IN VIVO AND IN VITRO EVALUATION OF IMMATURE SORGHUM GRAIN FOR POULTRY

by

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INTRODUCTION

Sorghum (Sorghum bicolor) is a popular multipurpose crop grown on all six populated continents. Originating in what is now Ethipoia and the Sudan, it is consumed by animals and humans as a cereal or fermented to be drunk as a beverage. Some varieties are raised to produce sugar and syrup; others provide forage and silage for livestock. Some are weeds.

In the United States the production of grain sorghum has increased rapidly since 1940. Between 1940 and 1968 the average yield rose seven-fold. In 1956, the crop was 170 million bushels; in 1976 the crop approached 710 million bushels. Between 1956 and 1976 the production in Kansas increased from 2,440,000 bushels to 153,750,000 bushels (1,2).

The reason for sorghum's success in cattle feeding areas such as Texas, Oklahoma and Kansas is its tolerance to adverse growing conditions in these areas. It thrives on less water than corn requires, making it a suitable crop for nonirrigated areas of the Southwest. Hybrids now available make it easier to suit the maturity time, insect and disease tolerance and other plant characteristics to the particular growing area.

Yet with all the advantages of the crop, an early freeze, delays in planting due to drought, muddy fields, or field infestations of any sort may result in a harvest of lower quality, low test weight sorghum. Such grain is discounted when sold, but little is known of the actual nutritive value. There is conflicting evidence as to the availability of the protein and to the amino acid balance. The information as to the energy availability is also limited.

The most common method of determining food value of grain is with animal feeding trials. These are difficult, entailing large numbers of animals for most accurate results, large sample sizes, and relatively long periods of time. This makes it impractical for the feeder or mill operator to evaluate grains prior to use.

In vitro tests are employed by some researchers in an attempt to cut the time and space required for feed evaluation. There are currently several in vitro tests which correlate well with ruminant nutrition, but few tests correlating to poultry, swine, humans and other monogastric animals exist.

This was the purpose of the study: to develop an in vitro test which could, when applied to feeds, give reasonable estimates of the protein, total dry matter, and energy digestibility for monogastrics. Such a test should correlate well with animal growth studies. Tests should then be applied to sorghums of different maturities to study possible differences in their nutritive values.

REVIEW OF LITERATURE

The term immaturity may encompass a multitude of conditions in cereal grains. Properly, immature grain is grain which has not achieved physiological maturity. However, depending on what the researcher called the material, the literature refers to light test weight grain, high moisture grain and frozen grain as well as immature and early harvested grain. Immature grain has a low test weight and when harvested will have more moisture that will mature grain. Grain may be mature or immature when frozen, but if frozen early in development will h ave the same characteristics as early harvested grain. The term immature will then be cautiously applied in this review to all forms of non-mature grain.

Accumulation of Nutrients During Growth

Vanderlip (3) divided growth of sorghum plants into nine stages. These stages vary somewhat in length with variety and environment. Grain formation begins in stage six. Dry matter in the head and grain continues to accumulate through stage nine, physiological maturity. Many authors use the cessation of dry matter accumulation as the point of maturity. In corn, it was found (4) that dry matter accumulation ceases at about 40% moisture, after which drying of the grain continues.

Protein also accumulates during grain development, but differences in expressing protein may lead to confusion in the literature. When expressed as percent of sample or percent of dry matter, protein levels are at a maximum early in development and decrease steadily throughout growth (5,6,7,8). Kersting (6) noted that in sorghum grain that while nitrogen as percent of sample was at a maximum 3 to 6 days after emergence,

it actually accumulates in the kernel as long as dry matter does. Both Hopper (5) and Brenne (7), working with corn, found the percent of protein decreased steadily until maturity. Flint and dent corn showed the same trends (5). Thorton et. al. (8) in work with corn, showed that as a percent of dry matter, protein decreased until mid-dent stage, after which it remained constant. The types of proteins accumulating during maturity were not all the same, however. In the same study on composition, Thorton et. al. (8) found that certain amino acids, notably lysine and tyrosine, decreased; serine, proline, leucine and glutamic acid increased.

Apparent decreases in percent protein in grains do not continue throughout development. Olson and Gastler (10) found that corn protein decreased from 20% to 13% in the first twenty days after silking, then remained constant. The protein content associated with cornstarch itself decreased until twenty-two days after pollenation, and the dry matter content continued to increase until forty-three days (11,12). Protein which actually increased in weight during the development of the grain (8, 13) may have reached a point where it was being laid down in a constant proportion to the starch and other nutrients. This last-laid-down protein was in the form of storage proteins, which formed the structure with which the starch associated (14).

These storage proteins are poorer biological quality than other proteins in the grain (14). The quality of total proteins would then be expected to decrease as the proportion of storage protein increased. Several researchers have reported that the percent of lysine, usually the limiting amino acid in cereal grains, decreases within the crude protein as the kernel matures. The results in wheat (15), corn (8), and grain sorghum (16, 17) were the same. Pomeranz et.al. (15) found

that in wheat aspartic acid, glycine, alanine and valine decrease, and glutamic acid and proline increase with maturity. Thorton et. al. (8) also reported increases in glutamic acid and proline in corn, as well as histidine, serine, leucine and phenylalanine, and decreases in tyrosine, aspartic acid, alanine and lysine. Sorghum analyses (16,17) showed decreases in lysine, aspartic acid and glycine and increases in proline, leucine, tyrosine and phenylalanine as grain matured. Some workers (15,18) have noted that free amino acids, nonprotein amino acids, and nonprotein nitrogen compounds were higher in immature than mature wheat.

Protein is, of course, only one of the components in grain whose proportion affects nutritional value. Starches and sugars, making up the so-called nitrogen free extract (NFE) portion of the proximate composition (19), represent energy available in the grain. The weight increases in later stages of development are largely starch. Willaman (13) in an early work with sorghum found that the total NFE of green tops increased steadily until about fifty days after the emergence of the panicles. This fifty day stage he called maturity. Olson and Gastler (10), using frozen immature corn, found the percentage NFE increased till 40 days after silking, the mid-dent maturity, and remained constant thereafter. Evans (11) reported a steady increase in percent of starch from 15-57 days after pollenation. Sugars, expressed as dextrose, decreased somewhat from 0.96% to 0.44% during this period. Leeson and Summers (20) confirm these findings and report a corresponding increase in metabolizabl energy of corn as fed to roosters. Sorghum reducing sugars were highest thirty days, and total sugars peaked at six to twelve days after pollenation, while starch content continues to increase till maturity (6).

The change during growth of fat, ash, fiber, individual minerals and some vitamins have been studied. It was generally agreed that minerals and fiber were laid down early in the development of corn (11,8,21) and sorghum (22,23). Willaman (13) alone reports an increase in ash content in developing sorghum. Ether extract, or crude fat, appeared to increase as long as the embryo developed (11, 23). There was a shift from saturated to unsaturated fatty acids in the total fat fraction of corn (11), but a trend from oleic to linoleic acid in the lipids associated with cornstarch (12). Little work has been done with vitamins in developing grain, but Adams (24) reports that vitamin E and carotene were low in immature corn.

One indication of damage to grain when it is frozen is a decrease in viability. The moisture content of the grain and temperature at which grain was frozen controlled the amount of damage to germinability. Robbins and Porter (25) found viability reduced from 94% to zero when immature (41% moisture) sorghum was exposed to -20° F. for 12 hours, but mature (15% moisture) grain was not affected at any temperature. Roschow (26) generalized that for sorghum moistures above 25% and temperatures below 20° F. were needed to reduce viability. Rossman (27) reported similar results with frozen corn, with the critical parameters also being 25% moisture and 20° F.

This lead to the hypothesis that perhaps it was the moisture, not the immature quality of the grain which changed its feeding value. Parrett and Riggs (28) and Riggs and McGinty (29) in two studies with sorghum, fed early harvested, 25-32% moisture sorghum grain, dry mature grain and mature grain which had been rehydrated to 30% moisture as part of steer rations. The rehydrated and immature grains gave equal gains and better feed conversions than the dry mature grain.

Animal Studies with Immature Grain

The reduction of amounts of starch and fat in a given volume of immature grain when compared to mature indicated lower energy values for light grains. Animal studies have been used to indicate the extent of difference between light and heavy grains.

Ruminant animals, particularly cattle and sheep, are capable because of their digestive systems of utilizing roughages. Thorton et.al. (9) fed immature corn, down to an early milk stage of maturity to lambs in a ration with alfalfa hay. They observed no differences in digestibility coefficients for protein or carbohydrate and slight increases in digestibility of fat, gross energy and TDN (total digestible nutrients). The digestible energy increased from 4030 kcal/kg for corn with a test weight of 451 g/liter to 4280 kcal/kg for grain weighing 747 g/liter.

Steers and lambs fed sorghum grain varying from 35-58 lb./bu. test weight gained slightly more weight on immature than mature grain when it was fed with pelleted alfalfa in one experiment (30). The feed efficiency (pounds of feed required to produce one pound of gain) was not significantly different throughout the range of test weights. Deyoe (31) stated that cattle utilized immature sorghum efficiently and suggested the principal difference among sorghum maturities was that the fiber was greater and NFE less in the immature. This work indicated energy to be lower but protein quality higher in light sorghums.

The higher fiber content in immature grains would make little difference in a ruminant system, where microbial cellulases break it down. The monogastric animals have essentially no gut cellulases and therefore cannot digest a high fiber diet. (The author acknowledges the current debate among human nutritionists as to the role of dietary

fiber; however, the assertion still remains that crude fiber does not contribute materially to the nutritive value of foods in the monogastric diet as it cannot be used for energy.) Waldroup et. al. (32) found a decrease in body weight and delay in sexual maturity among chickens fed 10% protein and 15% crude fiber in their diet when compared to birds fed 16% protein and low fiber.

There is disagreement in the literature as to the efficiency with which monogastrics utilize immature grain. Breuer and Dohn (33) correlated "nutritive value" (i.e., growth) for rats negatively with protein digestibility. Wheat, frozen when immature, was found to depress rat growth at 28 lb./bu. test weight but not at 44 lb./bu. (34). This was not due to B-vitamin deficiency as the addition of yeast did not improve growth. The difference was apparently due to wet bulk weight; addition of agar to the heavier test weight grain diets depressed growth-- the addition of cellulose did not. Antibiotics in the feed depressed rat growth at 28 lb./bu. grain, suggesting that intestinal flora might exist which did attack less digestible portions of the grain.

Whiting and Bezeau (35) also worked with frozen wheat. When fed to pigs, the low test weight grain (30-40 lb./bu.) produced lower protein and energy digestibilities. This lack of protein digestibility was offset by a higher biological value of the protein, resulting in net protein utilization (NPU) values which were not significantly different for any test weight of grain.

Poultry are of particular interest in the study of immature grain. The extremely short retention time of food in the avian gut make birds sensitive to anything which might tie up nutritents in their feed. Moreover, if high fiber was a factor in immature grain, the stimulative

effect of fiber on the gut might shorten the retention time even more. We might expect to find, therefore, that chickens grow more poorly on immature than mature grains.

Not all research has indicated this. Sunde (36) fed low test weight corn to chicks and found no reduction in growth except with test weights of 34 lb./bu. or less. Feed efficiency was not affected. Lambert (37) fed wheat, which was harvested 15-18 days before physiological maturity with corn and sorghum to chicks and found little difference in growth. However, when fed as the sole source of cereal, feed efficiency was better with the heavier grain. Immature corn has been found to have lower metabolizable energy for roosters (20) and immature sorghum has a lower ME in hen rations (16) than their mature counterparts. Immature sorghum caused chicks to grow more poorly than did mature and was found to have lower energy values and poorer conversion of feed (23).

In Vitro Analysis of Feedstuffs

Animal studies are expensive, time consuming, and tedious at best. Several workers have tried to develop laboratory methods which simulate *in vitro* the digestive systems of monogastric animals. The most successful of these *in vitro* systems have been tests for protein digestibility. Saunders and Kohler (38) used successive digestions with pronase, trypsin, and chick pancreas acetone powder to determine the protein digestibility and biological value of wheat mill feeds. Neudoerffer and Smith (39) used "various proteases" to degrade bran, not to study its protein availability but to render the bran more digestible to rats. Their experiment indicated that not all proteases are reliable in releasing the protein elements of value to rats; some of their digests were lethal to the rats.

One of the most widely used *in vitro* techniques is the pepsin-pancreatin digestion of Akeson and Stahmann (40) which was developed for leaf protein extracts. This procedure gave biological values comparable to literature values, according to the authors. Armstrong (41) modified this procedure to study grain sorghum proteins.

Not everyone agrees to the procedure's efficiency. Buchanen (42) tested several procedures for protein digestibility estimation and found pepsin-pancreatin to be poorly correlated with rat assay. Digestion with papain gave biological values which were closer to those from rat assays than pepsin-pancreatin.

The problem of estimating energy values from other than animal data is a large one and few have approached it. Titus (43) drew up a system of estimating metabolizable energy for poultry by the use of factors multiplied by the percent of protein, fat, fiber and NFE and summed.

Chick (44) attempted to estimate the value of wheat bran for humans by successive digestions of the substrate with saliva (alpha-amylase), pepsin and trypsin. They reported good correlations between this procedure and actual protein and dry matter digestion in humans. Booth and Moran (45) digested wheat mill feeds with saliva, pepsin, trypsin, and pancreatin. They determined nitrogen and dry matter dissolved. Clean bran lost 52% of dry matter and 79.5% of nitrogen, primarily from the aleurone layer. Since then, no one has reported working with this procedure.

Tamir and Alumot (46) used alpha amylase and trypsin digestions to demonstrate the inhibition of animal growth by tannins present in carobs. They concluded that the major effect of tannins, which are also a problem in certain strains of sorghum grain (47), significantly inhibited alpha amylase. Proteases were inhibited to a lesser degree.

In grain sorghum, tannin content of the so-called bird resistant or high tannin sorghum was higher at the dough stage than at full maturity (42).

Summary of Literature

In general, the literature shows a change in composition of grain and its nutritive value as it matures. Protein, which accumulates throughout development, is of high quality before the accumulation of storage proteins in later development. Crude fiber and minerals tend to be laid down early in development and do not accumulate later.

Very low test weight grains show a decreased general nutritive value, especially for poultry. The nutritive value improves as maturity approaches, but there appears to be a point after which further increases in the test weight of the grain are accounted for by starch accumulation and/or drying of the kernel. Little change in nutritive value is observed after that point.

In vitro estimation of nutritive value of grains has been most successful in estimating protein quality. Many procedures are available, and their effectiveness appear to depend mostly on the experimentor, the animal being evaluated, and the material. Few procedures are available for estimating energy or TDN, and their abilities to predict accurately are unknown.

METHODS AND MATERIALS

Quail Feeding Study

An initial feeding trial was undertaken to observe trends in nutritive value of immature or light weight grain sorghum for poultry. The sorghums used were of unknown variety, and were obtained from commercial channels. The light (29 lb./bu. test weight) sorghum had a protein content of 11.5%; the mature sorghum contained 8.5% protein. (Unless otherwise noted, all protein values are based on Kjeldahl nitrogen (19) and protein = N x 6.25.)

Japanese quail (Coturnix coturnix japonica) were used for this initial study. These quail are small and have a rapid growth rate, making them suitable for this study. The birds were housed in temperature controlled batteries with wire mesh floors. Initially they were fed from inside feeders; by the end of the experiment outside feeders and water pans were used. Ten one-week-old birds were allotted to each of the 20 groups divided among 4 batteries. Diets were assigned in a randomized block design, 4 replications per diet. The birds were weighed by groups once a week. They were allowed water ad libitum and their feed intake was monitored.

Five diets were fed. Diets 1-4 were calculated to be isonitrogenous and contained soybean flour, ground sorghum grain and fish meal as the protein sources. Vitamins, minerals and energy were calculated to meet the needs of the starting quail (49,50). Starter diets were calculated to 25% protein, later layer diets (begun at 4 weeks of age) were 20% protein. Complete composition and analysis appear in Table 1.

The 5 diets differed as follows:

Diet 1: Immature sorghum formed cereal fraction.

TABLE 1

COMPOSITION AND ANALYSIS OF QUAIL DIETS

Ingredient, %	Starter Diet 1	2	3	4	Layer Diet 1	2	3	4
Immature Sorghum	53.1	---	24.1	50.3	62.2	---	30.0	56.0
Mature Sorghum	---	48.25	24.1	---	---	57.8	30.0	---
Soy Flour	36.9	41.75	41.75	34.7	22.8	27.2	25.0	24.0
Fish Meal	2.5	2.5	2.5	5.0	2.5	2.5	2.5	2.5
Corn Oil	1.0	1.0	1.0	6.0	1.0	1.0	1.0	7.0
Limestone	0.65	0.65	0.63	1.2	7.0	7.0	7.0	7.0
Dicalcium Phosphate	2.8	2.8	2.8	1.9	2.3	2.3	2.3	2.3
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Mineral and Vitamin Premix*	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
TOTALS	99.25	99.53	99.48	101.4	100.1	100.1	100.1	101.1
Analysis:								
Moisture	9.9	10.3	9.1	8.8				
Crude Protein	28.63	30.1	30.4	28.6				
Ash	7.6	7.7	7.5	7.7				
Fat	2.9	2.7	2.6	8.4				
Fiber	3.2	2.2	2.9	3.3				
Calcium	1.0	1.0	1.01	1.0	2.5	2.5	2.5	2.5
Phosphorus	0.80	0.82	0.82	0.82	0.8	0.8	0.8	0.82
Metabolizable Energy** (kcal/kg)	2930	2900	2900	3180	2990	2800	2899	3110

* Includes for all rations: Trace minerals, 0.25 g/kg; sodium selenate, 0.4 mg/kg; vitamin A, 3500 IU/kg; vitamin D, 0.15 g/kg; vitamin E, 0.2 mg/kg; methionine, 0.5 g/kg; vitamin K, 0.06 g/kg; B-complex vitamins, 20 mg/kg; calcium pantothenate, 0.01 g/kg.

** Titus (43)

Diet 2: Mature sorghum formed cereal fraction.

Diet 3: Half of cereal immature, half immature sorghum.

Diet 4: Same as diet 1 with 10% extra calories as calculated provided by corn oil.

Diet 5: Commercial game bird feed.

The trial lasted five weeks. Birds were weighed weekly. Net gain and feed conversions were calculated for each group.

In Vitro Analysis

An attempt was made to produce a simple yet reliable test to determine protein and energy digestibility of a single ingredient or a complete feed. The procedure used was based on the pepsin-pancreatin index proposed by Akeson and Stahmann (40) as modified by Penner (51). The procedure was modified to include an alpha amylase digestion similar to Booth and Moran (45). Chick (44) reported a procedure for estimating nutritive value which entailed cooking the sample before digestion with saliva. This concept was also incorporated.

The initial pepsin-pancreatin procedure was as follows: 1.0 gram of finely ground sample was weighed into a 125 ml Erlenmeyer flask. Samples were run in replicate. Blanks containing no sample were used, and nonfat dry skim milk samples were run with each trial as a reference. Fifteen milliliters of 0.1 N HCl containing 1.5 mg pepsin (Nutritional Biochemical Corp., 3x crystalline) was pipetted into each flask, followed by 1.0 ml of 50 ppm gentian violet to retard mold growth. The flasks were stoppered and incubated for 6.0 hours at 37° C. in a thermostatically controlled water bath. The flasks were shaken frequently. At the end of 6 hours, 7.5 ml of 0.2 N NaOH was pipetted into each flask, neutralizing

TABLE 2

MODIFICATIONS OF BASIC PEPSIN-PANCREATIN IN VITRO DIGESTION

All modifications begin with 1.0 gram of sample, finely ground. Added to this is 1.0 ml 50 ppm gentian violet. Pepsin digestion entails adding 15 ml 0.1 N HCl containing 1.5 mg pepsin to flask, swirling the flask and incubating the sample at 37° C. for 6.0 hours, swirling frequently. Pancreatin digestion includes neutralizing the sample with 0.2 N NaOH, and adding 7.5 ml 0.2 M sodium phosphate buffer, pH 8.3, containing 4.0 mg pancreatin and swirling the flask to mix. Incubate this at 37° for 20.0 hours. Add TCA- filter indicated the addition of 6 ml 50% trichloroacetic acid, allowing mixed sample to settle 1.0 hour, and filter through previously dried and weighed #2 Watman filter paper.

Modifications:

A. Add 6.0 mg alpha amylase (AA) to pancreatin digestion. This action severely reduced dry matter and protein digestion.

B. Add 6.0 mg AA in pH 4.4 buffer for 1 hour between pepsin digestion and pancreatin digestion. Did not improve dry matter digestion over pepsin-pancreatin alone.

C. Add 60.0 mg AA in 6 ml pH 6.0 buffer between pepsin and pancreatin digestions. This improved dry matter disappearance somewhat.

D. Add 60.0 mg AA in 6 ml pH 6.0 buffer to 1.0 g sample, digest for one hour, then proceed with pepsin-pancreatin digestion. This gave no improvement over C.

E. Add 30 ml pH6.0 buffer to sample, cook in 70°C. water bath 30 min, proceed with AA digestion as in D, then pepsin-pancreatin digestions. This depressed protein digestibility and increased dry matter disappearance.

F. After pepsin-pancreatin digestions but before addition of TCA, cook samples in 70-75° C. water bath for 10,20,30,40 or 50 minutes, then cool sample back to 37°. Add 60 mg AA in 6.0 ml pH 6.0 buffer and digest mixture for 1,2,3, or 4 hours. This improved dry matter digestibility over pepsin-pancreatin alone, while maintaining protein disappearance. Optimum results occurred at: 40 minutes cooking time and 2 hours digestion.

TABLE 3

FINAL PROCEDURE FOR IN VITRO ANALYSIS OF FEEDS

Reagents

0.1 N H¢l-- 8.3 ml concentrated (12 N) HCl diluted to 1 liter.

0.2 N NaOH- 8.0 grams NaOH dissolved in water and diluted to 1 liter.

50 ppm Gentian Violet- dilute 1.0 ml 1% GV to 200 ml with ethanol.

0.2 M pH 8.3 Sodium phosphate buffer- 2.037 g NaH_2PO_4 and 49.657 g Na_2HPO_4 dissolved in water and diluted to 1 liter. Adjust to pH 8.3.

0.2 M pH 6.0 Sodium acetate buffer- 25.3 g sodium acetate and 2.0 ml glacial acetic acid dissolved in water and diluted to 1 liter. Adjust to pH 6.0.

50% TCA- 50 g tribloroacetic acid in 50 ml water.

Note: Do not store these reagents for a long period of time.

Procedure

To 1.0 g finely ground sample in a 125 ml Erlenmeyer flask add 1.0 ml 50 ppm gentian violet and 15 ml 0.1 N HCl containing 1.5 mg pepsin. Swirl flasks and digest in 37° C. water bath 6.0 hours, shaking frequently. At the end of 6.0 hours, add 7.5 ml 0.2 N NaOH to nautralize the mixture, then add 7.5 ml pH 8.3 0.2 M sodium phosphate buffer containing 4.0 mg pancreatin. Stopper, swirl, and digest at 37° for 20.0 hours, shaking occasionally.

Place the flasks in a 70-75° C. water bath for 40 minutes, shaking frequently. Care must be taken to prevent spillage. Let flasks cool in 37° water bath one to one and a half hours.

Pipette 6.0 ml pH 6.0 0.2 M sodium acetate buffer containing 60.0 mg alpha amylase into each flask. Stopper flasks, incubate 2.0 hours at 37° and shake frequently. Add 6.0 ml 50% TCA to each sample, shake, then let settle for one hour to precipitate any undigested proteins. Filter through previously dried and weighed #2 Watman filter paper, rinsing with distilled water. Dry at 70-100° C. overnight, weigh, and determine Kjeldahl nitrogen.

$$\% \text{ dry matter digested} = 100\% * \frac{\text{gm sample} - \text{gm recovered}}{\text{gm sample}}$$

$$\% \text{ protein digested} = 100\% * \frac{(\text{gm sample} * \% \text{ protein in sample} - \text{gm recovered} * \% \text{ protein in recovered fraction})}{(\text{gm sample} * \% \text{ protein in sample})}$$

Both these equations are figured on a dry matter basis.

the acid. Then 7.5 ml of 0.2 M pH 8.3 sodium phosphate buffer containing 4.0 mg pancreatin (Nutritional Biochemical Corp., 3x crystalline) was added, the flasks swirled and digestion continued at 37° C. for 20.0 hours, with occasional shaking. When all the digestions were completed 6 ml of 50% trichloroacetic acid (TCA) was added to each flask, swirled, and the sample was allowed to settle for one hour. The TCA precipitated undigested protein. Digests were filtered through #2 Watman paper which had been previously dried and weighed. The papers were dried, weighed and analyzed for nitrogen to determine the undigested protein. Digested protein and digested dry matter were determined by difference.

The alpha amylase digestion consisted of 60 mg alpha amylase (Wallerstein Co.) in 6 ml of 0.2 M sodium acetate buffer (pH 6.0), digested at 37° C. Several studies were tried as to the length and placement of the digestion. Both dry matter and protein disappearance were taken into account. It appeared the optimum disappearance occured if one proceeded with the pepsin-pancreatin digestions as above. Then after the pancreatin digestion, the flasks were placed in a 70-75° C. water bath for 40 minutes. This gelatinized starches. The flasks were swirled frequently. After cooking, the samples were allowed to cool 1 to 1½ hours at 37° C. Alpha amylase in buffer was added, and digestion proceeded as above.

Samples

Most grain sorghum samples used in this procedure were collected in the fall of 1975. Two, designated 75-237 and 75-268, were hybrid sorghum taken from a field in Riley Co., Kansas. Sample 237 was taken five weeks before sample 268, and was immature. The heads were cut by hand, dried in a forced air drier, threshed by a head thresher, and

further cleaned by a scour-aspirator. Sample 268 was combine threshed and harvested at maturity. A third sample, 76-24, was planted as that the grain would freeze before it matured. This sample was harvested, threshed and stored.

Ten other grain sorghum samples were obtained from various fields; all were low test weight sorghums ranging from 30-39 lb./bu. All samples were analyzed for protein, fat, ash, and fiber by the AOAC methods (19). Analysis of these samples are listed in Table 4.

An attempt was made to correlate the percent protein disappearance and percent dry matter disappearance in the in vitro procedure with sorghum test weight. Additional comparisons were made between the in vitro values and values for complete diets fed to poultry.

Chick Study

This study was conducted to estimate the metabolizable energy and nitrogen availability of grain sorghum at mature and immature stages in practical poultry diets. Three diets were used. They were composed of cereal grain (sorghum or corn), soybean meal, and dehydrated alfalfa as the protein sources. They were formulated to meet the needs of the starting chick (49), and were formulated to contain 24% protein. Diet 1 contained equal amounts of yellow corn and mature sorghum of unknown variety; diet 2 contained mature hybrid sorghum 75-268 described above; diet 3 contained immature sorghum 76-24 also described above. Complete compostiion of diets appear in Table 5.

Ten day-old male chicks (Hubbard strain) were randomly allotted to each group. There were three groups fed each diet. The birds were housed in wire mesh floored batteries; food and water were supplied

TABLE 4

COMPOSITION OF SAMPLES

Sample	Test Weight	Moisture %	Crude Protein,%	Ash %	Fat %	Fiber %	% Protein Dry Basis
75-268 Mature Riley Co.	60.6	10.5	11.2	1.3	2.8	2.0	12.51
75-237 Immature Riley Co.	52.8	14.1	9.8	1.9	2.4	3.6	11.41
75-229 Fiser & Wells	39	9.7	11.7	2.7	2.3	4.2	12.96
75-232 Blecha	38	10.3	10.9	2.8	2.0	4.4	12.51
75-234 Reed	38	13.0	10.3	2.6	2.0	5.3	11.84
76-24 Frozen Riley Co.	37.5	10.4	12.8	2.5	2.5	3.5	14.28
75-236 Fiser & Wells	37	9.0	10.9	2.9	2.8	4.9	11.98
75-233 Parrack	36	9.7	9.5	2.8	2.0	5.4	10.52
75-231 Holly	35	14.3	9.4	2.9	1.6	7.5	10.97
75-235 Brouse	33	12.5	11.5	2.4	2.4	4.4	13.17
75-230 Parrack	31	9.9	10.8	3.8	1.6	8.1	11.99

ad libitum. The birds were weighed individually once a week during the four week trial.

During the second week of the trial, total collection of feces and urine were made. A total collection procedure can be as accurate in estimating energy and other parameters as with the inclusion of chromic oxide in feeds as a marker (53). The feces were collected, dried and the feed and feathers removed. Samples were composited and analyzed for protein, fat, moisture,ash and fiber. Samples of feed and feces were also analyzed for gross energy by oxygen-bomb calorimetry, and for cell walls and cell contents by the procedures of Van Soest (56).

No attempt was made to seperate the feces and urine, which in the avian are voided together, thus the values found are for metabolizable

TABLE 5

COMPOSITION OF CHICK DIETS

Ingredient	Percent of Diet		
	Diet 1	Diet 2	Diet 3
Soybean Meal	34.5	34.5	34.5
Commercial Sorghum Grain	27.5	-----	-----
Mature Sorghum (75-268)	-----	55.5	-----
Immature Sorghum (76-24)	-----	-----	55.5
Yellow Corn	28.5	-----	-----
Dehydrated Alfalfa	2.5	2.5	2.5
Animal Fat	5.0	5.0	5.0
Dicalcium Phosphate	1.0	1.0	1.0
Limestone	1.0	1.0	1.0
Salt	0.3	0.3	0.3
Vitamin and Mineral Premix*	2.0	2.0	2.0

* Premix provides per cwt of diet: Vitamin A, 20g; vitamin D_3, 8 g; vitamin B_{12}, 1.25 g of 100%; B-complex vitamins, 45 g; choline chloride, 40 g; trace minerals, 23 g; remainder is sorghum carrier.

energy, and metabolizability coefficients rather than digestibility coefficients and digestible energy. The growth rate and feed efficiency of each diet were found. Since other ingredients in the diets were present in the same proportions, any differences in these values were due to the different cereal sources.

Samples of the 3 diets were analyzed according to the pepsin-pancreatin-alpha amylase procedure described previously. This data was used to determine correlations between the *in vitro* and *in vivo* values.

TABLE 6

ANALYSIS OF CHICK FEEDS AND FECES

	Percent of Sample					
	Diet 1	Diet 2	Diet 3	Feces 1	Feces 2	Feces 3
Moisture*	10.9	9.5	9.5	4.8	2.6	3.1
Protein	23.57	23.54	25.52	26.68	28.75	18.75
Ash	6.51	5.97	8.91	12.13	11.60	11.66
Fat	8.31	7.51	6.60	4.62	6.26	3.82
Fiber	4.04	3.76	10.01	11.34	10.78	18.68
Gross Energy (Kcal/Kg)	4844.	4907.	4767.	4194.	4341.	4291.
Nit. Free Extr.	46.67	49.72	39.86	45.23	42.61	47.09

* Expressed on as is basis; all others are on dry matter basis.

RESULTS AND DISCUSSION

Quail Study

A summary of the results of the quail study appear in Table 7.

TABLE 7

SUMMARY OF THE RESULTS OF QUAIL STUDY

	Diets				
	1	2	3	4	5
Initial No. Birds	40	40	40	40	41
Avg. Initial Wt./Bird, g	25.0	24.1	26.9	25.7	24.0
Final No. Birds	35	35	33	35	40
Avg. Final Wt./Bird, g	88.25	94.13	91.67	94.6	107.0
Avg. Gain/Bird, g	63.25[a]	70.03[a]	64.77[a]	68.90[a]	83.0
Avg. Feed Wasted, %	20	20	20	30	36
Avg Feed Consumed/Bird (adjusted for waste)	410.36	421.23	432.21	358.00	403.88
Feed Efficiency (g feed / g gain)	6.488[b]	6.015[b]	6.673[b]	5.199[c]	4.866[c]

[a,b,c] Values bearing the same superscript are not significant (LSD 0.05).

There were four replications within each diet group. Mortality ran between 12-18% in all the experimental groups.

During the first three weeks of the trial, inside feeders with metal covers were used. The feeders were necessary as the quail could not reach feed in outside pans; however, there was from 20-36% loss of feed due to the birds' habit of standing on the feeders and scattering feed. The losses were estimated by collection over a three day perios and weighing back dried wasted feed. The final figures in Table 7 reflect

the estimated feed losses. The greater wastage in groups 4 and 5 significantly changed the feed conversions with relationship to the other groups.

Data was analyzed using the methods of Snedecor (54) and were compared using an LSD at a significance level of 0.05. No significant differences were found in the gains among the experimental groups (groups 1-4). They were different from group 5. The differences in feed conversion ratios between groups 1,2 and 3 and groups 4 and 5 indicate that the higher energy levels of the latter groups caused more efficient growth. There were no significant differences among diets containing mature or immature sorghum or a mixture of the two.

There was no evidence in this study to indicate that immature sorghum either depressed gains or changed feed conversion in a complete quail feed.

Chick Feeding Trial

Three groups of ten birds were fed each diet. There were three diets; diet 1 contained corn and commercial mature sorghum grain; diet 2 contained mature sorghum; diet 3 contained immature frozen sorghum grain of the same variety.

All birds survived the four week trial. During the second week a few birds developed leg abnormalities similar to perosis. Since all the birds observed were in the groups being fed diet 3, and since Armstrong (41) had observed similar abnormalities in birds fed high tannin sorghum, the sorghums were compared for tanin content by the modified vanillin-HCl method of Burns (55). There was no difference by this methods in the sorghums' tannin contents.

From the first week the birds fed diet 3 grew less than those fed diet 1. After week 2, the diet 3 birds were significantly lighter (LSD 0.05) than birds fed diet 1 or 2. There was no difference in the growth rate between diet 1 and 2 birds. The results were similar for the feed conversions. Throughout the experiment, the birds fed diet 3 ate the same amount, grew less, and had a significantly higher feed per gain ratio than did the other birds. These results are summarized in Table 8.

TABLE 8

SUMMARY OF CHICK FEEDING TRIAL

	Diet 1	Diet 2	Diet 3
Initial No. Birds	30	30	30
Avg. Initial Wt./Bird, g	35.0	34.3	34.6
Final No. Birds	30	30	30
Avg. Final Wt./Bird, g	795.6	811.6	632.5
Avg. Gain/ Bird, g	760.60[a]	777.27[a]	579.90
Avg. Total Feed/Bird, g	1430.	1410.	1420.
Avg. G Feed/G Gain (Cum. for 4 Weeks)	1.880[b]	1.814[b]	2.374

[a,b] Figures with the same superscript are not significant (LSD 0.05).

Table 9 summarizes the apparent metabolizability coefficients for nutrients in the feeds. These were obtained by the formula of Harris(56):

$$\text{Apparent Metabolizability Coefficient} = \frac{\text{Nutrient Intake} - \text{Nutrient Excreted}}{\text{Nutrient Intake}}$$

or,

$$\text{App. Met. Coeff.} = \frac{\text{G Feed} \times \% \text{ Nutrient in Feed} - \text{G Excreta} \times \% \text{ Nutrient Excreta}}{\text{G Feed} \times \% \text{ Nutrient in Feed}}$$

TABLE 9

APPARANT METABOLIZABILITY COEFFICIENTS

	Diet 1	Diet 2	Diet 3
Gross Energy	.758	.743	,579
Crude Protein	.683	.647	.656
Ash	.480	.438	.387
Fat	.845	.759	.729
Crude Fiber	.216	.171	.126
NFE	.729	.752	.447
Dry Matter	.701	.681	.503
Estimated ME (kcal/kg)	3672.	3646.	2763.

No differences existed in apparent metabolizable protein among the diets. Because diet 3 was slightly higher in crude protein than the other two, the chicks actually received more protein from diet 3 than from diet 1 or 2.

The lower values for metabolizable ash, fat, fiber, and NFE foe diet 3 all contributed to its considerably lower metabolizable energy. Recalling the composition figures, (Table 6, p. 23) diet 3 was higher in ash and fiber than the other two. It also had twice the percent cell walls as did the corn-sorghum diet. Harris (56) explains that cell walls are an indigestible portion of the feed. Besides the crude fiber fraction, this figure also includes some materials which are not

digestible by the monogastric yet are hydrolyzed during the fiber analysis. Even 20% cell walls, however, fails to explain all the nondigested dry matter, but it does indicate that the available digestible material was considerably less than was indicated by the proximate analysis.

The high content of nondigestible materials accelerated the passage of the feed through the birds' systems. They comsumed copious quantities of water; they excreted far greater amounts of feces than did birds in groups 1 or 2. Whether this denied the opportunity for nutrients to be absorbed from the gut is not clear. If that were the case, all coefficients would probably be depressed, as most absorption of nutrients other than water occurs in the small intestine. The fact that the metabolizable protein was not lower for immature sorghum does not mean that it was not depressed. If the protein solubility were higher for immature than for mature sorghum, then it would be expected that the coefficient would be greater. It was not, and this might show a depression of protein as well as other nutrients.

So in this experiment, the diet containing immature sorghum performed poorer than diets containing mature sorghum or a mixture of sorghum and corn. The reasons for the poorer growth rate and feed conversion was that there was less available energy; the intake of feed was the same for the three diets. Protein metabolizability was the same for the three diets, but other studies have suggested that the metabolizable protein should have been higher for the immature grain. Cell walls were twice as great in the immature sorghum diet as in the other two. Besides the lessened NFE due to cell walls, the stimulative effect reduced the retnetion time of the feed in the gut, and this would depress absorption of nutrients from the feed.

The study showed that sorghum of test weight 37.5 lb./bu. did depress the growth of chicks and increased the feed conversion rate when fed as part of a complete diet. This appears to be due primarily to the higher fiber content of the immature sorghum grain.

In Vitro Analysis

Data presented in Table 10 compare three in vitro digestion procedures run on three materials. Skim milk powder was used as a reference for all experiments; its biological value was considered to be 95 (40). Sample 237 was the 52.8 lb./bu. immature sorghum described earlier; sample 268 was mature sorghum from the same field.

TABLE 10

MEAN VALUES FOR THREE IN VITRO PROCEDURES

	% Protein Digested			% Dry Matter Digested		
	Sk.Mk.	237	268	Sk.Mk.	237	268
Test Wt., lb./bu.	----	52.8	60.6	----	52.8	60.6
Pepsin-Pancreatin[1]	92.97	62.73	63.11	85.8	21.3	27.0
AA-P-Pn[2]	97.15	48.24	39.10	95.6	73.5	57.0
P-Pn-AA[3]	95.88	66.28	61.39	98.0	67.5	41.7

[1] Pepsin-pancreatin digestion after Penner (51).

[2] Cook 70° C. 40 minutes, 60 mg alpha amylase for 1 hour, then pepsin-pancreatin as above.

[3] Pepsin-pancreatin as above, cook 70° C. 40 minutes, 60 mg alpha amylase for 2 hours.

There were no differences between the percent protein digested from the two sorghums in any given procedure. However, the percent

dry matter disappearance was greater for immature sorghum than for the mature when the samples were cooked. This could be due to a higher proportion of tightly bound starches or other constit which were not released when the grains were cooked. The mono- and di-saccharides in immature grain would have been readily released.

Sample 237 did not have a lower set of values in these trials than did the mature samples. However, there were only 8 pounds per bushel difference in the grains. Sorghums of test weights in a range from 30-60 lb./bu. were digested using the three procedures. The results were analyzed using regression analysis (51,57) to estimate the linear relationships between digestibility and test weight. The resulting data and regression lines are shown on Fig. 1-3. The regression equations and statistics appear in Table 11.

TABLE 11

REGRESSION EQUATIONS FOR DIGESTION METHODS

Method	Regression Equation	R^2	F	Std. Error
Pepsin-Pancreatin	DM = 29.902-0.0957xTW	0.0260	0.615	6.263
	PRO = 92.919-0.528xTW	0.6683	46.343	3.983
AA-P-Pn	DM = 67.456-0.0965xTW	0.0099	0.261	8.198
	PRO = 48.52-0.1663xTW	0.0841	2.386	6.484
P-Pn-AA	DM = 64.214-0.300xTW	0.0644	2.273	11.842
	PRO = 78.157-0.293xTW	0.4369	25.599	3.452

There is variation within the data. Some of this is due to differences between varieties of sorghum; some variation also occurs between days when the analyses were run.

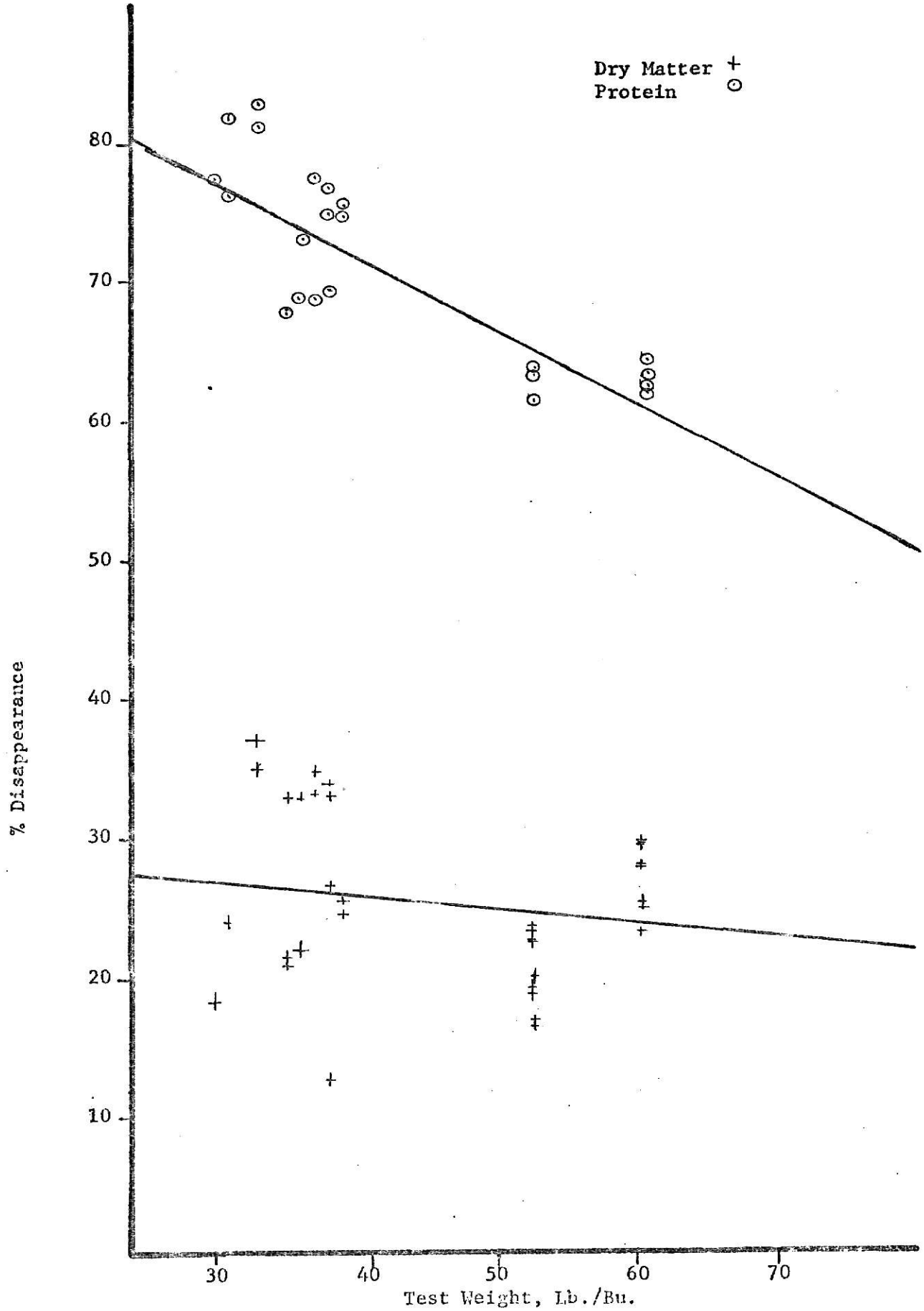

FIG. 1. PEPSIN PANCREATIN DIGESTION OF GRAIN SORGHUM

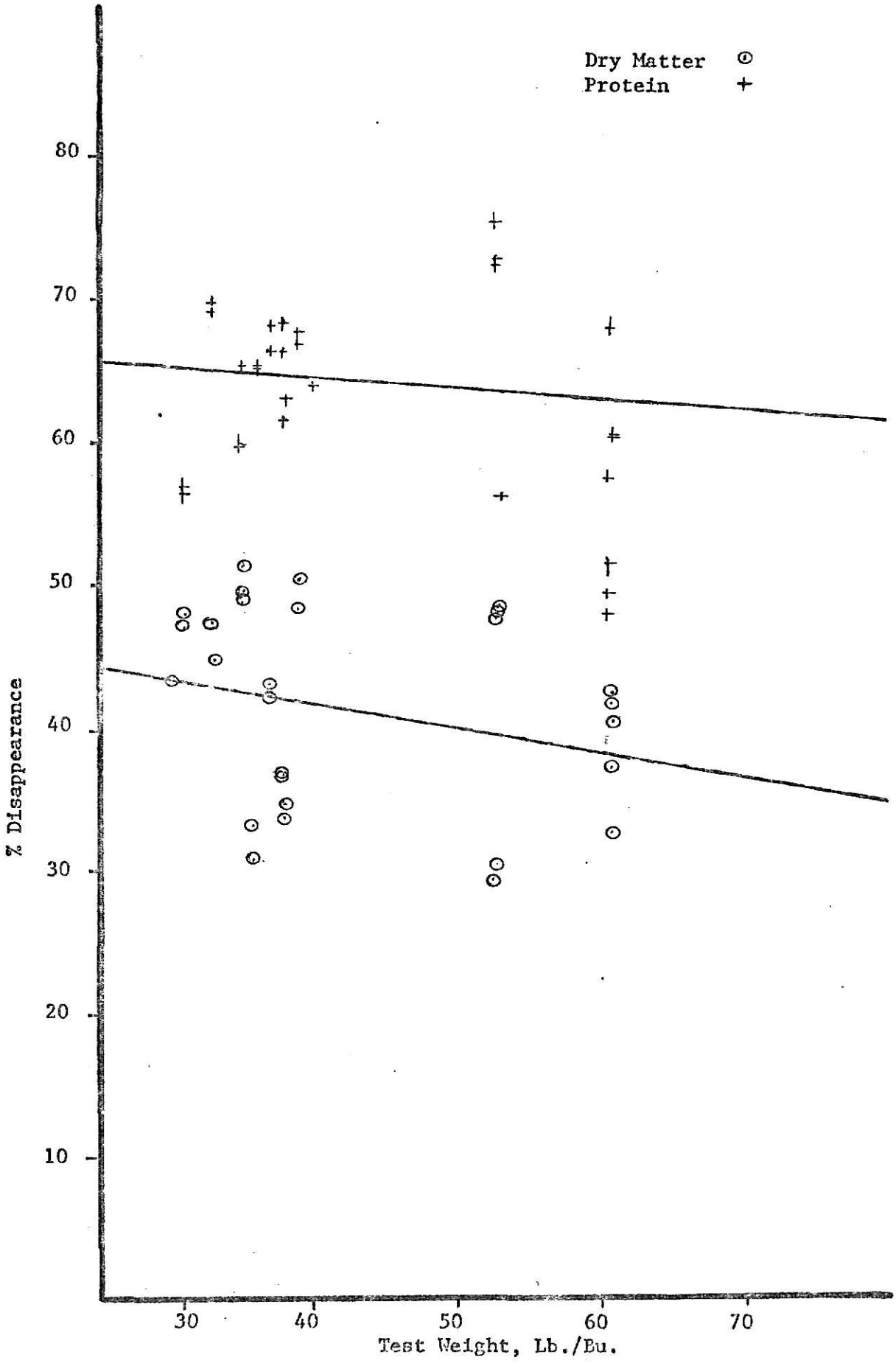

FIG.2. ALPHA AMYLASE PEPSIN PANCREATIN DIGESTION OF SORGHUM

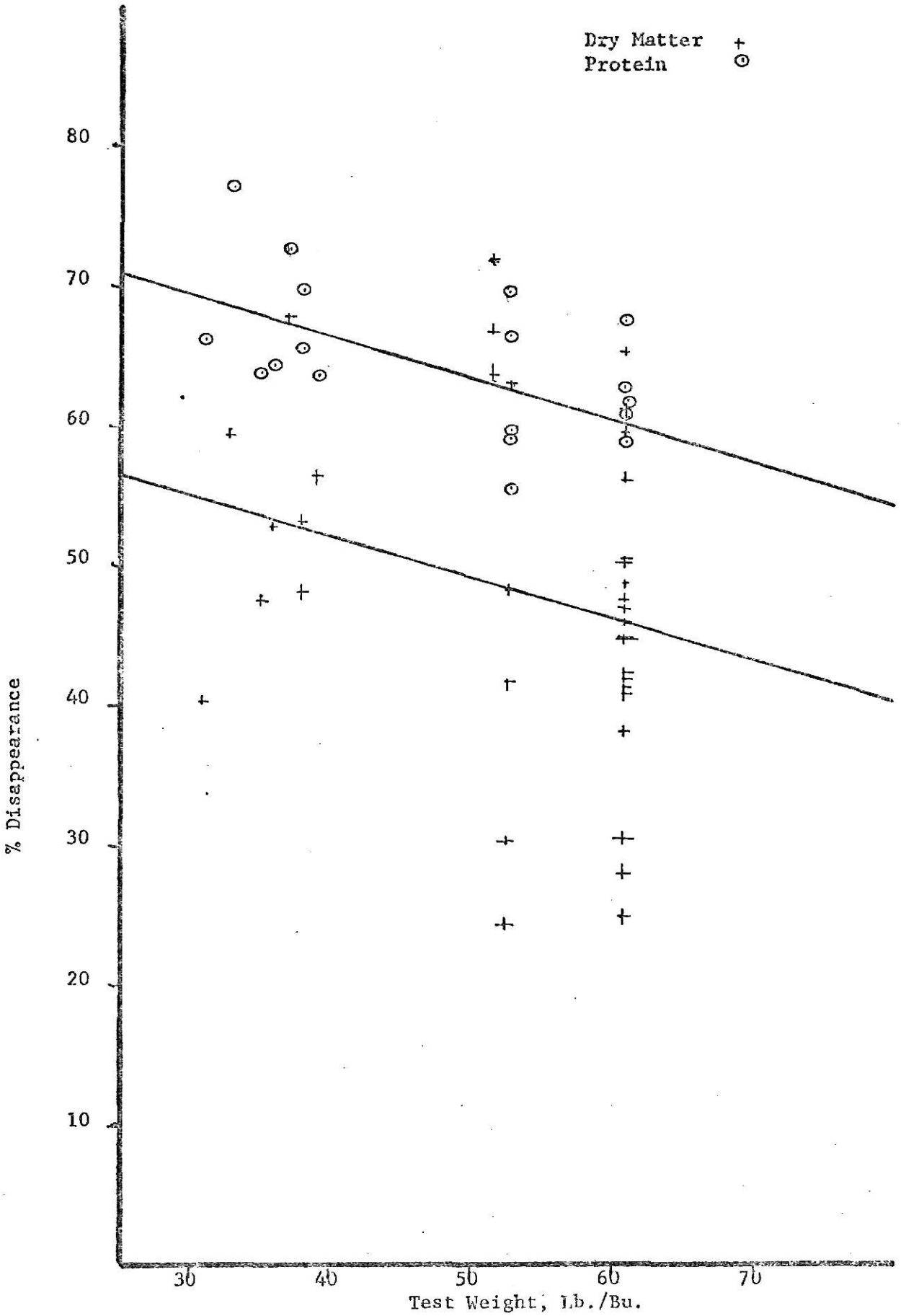

FIG. 3. PEPSIN PANCREATIN ALPHA AMYLASE DIGESTION OF SORGHUM

Generally the trends in analysis showed that as test weight increased, dry matter and protein digestibility decreased. The idea of loss of protein digestibility with advancing maturity is consistant with the literature. The F-tests for the protein disappearance on both the pepsin-pancreatin and pepsin-pancreatin-alpha amylase (P-Pn and P-Pn-AA respectivley) digestions show that there is a relationship between the test weight and the in vitro protein disappearance which is significant. F-tests for the other parameters, that is, for dry matter digestion for all three procedures and protein digestion for the alpha amylase-pepsin-pancreatin (AA-P-Pn) digestion, showed that coefficients in the regression equations were not significantly different from zero. Thus confining the discussion to the methods P-Pn and P-Pn-AA, no change in dry matter digestibility occurs as maturity approaches in sorghum. This is not consistent with findings for energy for sorghum in the literature (23). However, comparing dry matter digestibility with metabolizable energy may be like comparing oranges with tangerines. They may be related, but they are really not the same things. Han and coworkers (53) developed equations estimating metabolizable energy from apparent metabolizable dry matter, with fairly small errors of estimates. Because their equations are linear and based on dry matter, using their values would not indicate any change in energy among various test weights of grain sorghum.

To correlate the efficiency of the pepsin-pancreatin-alpha amylase digestion procedure in predicting <u>in vivo</u> values for complete chicken feeds, samples of the three diets used in the chick feeding trials were digested. A summary of the results appear in Table 12. The figures for protein digestibility were slightly higher than those for metabolizable protein in the chicks. If we were able to determine strictly digestible

protein in the chick study, the figures would have been somewhat closer to those for the *in vitro* results. However, the values do indicate that the *in vitro* procedure was capable of predicting relative differences in protein digestibility.

TABLE 12

SUMMARY OF IN VITRO DIGESTION OF CHICK FEEDS

	Avg. Protein Digested	Avg. D.M. Digested	Avg. Metabolizability Coefficients from Chick Study			
			Protein	G.Energy	D.M.	NFE
Diet 1	.7219	.4847	.683	.758	.701	.729
Diet 2	.6799	.3684	.647	.743	.681	.752
Diet 3	.7016	.3181	.656	.579	.503	.447

The dry matter digestibilities were not close to values for metabolizable energy, NFE or dry matter. However, the relative positions of dry matter disappearance, i.e. diet 1 greater than diet2 much greater than diet 3, are the same for the *in vitro* values and metabolizable dry matter coefficients from the chick study.

The P-Pn-AA procedure gave far higher values for dry matter disappearance and the same protein disappearance as the pepsin-pancreatin procedure. It is not as yet a sensitive indicator of the differences in energy values between ingredients in diets as are feeding trials. This may be due to the stimulative effect of the high fiber diet used in this particular study. It may be due to the fact that only proteolytic and amylolytic enzymae were used. It may reflect the lack of shaking and mixing of samples during digestion or the fact that the products of digestion remained in the reaction flask. This might cause the reactions

catalyzed by the enzymes to come to equilibrium sooner than would occur *in vivo*.

The procedure has potential. It is now a good indicator of digestible or metabolizable protein in the feed or ingredient. It is not so good an estimator of energy, but quite possibly it could be made more sensitive to differences in feed energy.

SUMMARY

There are conflicting reports in the literature as to the feeding value of immature sorghum and other grains for monogastric animals. This research was conducted to contribute to clarifying the issue. In the quail study, the different diets were isonitrogenous, with three diets intented to be different only in their sorghum sources, and the fourth diet contained extra energy. The quail grew better on the diet containing extra energy, but statistically no better on mature sorghum than on ommature. There were indications that the birds fed mature sorghum grew slightly more and had beeter feed conversion than those fed immature sorghum.

In the feeding trial with chickens, the diets were not calculated to be isonitrogenous, but rather with equal proportions of ingredients. The birds fed immature sorghum gained less on the same amount of feed and had poorer feed conversion than birds fed other diets. This poorer growth was due to a lack of metabolizable energy in the immature sorghum diet. The immature sorghum used weighed 37.5 lb./bu.; in the quail study the sorghum weighed 29 lb./bu. Thus the poorer growth could not be attributed directly to the fact that the sorghum fed to the chicks was less mature than that fed the quail, at least as measured by test weight.

The major differences in the diets containing immature sorghum was in fiber and energy. In the quail s dy, the fiber content was only slightly higher than that of the mature grain diets (3% vs. 2%). In the chick study, the fiber content was 10% for the immature diet as compared to 4% for the mature diet. The corresponding decrease in starch made less energy available to the birds. The high content of

indigestible material gave the chicks very loose droppings. It is unclear from the evidence whether this kept the birds from utilizing the nutrients which were available from the immature grain, or whether the nutrients simply were not available in the first place. Whichever the case, the immature sorghum with the high fiber content had lower metabolizable energy than the other cereal sources and the birds fed it grew poorly. When the fiber content of mature and immature sorghum diets were similar, the quail performed the same.

In an attempt to make an evaluation of feeds and ingredients for poultry and other monogastrics, an in vitro simulation of the digestive system was developed. This system, the pepsin-pancreatin-alpha amylase digestion, accurately predicted the relative differences in the metabolizable protein in the chick feeds. It closely predicted the actual values. The procedure also predicted relative differences in the metabolizable dry matter, but was not nearly so accurate in predicting metabolizable energy values. A series of sorghum samples of various test weights were digested using the procedure and regression equations were developed for the results. The equations showed that as test weight increased, protein disappearance decreased. There was a slight decrease in dry matter disappearance with increasing test weights, but the regression coefficients were not significantly different from zero. This agrees with the results of the quail feeding trial, but not with the chick study.

Generally, the in vitro procedure predicted protein utilization and relative differences in dry matter disappearance among feeds, but it was not sensitive to conditions such as fiber which might complicate metabolizability of energy. The actual values for dry matter digestibility

obtained were much lower by the *in vitro* digestion than were the corresponding values from the chick study. However, further modifications such as constant shaking of the samples or removal of the products of digestion from the reaction might bring these values closer to their *in vivo* values. Additionally, it is not clear from the data whether the values are for digestible or metabolizable nutrients.

In conclusion, this research has developed an *in vitro* digestion simulation which makes a significant effort toward a laboratory method for evaluation of feeds. It was applied to test the feeding value of sorghum grain of various test weights. The results indicate that as test weight increases, protein digestibility decreases and dry matter or energy does not change. Results of birds feeding trials with quail agree with the *in vitro* tests, and indicate no difference in immature and mature sorghum. A chicken feeding trial indicated a much lower growth rate and poorer feed conversion among birds fed immature sorghum as compared to mature. There appeared to be less energy in immature grain. This may be due to indigestible fiber or to an increased rate of passage through the avian gut.

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APPENDIX

APPENDIX TABLE 1

QUAIL STUDY - WEIGHT LOG

JUNE 9 - JULY 15, 1975

		Initial		Week 1		Week 2	
Diet	Group	No. Birds	Wt. Gm.	No. Birds	Wt. Gm.	No. Birds	Wt. Gm.
1	A	10	247	10	385	10	571
	B	10	231	10	390	10	579
	C	10	254	10	438	10	660
	D	10	235	10	414	9	522
2	A	10	236	10	415	10	588
	B	10	249	9	342	8	481
	C	10	245	10	455	10	648
	D	10	249	10	379	9	558
3	A	10	250	10	406	10	561
	B	10	236	10	386	9	511
	C	10	264	9	314	8	432
	D	10	232	10	374	10	511
4	A	10	237	10	471	9	558
	B	10	255	10	399	10	589
	C	10	239	10	381	10	558
	D	10	232	9	388	9	519
5	A	11	239	11	503	11	779
	B	10	227	10	472	10	695
	C	10	245	10	491	10	716
	D	10	243	10	483	10	742

TABLE 1

QUAIL STUDY - WEIGHT LOG - CONTINUED

JUNE 9 - JULY 15, 1975

Diet	Group	Week 3 No. Birds	Week 3 Wt. Gm.	Week 4 No. Birds	Week 4 Wt. Gm.	Week 5 No. Birds	Week 5 Wt. Gm.	Total Gain Per Bird
1	A	10	625	8	587	8	753	69.42
	B	10	644	10	618	9	723	57.2
	C	10	748	10	678	9	816	65.27
	D	9	655	9	702	9	848	70.72
2	A	10	708	10	648	9	817	67.18
	B	8	541	8	653	8	773	71.7
	C	10	710	10	751	9	851	69.96
	D	9	666	9	728	9	859	70.5
3	A	10	650	8	638	8	732	66.5
	B	9	575	9	623	8	742	69.15
	C	8	490	8	594	8	745	66.7
	D	9	683	9	733	9	842	70.36
4	A	9	710	9	785	9	937	80.4
	B	10	740	10	768	10	940	68.5
	C	10	659	10	653	7	574	58.1
	D	9	664	9	740	9	861	72.47
5	A	11	936	11	873	11	1145	82.36
	B	10	839	10	781	9	931	80.7
	C	10	879	10	900	10	1129	88.4
	D	10	884	10	878	10	1077	83.4

APPENDIX TABLE 2

QUAIL STUDY

FEED CONSUMED, IN GRAMS, UNCORRECTED FOR WASTE

Diet	Group	Week 1	Week 2	Week 3	Week 4	Week 5	Total
1	A	850	1075	950	900	1362	5137
	B	860	950	870	900	1304	4884
	C	775	975	975	900	1306	4931
	D	800	750	800	900	1183	4433
2	A	850	925	925	900	1362	4962
	B	900	900	725	900	1292	4717
	C	775	975	900	900	1292	4842
	D	935	900	875	875	1291	4876
3	A	850	1000	1075	900	1239	4771
	B	800	885	793	900	1363	4741
	C	800	875	700	875	1344	4594
	D	800	900	932	900	1293	4771
4	A	875	800	875	900	1344	4794
	B	850	875	1000	900	1344	4969
	C	750	775	875	900	1319	4619
	D	800	800	875	900	1217	4592
5	A	1060	1250	1425	1125	1957	6817
	B	1050	1175	1375	1125	1770	6495
	C	950	1075	1250	1125	1875	6275
	D	865	1100	1275	1150	1896	6286

APPENDIX TABLE 3

SUMMARY OF DRY MATTER AND PROTEIN DIGESTED FROM SAMPLES BY PEPSIN - PANCREATIN DIGESTION

Sample	Test Weight	% Dry Matter Digested	% Protein Digested
Skim Milk	---	90.93, 92.76 89.62, 79.79 91.13, 76.64 79.87	92.25, 94.17 92.19, 93.85 93.85, 91.09 93.38
Sorghum 75-268	60.6	25.67, 23.37 29.65, 29.47	62.57, 62.33 63.28, 64.26
Sorghum 75-237	52.8	22.70, 23.41 16.69, 23.52 20.12	63.28, 63.27 63.23, 61.20 63.48
Sorghum 75-236	37	34.98, 33.15	68.79, 77.34
Sorghum 75-235	33	37.10, 35.00	82.81, 81.10
Sorghum 75-234	38	26.80, 26.04	69.34, 69.33
Sorghum 75-233	36	22.03, 33.02	73.07, 68.72
Sorghum 75-232	38	33.94, 12.77	76.73, 74.80
Sorghum 75-231	35	20.81, 21.45	67.95, 67.92
Sorghum 75-230	31	23.84, 18.49	76.13, 81.57
Sorghum 75-229	39	25.42, 24.73	74.61, 75.28
Sorghum 75-227	less than 30	23.32, 19.80	65.83, 60.16
Sorghum 75-225	less than 30	19.87, 17.53	61.80, 62.75

APPENDIX TABLE 4

SUMMARY OF DRY MATTER AND PROTEIN DIGESTED FROM SAMPLES

BY COOK-ALPHA AMYLASE-PEPSIN-PANCREATIN DIGESTION

Sample	Test Weight	% Dry Matter Digested	% Protein Digested
Skim Milk	---	93.41, 92.53 98.15, 96.05 97.05, 96.58	95.14, 95.53 97.73, 97.76 96.67, 96.43
Sorghum 75-268	60.6	85.52, 48.10 67.73, 51.66 49.46, 67.96 57.34, 60.08	37.38, 38.31 40.16, 32.81 42.10, 40.31 40.09, 41.63
Sorghum 75-237	52.8	56.06, 72.92 72.07, 75.55	29.45, 30.38 48.29, 48.19
Sorghum 75-236	37	68.10, 66.31	43.17, 42.40
Sorghum 75-235	33	69.00, 69.85	47.43, 44.92
Sorghum 75-234	38	61.68, 63.15	33.85, 34.85
Sorghum 75-233	36	65.16, 65.10	33.17, 31.10
Sorghum 75-232	38	68.28, 66.26	36.49, 36.61
Sorghum 75-231	35	59.85, 65.18	49.30, 51.41
Sorghum 75-230	31	56.90, 56.70	47.98, 47.02
Sorghum 75-229	39	67.84, 66.64	48.39, 50.12
Sorghum 75-227	less than 30	69.16, 67.89	41.95, 36.51
Soeghum 75-225	less than 30	64.17, 66.27	37.38, 37.40

APPENDIX TABLE 5

SUMMARY OF DRY MATTER AND PROTEIN DIGESTED FROM SAMPLES

BY PEPSIN-PANCREATIN-COOK-ALPHA AMYLASE DIGESTION

Sample	Test Weight	% Dry Matter Digested	% Protein Digested-
Skim Milk	----	99.62, 95.52	96.46, 93.21
		99.32, 98.37	98.55, 98.55
		89.55, 95.81	91.61, 89.58
		92.23, 90.34	93.06, 92.48
		97.14, 98.84	95.37, 96.38
		89.96, 95.03	93.67, 95.20
		95.50	95.68
Sorghum 75-268	60.6	47.82, 41.34	62.71, 61.92
		47.22, 46.07	67.56, 59.48
		44.94, 48.97	62.76, 61.68
		59.60, 65.33	59.44, 59.52
		56.03, 50.45	59.48, 61.16
		50.04, 42.66	58.64, 59.50
		30.45, 28.03	57.86, 59.05
		41.91, 45.00	59.22, 59.09
		41.59, 24.96	61.10, 61.98
		38.38, 60.74	61.10, 59.56
Sorghum 75-237	52.8	48.50, 63.84	55.76, 62.78
		66.75, 71.99	69.78, 66.29
		30.46, 41.91	59.05, 59.22
		24.66	59.09
Sorghum 75-236	37	67.91	72.63
Sorghum 75-235	33	59.79	77.19
Sorghum 75-234	38	48.40	65.50
Sorghum 75-233	36	52.87	64.65
Sorghum 75-232	38	53.39	69.27
Sorghum 75-231	35	47.52	63.87
Sorghum 75-230	31	41.46	66.04
Sorghum 75-229	39	56.58	63.87
Sorghum 76-24	37.5	41.67, 38.39	67.48, 64.47

TABLE 5 - CONTINUED

Sample	Test Weight	% Dry Matter Digested	% Protein Digested
Chick Feed #1	---	28.66, 48.47 40.15	70.63, 70.70 72.19
Chick Feed #2	---	38.84, 36.84 40.23	67.66, 71.20 67.99
Chick Feed #3	---	40.48, 31.81 29.05	73.32, 78.10 71.05

APPENDIX TABLE 6

SUMMARY OF WEIGHTS OF CHICKENS

TRIAL FROM SEPT. 13 TO OCT. 11, 1976

	Avg. Wt./Bird, Grams, 10 Birds per Group					
Group	Sept. 13	Sept. 20	Sept 28	Oct. 4	Oct. 11	Avg. for Diet
Diet 1						
Lot 3	35.8	111.3	271.5	514.5	813.2	
Lot 8	34.8	107.8	256.7	494.1	777.6	
Lot 33	34.4	110.7	269.4	500.8	796.2	795.7
Diet 2						
Lot 10	34.0	110.5	270.3	517.4	830.2	
Lot 11	34.6	107.2	249.7	481.1	789.8	
Lot 13	34.3	115.1	271.2	514.5	814.7	811.6
Diet 3						
Lot 6	34.6	106.5	257.0	465.2	658.6	
Lot 17	34.5	101.3	238.8	439.7	639.8	
Lot 34	34.6	101.4	215.4	409.2	594/0	630.8

APPENDIX TABLE 7

SUMMARY OF WEIGHT GAINS MADE BY CHICKENS

TOTAL IN GRAMS FOR 4-WEEK PERIOD ENDING OCT. 11, 1976

Bird	Diet 1			Diet 2		
Lot No.:	3	8	33	10	11	13
1	731	723	733	760	699	790
2	797	834	581	830	729	732
3	752	834	581	830	729	722
4	768	770	794	837	603	845
5	642	667	797	812	757	714
6	790	610	762	739	745	781
7	850	775	775	769	758	786
8	748	778	862	800	891	740
9	823	756	828	842	843	805
10	873	767	846	773	782	763

	Diet 3		
Lot No.:	6	17	34
1	587	618	555
2	588	630	560
3	636	628	518
4	664	576	628
5	669	628	553
6	557	681	566
7	620	600	423
8	646	679	632
9	659	566	559
10	664	477	600

APPENDIX TABLE 8

SUMMARY OF FEED CONSUMED BY CHICKENS

WEEKLY, IN GRAMS, SEPT. 13 TO OCT. 11, 1976

ADJUSTED FOR WASTAGE WHERE APPLICABLE

	For Week Ending:				
	Sept.20	Sept. 28	Oct. 4	Oct. 11	Total
Diet 1					
Lot 3	1009	2431	5717	5897	15054
Lot 8	956	2363	5112	5339	13770
Lot 33	986	2381	5655	5607	14629
Diet 2					
Lot 10	1037	2436	5075	5988	14536
Lot 11	976	2229	5412	5932	14549
Lot 13	1044	2344	4537	5272	13197
Diet 3					
Lot 6	1028	2690	5317	6002	15037
Lot 17	988	2542	4801	4919	13250
Lot 34	1042	2242	4994	5628	13906

APPENDIX TABLE 9

FEED EFFICIENCY

GRAMS FEED PER GRAM GAIN IN CHICKENS, WEEKLY

	For Week Ending:				
	Sept. 20	Sept. 28	Oct. 4	Oct. 11	Cum. Total
Diet 1					
Lot 3	1.336	1.518	2.35	1.974	1.867
Lot 8	1.310	1.587	2.15	1.883	1.854
Lot 33	1.296	1.500	2.44	1.899	1.920
Diet 2					
Lot 10	1.350	1.524	2.05	1.914	1.824
Lot 11	1.344	1.564	1.91	1.922	1.826
Lot 13	1.292	1.502	1.86	1.756	1.691
Diet 3					
Lot 6	1.430	1.788	2.09	3.103	2.410
Lot 17	1.479	1.849	2.06	2.458	2.189
Lot 34	1.560	1.967	2.20	3.045	2.522

APPENDIX TABLE 10

MANURE COLLECTED FROM CHICKENS

DRY WEIGHT, IN GRAMS, COLLECTED SEPT. 21-28

	Day 3	Day 7	Total	Feed Removed	Net Feces
Diet 1					
Lot 3	303.6	625.5	929.1	280.5	648.6
Lot 8	263.9	763.1	1027.0	425.2	601.8
Lot 33	438.1	800.8	1238.9	484.9	754.0
Diet 2					
Lot 10	270.0	736.7	1006.7	338.4	668.3
Lot 11	356.6	813.6	1170.2	454.7	715.5
Lot 13	252.8	571.8	824.6	180.5	644.1
Diet 3					
Lot 6	521.6	113.0	1634.6	280.6	1354.0
Lot 17	386.1	977.4	1363.4	254.7	1108.7
Lot 34	474.3	980.8	1455.1	417.1	1038.0

APPENDIX TABLE 11

METABOLIZABLE ENERGY

BASED ON COLLECTIONS MADE SEPT. 21-28, 1976

ALL FIGURES ON A 100% DRY MATTER BASIS

Replication:	1	2	3
Diet 1			
Feed/bird, g	243.15	236.3	238.1
Gross energy, kcal/kg	4844.	4844.	4844.
Kcal consumed per bird	1177.8	1144.6	1153.4
Urine & feces/bird,g	64.86	60.18	75.40
Gross energy, kcal/kg	4194.	4194.	4194.
Kcal excreted per bird	272.0	252.4	316.2
Kcal retained per bird	905.8	892.2	837.2
Percent energy retained	76.9	77.9	72.6
Metabolizable energy, kcal/kg	3725.	3776.	3516.
Avg. for diet			3672.
Diet 2			
Feed/bird, g	243.6	222.9	234.4
Gross energy, kcal/kg	4907.	4907.	4907.
Kcal consumed per bird	1195.3	1093.8	1150.2
Urine & feced/bird, g	66.83	71.55	64.41
Gross energy, kcal/kg	4341.	4341.	4341.
Kcal excreted per bird	290.1	310.6	279.6
Kcal retained per bird	905.2	783.2	870.6
Percent energy retained	75.7	71.6	75.7
Metabolizable energy, kcal/kg	3716.	3514.	3714.
Avg. for diet			3646.
Diet 3			
Feed/bird, g	269.0	254.2	224.2
Gross energy, kcal/kg	4769.	4769.	4769.
Kcal consumed per bird	1282.9	1212.3	1089.2
Urine & feces/bird, g	135.4	110.87	103.8
Gross energy, kcal/kg	4291.	4291.	4291.
Kcal excreted per bird	581.0	475.4	445.4
Kcal retained per bird	701.9	736.6	623.8
Percent energy retained	54.7	60.8	58.3
Metabolizable energy, kcal/kg	2609.	2898.	2782.
Avg. for diet			2763.

APPENDIX TABLE 12

METABOLIZABILITY COEFFICIENTS

BASED ON COLLECTIONS SEPT. 21-28. 1976

	Dry Matter	Crude Protein	Ash	Fat	Fiber	NFE
Diet 1						
Avg feed/bird, g --- 239.18						
Percent in feed	89.1	23.57	6.51	8.31	4.04	46.67
Grams in feed	213.309	56.375	15.571	19.876	9.663	111.625
Avg. feces & urine/bird, g --- 66.81						
Percent in feces	95.2	26.68	12.13	4,62	11.34	45.23
Grams in feces	63.603	17.845	8.104	3.087	7.576	30.218
Grams retained	149.706	38.530	7.467	16.786	2.087	81.407
Metabolizability coefficient	0.701	0.683	0.480	0.845	0.216	0.729
Diet 2						
Avg. feed/bird, g --- 233.64						
Percent in feed	90.5	23.54	5.97	7.51	3.76	49.73
Grams in feed	211.435	54.997	13.948	17.546	8.785	116.181
Avg. feces & urine/bird, g --- 67.60						
Percent in feces	97.4	28.75	11.60	6.26	10.78	42.61
Grams in feces	65.842	19.435	7.842	4.332	7.282	28.804
Grams retained	145.593	35.562	6.106	13.314	1.503	87.357
Metabolizability coefficient	0.681	0.647	0.438	0.759	0.171	0.757
Diet 3						
Avg. feed/bird, g --- 249.13						
Percent in feed	90.9	25.52	8.91	6.60	10.01	39.86
Grams in feed	226.459	63.578	22.197	16.443	24.938	99.303
Avg. feces & urine/bird, g --- 116.03						
Percent in feces	96.6	18.75	11.66	3.82	18.68	47.09
Grams in feces	113.014	21.868	13.599	4.455	21.788	54.921
Grams retained	113.445	41.710	8.598	11.988	3.152	44.383
Metabolizability coefficient	0.503	0.656	0.387	0.729	0.126	0.447

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IN VIVO AND IN VITRO EVALUATION OF IMMATURE SORGHUM GRAIN FOR POULTRY

by

REBECCA ANNE KENYON LONGBOTTOM

B. S., Kansas State University, 1974

AN ABSTRACT OF A MASTER'S THESIS

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Immature sorghum was examined in this research to determine its feeding value for poultry. Proximate analysis of several varieties of immature sorghum grain of different test weights were made. As the test weight of the grains increased, from 30 to 60 lb./bu., the percent ash and percent fiber decreased, while the percent of fat increased slightly. Percent protein, which was expected to decrease as test weight increased, remained relatively the same in the samples.

An initial feeding trial was made using Japanese quail. The birds were fed various diets which were calculated to be isonitrogenous. The birds fed immature sorghum as the cereal fraction of the complete feed gained the same and had the same feed conversion ratio as those birds fed mature sorghum or a combination of mature and immature grain. Birds fed a fourth diet in which the energy supplied by immature sorghum grain was supplemented by corn oil gained better on less feed than the birds in the other three groups.

A feeding trial using broiler chicks compared immature and mature sorghum grain from the same field. In this study the birds fed the low test weight grain diets gained less weight and had poorer feed conversion than birds fed mature grain. In these diets the composition was based on equal percent substitution of the immature or mature experimental grains for a mixture of sorghum and yellow corn.

An <u>in vitro</u> method of determining the feeding value of grains for poultry was developed. Samples of the grains and complete feeds were digested with pepsin, pancreatin, and alpha amylase. Disappearance of protein and dry matter were measured. The protein disappearance had a negative correlation with test weight, but no correlation which was statistically significant was found between in vitro dry matter disappearance and test weight.

When the diets from the broiler chick study were analyzed using the *in vitro* technique, the percent protein disappearance correlated well with the apparent metabolizable protein of the feeds. The dry matter disappearance values were not good estimates of the values for metabolizable energy, dry matter, or NFE found for the birds, but the relative positions of the three feeds were the same. That is to say, the feed which had the highest apparent metabolizable dry matter coefficient was the feed which had the highest *in vitro* dry matter disappearance.

This research indicates that immature sorghum is probably not as well utilized by birds as is mature sorghum. This may be due to the higher fiber content of the immature grain. This higher fiber content resulted in less metabolizable energy in the light test weight grain. There may have been a stimulative effect of the fiber, causing more rapid food passage through the birds' digestive tracts.