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Two non-bird resistant grain sorghums exhibited higher in vitro protein digestibility values than two bird resistant varieties. Extraction of tannins from a bird resistant variety resulted in increased in vitro protein digestibility as compared to the non-extracted grain, whereas the extraction process had little influences on the protein digestibility of a low tannin non-resistant grain sorghum.

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IN VITRO STUDIES OF THE PROTEIN DIGESTIBILITY OF SORGHUM GRAIN[1]

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ABSTRACT Two non-bird resistant grain sorghums exhibited higher *in vitro* protein digestibility values than two bird resistant varieties. Extraction of tannins from a bird resistant variety resulted in increased *in vitro* protein digestibility as compared to the non-extracted grain, whereas the extraction process had little influence on the protein digestibility of a low tannin non-resistant grain sorghum.

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PREVIOUS studies in this laboratory have shown that bird resistant sorghum grain does not support comparable chick growth or feed efficiency to that observed with non-resistant sorghum grain (Rostagno *et al.*, 1973a; Armstrong *et al.*, 1973). Further research by Rostagno *et al.* (1973b) suggested that the protein digestibility of the bird resistant sorghum grain was lower than that of the non-resistant grain. Studies by Armstrong *et al.* (1974) indicated that extraction of the tannin from bird resistant sorghum grain overcame the growth depressing effects of these grains. The present report describes

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attempt to develop an *in vitro* digestion assay for use as a possible rapid screening test of the biological value of various varieties of sorghum grain.

The *in vitro* method studied in this experiment was a pepsin followed by a pancreatin digestion as described by Akeson and Stahmann (1964). Two sets of conditions were studied to evaluate the effect of differences in enzyme concentration. Method 1 used 500 mg. sorghum grain, 1.5 mg. pepsin in 37.5 ml. of 0.1 N hydrochloric acid and 4.0 mg. pancreatin in 18.75 ml. of pH 8.0 phosphate buffer. Method 2 used 100 mg. sorghum grain, 6.0 mg. pepsin in 37.5 ml. of 0.1 N hydrochloric acid and 20.0 mg. pancreatin in 18.75 ml. of pH 8.0 phosphate buffer.

The finely ground sorghum sample was weighed and placed in a 125 ml. Erlenmeyer flask and incubated with 37.5 ml. of the pepsin solution at 37° C. for 3 hours. After neutralization with 18.75 ml. of 0.2 N sodium hydroxide and addition of 18.75 ml. of the pancreatin solution, the digestion mixtures were incubated for an additional 24 hours at 37° C. Thimerosal was added to the digestion mixtures to a final concentration of 50 p.p.m. to prevent growth of microorganisms. Enzyme blanks were prepared by incubation under the described conditions with the sorghum grain omitted. Sorghum grain blanks were also prepared by incubation at 0.° C. using the solutions described earlier with the enzymes omitted. The digestion flasks were covered with a rubber stopper and lightly swirled occasionally during incubation.

Following incubation, the digestion mixtures were stirred on a magnetic stirrer, an aliquot poured into a centrifuge tube and then centrifuged for 10 minutes at 34,800 × *g* to remove the solid material. The free amino acid concentration of the supernate was determined using a ninhydrin assay based on the procedure described by Clark (1964). The standard amino acid stock solution was prepared in similar solutions as the sample being analyzed. Different sample sizes were analyzed so a small range in concentrations could be maintained for more accurate spectrophotometric readings.

The total amino acid concentration of the sorghum grain was determined by first hydrolyzing 500 mg. of sorghum grain with 250 ml. of 6 N hydrochloric acid for 8 hours at 250° C. in an autoclave. After hydrolysis, the sample was immediately filtered through a fine fritted glass filter and brought to a volume of 400 ml. with distilled water. A 10 ml. portion of this solution was evaporated to dryness using a rotary vacuum evaporator and brought to a 10 ml. volume with pH 2.75 sodium citrate buffer. The total concentration of amino acids was determined using the ninhydrin assay.

The *in vitro* digestibility values (IVDV) of the sorghum protein were calculated from the following equation:

$$\text{IVDV (\%)} = \frac{\text{(amino acid concentration of digested sorghum sample)} - \text{(amino acid concentrations of enzyme blank + sorghum grain blank)}}{\text{total amino acid concentration of hydrolyzed sorghum grain sample}} \times 100$$

A preliminary study was conducted to compare the IVDV values of 4 grain samples determined as described above using ninhydrin to measure the amino acid concentration in the entire sample with those obtained by determining the individual amino acids by ion exchange chromatography. The IVDV values obtained by using the sum of the individual amino acid determined by ion exchange chromatography compared favorably to the values obtained from the ninhydrin assay without chromatographic separation.

TABLE 1.—*In vitro digestibility values (IVDV) of bird resistant (BR64, IS8260) and non-resistant (RS671, RS610) sorghum grain*

Sorghum grain	Number of determinations	IVDV[1] %
RS610	6	12.97 ± 0.64
RS671	6	13.42 ± 0.96
RS671E[2]	5	12.80 ± 0.79
BR64	6	4.81 ± 0.49
IS8260	7	4.35 ± 0.42
IS8260E[2]	6	17.82 ± 1.73

[1]Mean ± standard error of mean. The concentration of pepsin and pancreatin used in Methods 1 and 2 had no influence on the IVDV; therefore, figures listed are the mean values for the two methods combined.

[2]The tannins were extracted by an alkaline treatment of the whole grain followed by hot water washing as described by Armstrong *et al.* (1974).

A summary of the *in vitro* digestibility values of two non-resistant sorghum grains (RS610 and RS671), two bird resistant sorghum grains (BR64 and IS8260), as well as non-resistant and resistant grains (RS671 and IS8260) from which the tannins were extracted (Armstrong *et al.*, 1974) is shown in Table 1. The IVDV for the two non-resistant grains as well as the extracted non-resistant grain were all close to 13%. The two bird resistant grains had IVDV of around 4-5% while the bird resistant grain from which the tannins were extracted exhibited a value of 17.8%. These results demonstrate similar trends with those noted in chick feeding studies in that the bird resistant sorghum grains show lower biological values than the non-resistant grains. Also, the extraction of the tannins from the bird resistant sorghum grain resulted in an improvement in the biological value, whereas the extraction process had little influence on the values for the non-resistant sorghum grain. The results suggest that this method may have value for screening purposes.

The low values observed raise questions concerning the validity of the test. Certainly the chick utilizes more of the grain than is indicated here and the bird resistant grains do not appear to be as low in value for chick growth in relation to the non-resistant grain as is indicated in this study. It may be that the optimal conditions for *in vitro* digestion have not been determined. The second set of conditions mentioned above resulted in no higher values than noted with the first set of conditions, indicating that the levels of pepsin and pancreatin used initially were not responsible for the low values. However, it would appear that the method is still valid since relative values are more important than absolute values; i.e.—the digestibility of bird resistant sorghum relative to that of non-resistant sorghums.

The need for a rapid screening procedure for determining the biological value of sorghum grain varieties is indicated by the wide differences noted in the performance of chicks and other animals fed different grain sorghums. This need is evident both to the plant breeder as well as the nutritionist. The need is further emphasized by the observations of several workers that seed color does not serve as a valid indicator of nutritional quality. Also, Cummings (1973) recently showed that the presence or absence of the testa of the grain cannot be used as an indication of level of tannin or nutritional quality.

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ENERGY METABOLIZED BY BANTAM CHICKENS AND BLUE-WINGED TEAL

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ABSTRACT Energy metabolized by bantam chickens was compared with that by Blue-winged Teal (*Anas discors*) in six diets, five of which contained wild duck foods. Bantams metabolized significantly more energy in four diets; teal metabolized significantly more in only one diet containing natural duck food. Differences did not exceed 11 percent. Results from this and published studies show that calculated metabolizable energy values for a given diet cannot be transferred from one kind of bird to another with confidence.

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IT has been suggested (Sugden, 1971) that metabolizable energy (M.E.) of foods eaten by wild ducks might be predicted from chemical data and equations developed for chickens (Carpenter and Clegg, 1956; Sibbald *et al.*, 1963). To do so, one must know that the abilities of chickens and ducks to metabolize energy are similar. This note describes results of one experiment to measure the M.E. in diets fed to bantams and Blue-winged Teal (*Anas discors*).

MATERIALS AND METHODS

M.E. was measured in six diets fed to full grown male bantams and male teal using methods described by Sibbald *et al.* (1960) and Sugden (1973). Laboratory conditions and metabolism pens have also been described (Sugden, 1971). Each diet was fed to 8 to 12 bantams and 6 to 9 ducks and collections were made from individual birds. The number of birds used was governed primarily by the amount of natural food available for inclusion in the diet. Proximate composition of diets containing natural foods was estimated from compositions for the basal and natural foods and the proportion of each in the experimental diet.

RESULTS AND DISCUSSION

Both bantams and teal adapted well to the diets and weight changes were small. In most tests, some birds had small gains while others had small losses. Mean daily weight changes of bantams as percentage of starting weight was less than 0.1 percent and ranged from −0.2 to 0.4 percent. Corresponding values for teal were 0.6 percent and ranged from −0.7 to 2.5 percent.

Bantams metabolized significantly more energy than teal in four diets (Table 1), basal, basal plus slough grass (*Beckmannia syzigachne*) seeds, basal plus pondweed foliage (*Potamogeton pusillus*), and basal plus duck

TABLE 1.—*Comparison of energy metabolized by bantams and teal*

Diet	Composition on a dry basis				Metabolizable energy		T-test between groups
	Protein	Fat	Fiber	Ash	Bantams	Teal	
	%	%	%	%	Kcal./gm.	Kcal./gm.	P
Basal (100%)	19.7	2.7	5.4	5.4	3.00 ± 0.03	2.84 ± 0.03	<0.001
Basal (54%), duck weed (46%)	25.5	3.0	9.3	7.4	2.29 ± 0.03	2.25 ± 0.01	N.S.
Basal (60%), slough grass (40%)	14.8	3.7	15.4	6.6	2.52 ± 0.03	2.44 ± 0.04	0.05
Basal (50%), pond-weed (50%)	15.8	2.6	14.8	12.2	1.71 ± 0.04	1.54 ± 0.06	<0.01
Basal (65%), green alga (35%)	15.5	2.0	9.8	11.6	2.25 ± 0.01	2.12 ± 0.00	<0.001
Basal (49%), shrimp (51%)	34.1	5.3	7.6	14.3	2.61 ± 0.03	2.67 ± 0.02	<0.05

weed (*Lemna minor*). Teal metabolized significantly more energy than bantams in the diet containing shrimp (*Gammarus* sp.). Differences in M.E. values derived for the two groups did not exceed 11 percent. Reasons for the differences are unknown, though there is some suggestion that the ducks metabolized more energy relative to bantams with increasing percentage of dietary protein.

Published results of comparisons between different kinds of birds are variable. Chicks metabolized more energy from a high-energy diet than turkey poults (Slinger *et al.*, 1964). Conversely, poults metabolized more energy from a low-energy diet than chicks. Also, a slow-growing breed of chicks metabolized more energy from a high-energy diet than a fast-growing breed. These differences were less than 5 percent. Hill *et al.* (1968) reported that the M.E. of cellulose eaten by Ruffed Grouse (*Bonasa umbellus*) was twice that measured for chickens. The M.E. in wheat bran eaten by grouse was 56 percent higher as compared with chickens. On the other hand, Japanese Quail (*Coturnix coturnix japonica*) and a light breed of chickens were equal in their abilities to metabolize energy from high and low-energy diets (Begin, 1968). Similarly, the M.E. of a commercial duck starter fed to young Black Ducks (*Anas rubripes*) and American Coots (*Fulica americana*) was the same for both species (Penney and Bailey, 1970).

The results from this experiment provide additional evidence that M.E. values measured with one kind of bird cannot be applied to another kind with confidence.

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