9% for crab (Lubitz *et al.*, 1943), 12.3% for salt-water crayfish (Black and Schwartz, 1950), and 7.6% for shrimp (Brown, 1959).

By comparing chitin percentage with that of crude fiber as determined in proximate analysis (Table II), crude fiber apparently is an accurate measure of chitin in crayfish meal. Black and Schwartz (1950) and Lubitz *et al.* (1943) reported crude fiber to be a good estimator of chitin.

Protein Digestibility. Table V shows the crude protein percentages and digestibility coefficients for the experimental rations. Uncorrected crude protein was calcu-

Table III. Chitin in Crayfish Meal Determined by Three Methods and Expressed as Ash-Free Residue Following Decalcification and Extraction of Nonchitinous Organic Matter

Method	% Ash-Free Residue		
	Av.[a]	S. D.	F test[b]
Formic acid	16.71	4.48	$P < 0.05$
Cold HCl	14.06	0.98	
Hot HCl	15.78	2.08	

[a] Average of 10 samples.
[b] F test $P < 0.05$ means that the three means are significantly different.

Table IV. Mean Percentages of Ash, Nitrogen, and Acetic Acid-Soluble Material in the Chitinous Residues Using Three Chitin Determination Methods

Method	% Ash			% Nitrogen			% Acetic Acid Soluble		
	Av.[a]	S. D.	F test[b]	Av.	S. D.	F test	Av.	S. D.	F test
Formic acid	3.04	1.00	$P > 0.01$	4.9	0.97	$P > 0.01$	8.38	6.67	$P > 0.05$
Cold HCl	1.65	0.23		6.7	0.14		1.65	1.83	
Hot HCl	1.24	0.14		6.5	0.22		8.86	6.25	

[a] Average of 10 samples.
[b] F test $P > 0.01$ or $P > 0.05$ means that the three means are significantly different.

Table V. Per Cent Crude Protein and Mean Digestibility Coefficients for Crayfish Meal and Soy Protein Rations[a]

Ration	% Uncorrected Crude Protein	% Corrected Crude Protein	% Digestibility			
			Uncorrected Protein		Corrected Protein	
			Av.	F test[b]	Av.	F test
Crayfish, 4%	4.70	3.77	72.56	$P > 0.01$	88.52	N.S.
Crayfish, 8%	8.40	6.74	70.24		86.55	
Soy protein, 4%	5.10	5.10	87.37		87.37	
Soy protein, 8%	8.50	8.50	85.74		85.84	

[a] Eight animals used in each test.
[b] F test $P > 0.01$ means that there is a significant difference among the four means; N.S. means that the four means are not significantly different at $P > 0.05$.

lated as total nitrogen × 6.25. Corrected crude protein was calculated on the premises that all of the nitrogen in the rations was contributed by crayfish meal or soy protein, and 100% of the nitrogen in soy protein and 80.2% of the nitrogen in crayfish meal was in the form of protein. The uncorrected crayfish protein was approximately 82.5% as digestible as amino acid–supplemented soy protein. When corrected for chitinous nitrogen, the crayfish protein was essentially equal in availability to the soy protein (assuming that chitin is indigestible to the rat).

These chemical and biological data indicate that crayfish waste meal has significant value as an animal feed supplement. A more precise evaluation of its nutritional value will be possible with amino acid analyses, which will be pursued here in the future. Another area which needs further study concerns the fact that the amount of crayfish meal which can be added to animal rations will be limited by the calcium content of the meal. As crayfish meal contains approximately 18% calcium, the maximum percentage of meal recommended in a productive ration formula should perhaps not exceed 10% of the total composition; however, more exact values should be determined for various feeding regimes.

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