nonirradiated samples, in both Visking and saran casing, was stored for 76 days. At the end of this time the sample in Visking casing was brown, dried out, and completely unacceptable. The sample in saran casing was a somewhat light purple color, which changed to a good bright red color when exposed to air.

Spectral curves showed the samples in saran to contain mainly oxymyoglobin, while those in Visking contained mainly metmyoglobin. Presumably any reduced myoglobin obtained from samples stored in saran was converted to oxymyoglobin in the course of preparing the extracts.

The browning (formation of metmyoglobin and related compounds) of the irradiated meat in Visking casing during storage under these conditions is presumably due in part to dehydration of the samples. The shelf life of fresh beef irradiated with low dosages of gamma rays (60,000 rep) and stored in a moist chamber, however, was extended fivefold over controls held under identical conditions (Felton and Niven, *11*).

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FEED DIGESTIBILITY

Chemical Method for Measuring Relative Digestibility of Animal Protein Feedstuffs

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A simple enzymatic method is useful in determining the relative digestibility of meat and fish by-products used as feedstuffs. The indigestible residues may be studied microscopically for identification of most of their constituents, which consist predominantly of vegetable fiber, hoof, colloidal organic matter, hair, fuzz, and charred meat in various proportions. Indigestible residues in meat scrap range from 2 to 25%, seldom exceeding 10% in samples of good quality. Limited studies on fish meal show a high degree of digestibility unless the product has been overheated. Blood meal is highly digestible when properly processed, but may be much less digestible if overheated in the drying process. Reproducibility of the method is good, with less than 1% difference between duplicate samples.

FEED MANUFACTURERS throughout the country are becoming increasingly concerned over quality of ingredients going into manufactured feeds. It is common for feedstuffs to be bought on specification and for feed manufacturers to analyze them for moisture, protein, fat, fiber, and in many cases mineral elements and certain vitamins. Microscopic examination of feed ingredients is being used increasingly as a tool in improving quality of manufactured feeds.

However, the usual chemical analyses and vitamin assays do not tell the whole story of feedstuff quality and much effort is being expended by feed manufacturers to find other measures of quality control.

Meat scrap, meat and bone scrap, and tankage are by-products of the packing and rendering industries and their specifications have been concerned mainly with crude protein, fat, fiber, and phosphorus. Useless or contaminating ingredients such as hoof, hair, manure, and stomach contents have been covered by the stipulation that the maximum permissible quantities must not be greater than "might occur unavoidably in good factory practice." Such specifications do not mention digestibility of the protein, which is one of the determining factors in the nutritional value of the feedstuff. This is especially important in those meat scrap and tankage samples which contain

appreciable quantities of hoof and hair, as these contaminants are very high in protein but nevertheless indigestible.

In an effort to provide a rapid method for determining the amounts and nature of indigestible fractions of animal proteins, a method was developed involving the action of a warm acid solution of pepsin on the fat-free sample. This pepsin solution dissolves the digestible protein of the meat, leaving indigestible residue, which can be studied further.

The method used in this laboratory is a modification of those of Sterling (*2*) and Almquist and coworkers (*1*). The Sterling method is concerned primarily with the detection and quantitative evaluation of hoof meal in animal by-products, with little attempt to identify other indigestible components; the heavy fraction of the sample is not studied. The Almquist method involves a pepsin digestion as a part of a system for evaluating protein quality, but no attempt is made to study the nature of the indigestible residues—an important feature of the method described below.

The entire procedure consists of four major steps:

1. Extraction of the fat from the original sample.
2. Separation of meat and light impurities from bone and heavy impurities by flotation in carbon tetrachloride.
3. Digestion of the meat fraction (including light impurities) and determination of residue.
4. Determination of heavy impurities.

Apparatus

Glassware needed for these analyses consists of 125-ml. Erlenmeyer flasks and 15- and 50-ml. centrifuge tubes. A small funnel of the Büchner or Hirsch type, to which suction may be applied, is used for final filtration of the indigestible residue. For evaporation of the solvents and for suction filtration an aspirator is needed.

A centrifuge is very desirable for washing the residue, as the digestion mixture is much easier to centrifuge than to filter. The centrifuge is also convenient for making the preliminary fat extraction. However, any accepted method of fat extraction may be used.

Procedure

Fineness of Sample. Because very fine grinding may increase the digestibility of the indigestible portion, it is best for the sample to be in approximately the same state of subdivision as it will be when incorporated into the mixed feed or concentrate.

Most protein ingredients are ground by the manufacturer to pass an 8-mesh sieve and are mixed into the feeds without further size reduction. As this is sufficiently fine to permit fairly accurate sampling, these analytical samples are not ground further. Avoidance of fine grinding also facilitates microscopic identification of the residue.

Fat Removal. Place 1.00 gram of the sample in a 15-ml. centrifuge tube and add 10 ml. of diethyl ether, such as is used in fat determinations. Allow to stand 10 minutes with frequent agitation and centrifuge 5 minutes at 2000 to 3000 r.p.m. Pour off the clear supernatant liquid into a weighed 125-ml. Erlenmeyer flask. Repeat the extraction with four 5-ml. portions of ether, stirring and centrifuging each time and pouring all extracts into the Erlenmeyer flask. Save both the residue and ether extract. Evaporate the ether from the extract under vacuum from a warm water bath (40° to 50° C.) and drive off the last traces of solvent and moisture by placing the flask in an oven at 100° to 110° C. for 30 minutes. Cool in a desiccator and weigh the flask and contents.

Nature of Fatty Extract. This material represents the ether-soluble portion of the sample—i.e., crude fat. It is not claimed that this method of fat determination will be as accurate as the long-time extraction used in the official method. However, experience has indicated that the methods agree well. Removal of the fat prior to the pepsin digestion eliminates the possibility that some of the fat might appear in the indigestible residue.

Separation of Meat from Bone and Heavy Impurities. Air-dry the defatted sample in the centrifuge tube until free from ether odor (allow it to remain in the tube), then add 10 ml. of carbon tetrachloride (purified or N.F. grade). Agitate until completely suspended and centrifuge 5 minutes. Pour off the upper layer into a dry 125-ml. Erlenmeyer flask and repeat the separation with another 10-ml. portion of carbon tetrachloride, again adding the upper layer to the Erlenmeyer flask. Save both light and heavy fractions.

Nature of Fractions. The material floated by the carbon tetrachloride consists of meat, hoof, fuzz, hair, vegetable fiber (paunch or stomach content), and any other material lighter than carbon tetrachloride. The heavy residue is made up of bone, sand, glass, and other heavy materials.

Treatment of Light Fraction (Meat and Light Impurities). Evaporate the solvent under vacuum in a warm (60° to 70° C.) water bath until the residue is free of solvent odor. Add 50 ml. of a freshly prepared solution of 0.1*N* hydrochloric acid containing 0.1 gram of pepsin (U.S.P or N.F.) Stopper and incubate at 37° C. for 40 to 48 hours. Agitate at least once every 12 hours by swirling the flask. Transfer quantitatively to a tube and centrifuge 5 minutes; decant and wash the residue twice with warm water and once with denaturated alcohol (such as Shellacol or Solox). Pour off and drain. Add 5 to 10 ml. of alcohol and filter quantitatively on a Hirsch funnel. Wash with alcohol and suck dry. Transfer the residue and filter paper to a 50-ml. beaker and dry in the oven 30 minutes at 100° to 110° C. Brush or gently scrape the residue from the filter paper onto a watch glass and weigh. Examine under the microscope, and if desired determine protein by the Kjeldahl method.

Nature of Residue. This residue is the pepsin-indigestible portion of the light fraction. It may consist of hoof, fuzz, hair, vegetable fiber, colloidal organic matter, and what appears to be charred or overheated meat. Feeding tests of this residue were made with weanling rats, using a semipurified diet complete in known vitamins and minerals and containing 10% total protein. Fifty per cent of the protein was derived from the test material, 25% from corn, and 25% from soybean oil meal. Feed and water were given *ad lib.* Results after 3 weeks, shown in Table I, indicate conclusively that the indigestible residue had no value for growth.

Table I. Feeding Test on Pepsin-Indigestible Residue

(Rats, 3 weeks)

Protein Tested	Protein, % From basal	Protein, % From test source	Total Feed per Rat	Av. Initial Weight	Total Gain, 3 Weeks
Negative control	5	0	89.0	47.0	−1.0
Pepsin-indigestible residue, from meat scrap (below)	5	5	101.3	47.3	3.0
Meat scrap	5	5	146.0	48.3	24.3
50% solvent-extracted soybean oil meal	5	5	159.0	48.0	41.3

Treatment of Heavy Fraction. Air-dry the heavy residue from the carbon tetrachloride flotation in the centrifuge tube. Add 10 ml. of 1 to 1 hydrochloric acid, agitate thoroughly, and heat 30 minutes in a boiling water bath. Remove from the water bath and centrifuge 5 minutes. Decant and add 10 ml. of distilled water. Stir, centrifuge, decant, and drain. Wash once more with water and once with alcohol, centrifuging each time. Drain, dry in the oven (110° C.), and weigh the residue. Examine under a microscope.

Nature of Residue. The 1 to 1 hydrochloric acid completely dissolves the bone, leaving only the heavy impurities, usually glass and sand. A few samples have shown some black cinderlike material which is believed to be thoroughly scorched meat. These materials, including the charred meat, are valueless, as has been shown by rat feeding tests.

Precision of Method

Reproducibility of the results has been checked by analyzing in duplicate 39 samples including meat scrap, fish meal, and blood meal. The percentage of pepsin-indigestible residues was found to vary by less than 1% between duplicates. This agreement is satisfactory, considering the possible introduction of a slight sampling error due to the relative coarseness of the feedstuff.

Results

In this laboratory more than 500 samples have been examined by this method, including meat scrap, tankage, fish meal, and blood meal. Some typical analytical results are shown in Table II.

Wide variations among samples, both quantitative and qualitative, were observed. Indigestible residues in meat scrap ranging from 2 to 25% have been found; however, a product of good quality will seldom have more than 10% of indigestible material. The composition of the residues also varies with the producer and sometimes with different shipments from the same producer. The residues from some suppliers consist mainly of vegetable fiber and fuzz; those from other suppliers contain predominantly hoof and hair with very little vegetable fiber. Photomicrographs of the various components of the residues are shown in Figure 1.

As shown in Table II, fertilizer tankage was high in indigestible matter, with vegetable fiber, hoof, and horn making up practically the entire residue.

Limited studies showed fish meal to be low in indigestible matter when properly processed. However, several samples which had overheated spontaneously during railway transit contained extremely high percentages of indigestible material (see Table II).

As might be expected, blood meal is highly digestible when properly processed. However, a few samples contained appreciable quantities of the common meat scrap contaminants—hair, hoof and horn, vegetable fiber, etc.—as also shown in the table. Recently a series of studies has been begun, which indicates that excessive heating of blood in the drying process may cause a serious decrease in its digestibility as measured by the above test. This work is being checked with feeding tests and will be reported if proved to be correct.

Discussion

During the early part of the investigation the pepsin-meat scrap mixture was

Table II. Typical Results of Pepsin Digestion Test

	Meat Scrap				Fish Meal		Blood Meal	
	Good quality	Fair quality	Poor quality	Fertilizer Tankage	Properly cooked	Overheated in transit	Good quality	Poor quality
Fat, %	8.6	12.1	5.7	10.8	9.9	6.7	0.1	0.3
Pepsin-indigestible residue (from light fraction), %	4.2	11.8	20.6	30.0	8.5	43.8	0.3	13.8
Acid-insoluble residue (from heavy fraction), %[a]	1.1	0.3	2.4	1.1	0.8	0.6	0.1	0.7
Protein, %								
In original sample	52.8	57.1	52.0	49.9	60.9	61.7	89.3	75.3
In indigestible residue	45.8	37.5	63.0	52.5	59.7	80.6	...	88.2
Feedstuff protein digested by pepsin, %[b]	96.4	92.3	75.0	68.4	91.6	43.0	99.7	83.9
Approximate Percentage Composition of Pepsin-Indigestible Residue (Microscopic Estimation)								
Vegetable fiber[c]	50	55	25	60	0	0	...	5
Brown colloidal matter[d]	15	15	20	0	100	0	...	5
Hoof and horn	5	10	25	30	0	0	...	5
Hair	5	15	10	Trace	0	0	...	80
Fuzz (fluff)[e]	25	5	Trace	0	0	0	...	5
Charred meat	0	0	20	10	0	100	...	0

[a] Any material from heavy fraction not dissolved by 1:1 HCl. Glass and sand predominate in most samples, although a few contain black cinderlike material, believed to be thoroughly scorched meat.

[b] Calculated from data in table as follows:

From meat scrap, good quality

4.2% (pepsin-indigestible residue) × 45.8% (its protein content) = 1.9% (indigestible protein)

52.8% (total protein in sample) − 1.9% = 50.9% (digestible protein)

Then $\frac{50.9\%}{52.8\%} \times 100 = 96\%$ feedstuff protein digested by pepsin

[c] Primarily straw and other fibrous material from paunch or intestinal tract of animal, introduced by improper cleaning of paunch and intestines prior to cooking.

[d] Proteinaceous material, probably indigestible parts of carcass which have lost their cellular or fibrous structure in cooking process.

[e] Very fine individual transparent fibrous material, usually colorless but sometimes slightly amber. It is much finer than hair and does not possess irregular lateral microscopic structure of wool. High in protein (65 to 70%).

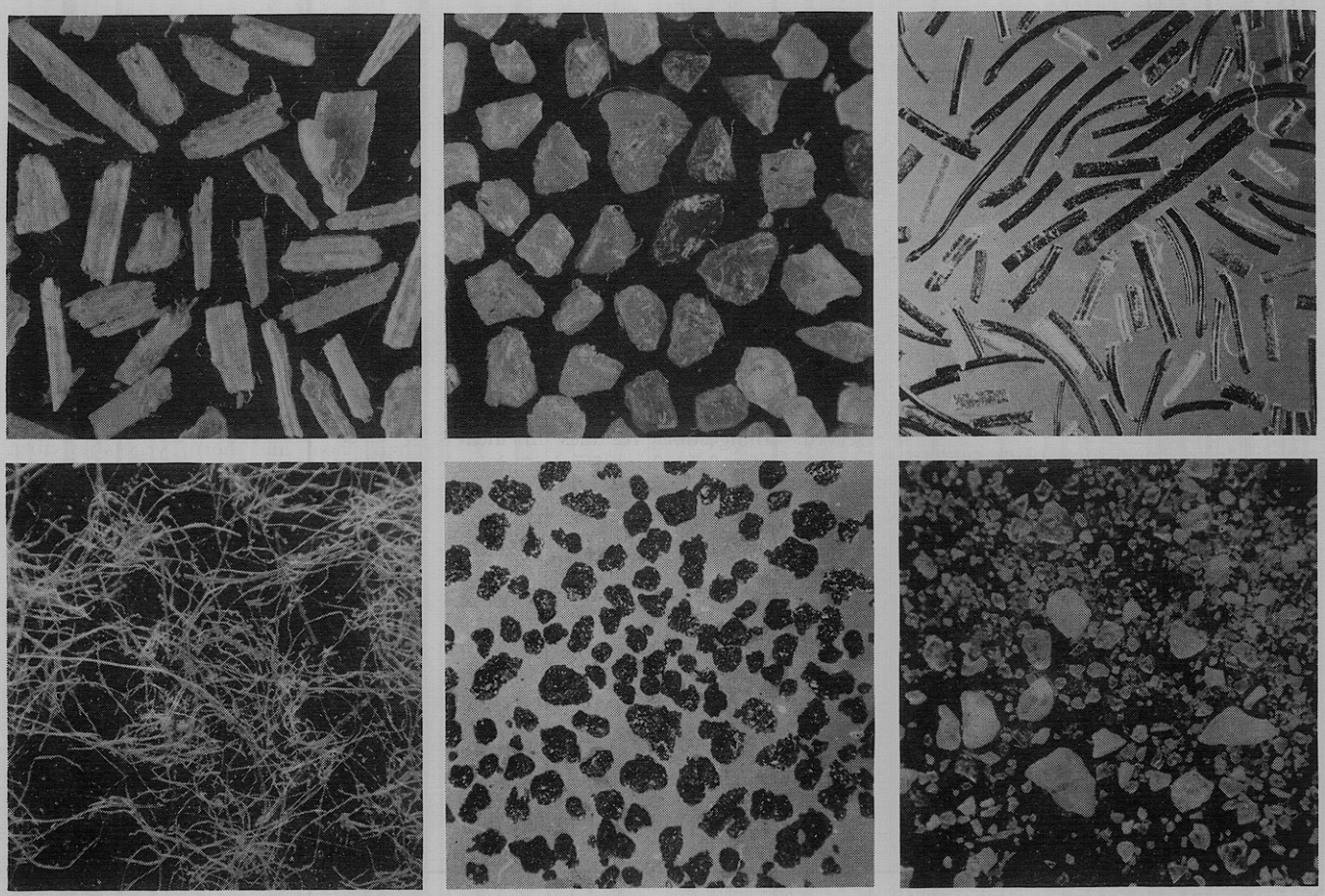

Figure 1. Photomicrographs showing common indigestible components of meat scrap and tankage (X7.5)

Top row, left to right. Vegetable fiber. Hoof and horn. Hair, showing some bulb formation at root end
Bottom row. Fuzz (fluff). Scorched meat. Glass and sand

neutralized after incubation, as this was a regular part of the Sterling (*2*) procedure. However, in certain samples neutralization of the mixture precipitated material that had been dissolved by the acid-pepsin solution. To study this phenomenon, approximately 20 samples from various suppliers were run in duplicate. After incubation, one member of each pair was neutralized and centrifuged, while the other was centrifuged before neutralization to separate the acid-insoluble fraction from the neutral-insolubles. The supernatant liquid from the unneutralized sample was transferred to another centrifuge tube and neutralized, and the precipitated residue separated by centrifuging. The results of this study indicated that the neutralization has a rather small effect on approximately two thirds of the samples. However, in six of the meats tested a considerable quantity of additional material precipitated upon neutralization. Microscopic examination of this material (acid-soluble, neutral-insoluble) showed it to be an amorphous powder, tan to medium brown when dry. It was analyzed chemically and found to contain 41% protein and 38% ash. The ash contained 18.7% phosphorus, equivalent to 93.7% $Ca_3(PO_4)_2$. The composition of this powder and the conditions by which it was obtained indicate that it consists of colloidal proteins plus calcium phosphate which was carried into the light fraction as finely ground bone covered sufficiently by meat to be floated by the carbon tetrachloride. The bone would be dissolved by the hydrochloric acid during the pepsin-digestion and as it reprecipitates on neutralization of the acid, it would carry down some of the colloidal organic matter to yield a product similar in appearance and composition to the powder described above.

Although the method described in this paper does not duplicate exactly the digestive processes as they occur in the animal body, it has proved to be a valuable tool in comparing the quality of meat scrap from various producers. Microscopic examination of the indigestible residues allows positive identification of most of the constituents present, which is the first step in understanding the problems involved in their reduction.

The quality of a given meat scrap is not solely dependent on its percentage of digestible matter. Differences in amino acid content or availability are fully as important as differences in digestibility. Furthermore, heating in excess of that required for production of a sterile and stable product or overheating during the grinding operation may reduce the nutritional value. For these reasons it is not to be expected that this or any similar method will correlate perfectly with animal feeding tests. Nevertheless, any method which gives a quantitative measure of the indigestible and therefore valueless portions of a meat scrap, and which makes possible their identification by microscopic techniques, has a real place in the feedstuffs laboratory. The test described in this paper furnishes such a method, and is proving to be a valuable tool in the study and improvement of animal protein feeds.

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