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DETERMINATION OF TRUE METABOLIZABLE ENERGY CONTENT OF BOBWHITE FOODS

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Abstract: True metabolizable energy (TME) and nitrogen-corrected true metabolizable energy (TME_n) bioassays were used to determine available energy content of several northern bobwhite (*Colinus virginianus*) foods. A proximate analysis and trypsin inhibitor (TI) activity were also determined for each food. Corn (*Zea mays*) was found to contain the highest amount of TME_n (4.37 kcal/g dry matter) compared with Fayette soybeans (*Glycine max*; 3.93 kcal/g), Korean lespedeza (*Kummerowia stipulaceae*; 3.73 kcal/g), Marion lespedeza (*K. striata*; 3.71 kcal/g), tick-trefoil (*Desmodium* spp.; 3.51 kcal/g), and wild trailing (WT) soybeans (3.24 kcal/g). The higher TME_n value of corn was attributed to its high digestible carbohydrate content and lack of appreciable TI activity.

Key words: bobwhite, corn, lespedeza, metabolizable energy, nutrition, soybeans, tick-trefoil, trypsin inhibitor.

Habitat improvement, in particular establishment and maintenance of food plots, is an important management practice employed by wildlife conservationists to help sustain game bird populations at desirable levels. However, in such programs only the most suitable feedstuffs are usually planted to provide foods in winter. For several years, a food plot mix distributed by the Missouri Department of Conservation to landowners for habitat improvement plantings contained a strain of reseeding annual soybeans, the WT soybean. However, higher costs are encountered in the production of WT strain soybeans for seed, and only limited information is available on their nutritional value. Since overwintering of viable seed is such a desirable characteristic for wildlife food plot plantings, we thought information on the nutritional content of WT soybeans would be helpful in appraisals of their potential value as a component of food plot mixes for bobwhite. Since energy is the most critical need during winter, determining the metabolically available energy content of WT soybeans and relating it to their nutrient composition was the primary objective of the study. For comparative purposes, similar nutrients were measured in 5 other foods consumed in appreciable quantities by bobwhite during winter months.

Apparent metabolizable energy (AME) values of foods are typically determined by subtracting gross excreta energy (EE) from energy consumed (NRC 1966, Sibbald 1977). In the AME procedure no correction is made for EE of endogenous origin such as bile, digestive secretions, abraded cells from the alimentary mucosa, uric acid, and other products of tissue catabolism (Sibbald 1977).

Sibbald (1976) used chickens to devise a biological assay, the TME assay, in which a fasted control is used to quantify the endogenous portion of the EE. Fundamental to development of the TME assay was the recognition that EE is a linear function of food intake and the intercept of the regression line on the ordinate axis represents endogenous EE (Sibbald 1982).

The TME assay involves gavaging a previously fasted experimental bird with a weighed quantity of the test food then quantitatively collecting excreta over a sufficient period of time to allow digestion of the food and excretion of its indigestible fraction. The endogenous portion of the EE is determined via the fasted control and is subtracted from EE of the fed bird. Therefore, the error induced by inclusion of endogenous EE, as in the AME assay, is eliminated. This is of greater significance at low levels of food intake because the endogenous EE constitutes a larger proportion of the total EE at low intake levels (Guillaume and Summers 1970). Sibbald and Price (1975) measured the variation in AME values of 2 foods and reported that values varied from day to day in a "saw-tooth" manner. Fluctuating food intake was suggested to be the most probable explanation for the variation.

Advantages associated with use of the TME bioassay over the traditional AME scheme are numerous. Reductions in variation, costs and labor requirements, shorter determination times, and use of a smaller quantity of food are the major

advantages of the TME system (Sibbald 1977). Furthermore, feeding via gavage assures an exact measure of food ingested and reduces errors associated with *ad libitum* feeding.

Nitrogen corrected TME_n values are calculated by adjusting EE to reflect a zero nitrogen balance. This is of particular importance in the TME assay where food intake is limited, thus increasing the rate of tissue protein catabolism (Parsons et al., 1982). A correction factor of 8.73 kcal/g nitrogen excreted was suggested by Titus et al. (1959) as best representing energy content of the nitrogenous excretory products of the chicken. Sibbald and Morse (1983) reported that TME_n values were 6-7% less than corresponding TME values. Nitrogen correction reduced EE of the unfed controls by 56%. Also, the variation in TME_n values was less than when nitrogen balances of fed and fasted bobwhite were not equilibrated.

Objectives of the research described herein were to: (1) determine if TME and TME_n bioassay techniques (gavaging, short assay periods, etc.) could be used in the bobwhite to establish ME values of selected foods and (2) compare TME and TME_n values determined with nutrient composition and TI activity present in these foods.

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METHODS

Foods assayed included corn, Fayette soybeans, WT soybeans (a reseeding strain developed by the Missouri Department of Conservation), Korean lespedeza, Marion lespedeza, and tick-trefoil. Seeds were fed unground and the lespedezas and tick-trefoil were dehulled.

Adult male northern bobwhite, weighing 175-210 g, were housed in individual wire mesh cages, 20-cm wide x 25-cm long x 15-cm high. The room was maintained at 24 ± 2 C temperature, 55% relative humidity, and a 14L:10D photoperiod. Bobwhite were fed *ad libitum* a diet containing 16% crude protein (CP) and 2,737 kcal ME/kg during maintenance periods. A higher protein and energy repletion diet (26% CP and 2,900 kcal ME/kg) was fed after each assay period to expedite the recovery of weight lost during the assay. Water was continuously available.

Bioassays were conducted according to the method described for chickens by Sibbald (1976)

with the following modifications. Fed and fasted bobwhite were not paired by weight since Arvat et al. (1980) found no correlation between body weight and EE. Instead, an average EE value was calculated for the fasted bobwhite and used to compute TME values. A 24-hour fasting period was used rather than 21 hours, and the excreta collection period was extended to 72 hours.

Prior to each assay, bobwhite were weighed and randomly assigned to the fasted control or fed groups. After a 24-hour fasting period, precision-feeding was accomplished by passing a funnel, having a stem measuring 7.5 cm in length and 8 mm in diameter, via the esophagus into the crop. The funnel was lubricated with water prior to insertion into the esophagus.

A blunt glass rod was used to push the seeds from the funnel into the crop. The few seeds larger than the funnel opening were manually placed in the esophagus and then pushed into the crop with the glass rod. Care was taken to ensure that adequate ventilation was maintained. Due to the small size of the desmodium and lespedeza seeds, they were administered in gelatin capsules (No. 000) to ensure accurate delivery of the preweighed quantity to the crop and to prevent regurgitation. The fasted control bobwhite were given an equal number of empty capsules to allow for correction of the energy contained in the capsules. All birds were fed 3-5 g of test foods.

Excreta samples were stored at -7 C until analyzed. Gross energy of the foods and excreta samples was measured in an adiabatic bomb calorimeter according to procedures outlined in Oxygen Bomb Calorimetry and Combustion Methods (Parr Inst. Co. 1960). Nitrogen content was determined by the Kjeldahl procedure (AOAC 1984).

Proximate analyses were carried out on all foods. AOAC (1984) procedures were used except for crude fiber and ash which were determined simultaneously by the method of Whitehouse et al. (1945).

Trypsin inhibitor activity was assayed by the method of Sandholm et al. (1976). Relative TI contents were compared based on the most dilute solution which contained sufficient inhibitor activity to suppress the enzymatic digestion of the casein contained in calcium-caseinate agar plates.

Procedures described in SAS (1982) were used for statistical analysis. The TME and TME_n values of foods were compared by analysis of variance, and significant differences among treatment means were determined using Fisher's

Least Significant Differences (Snedecor and Cochran 1980).

RESULTS

The TME and TME$_n$ bioassays were successfully carried out with bobwhite (Spurlock 1987). Weight loss (data not shown) during an assay varied from 5 to 15% of initial body weight and was generally recovered by the end of a 14-day repletion period. No detrimental effects were observed when the same bobwhite were used in repeated assays.

As shown in Table 1, whole corn had the highest nitrogen free extract (NFE) content (74.9%, air dry basis) but less protein, fat, and fiber than other foods. Of particular interest was the higher fiber and lower fat content of WT soybeans compared to the Fayette variety. Tick-trefoil contained substantially more fiber than did other foods. The lespedezas were similar to the soybean varieties in protein but lower in fat and higher in fiber.

With the exception of corn, TI activity was detected in all foods. The soybean varieties required a dilution of 1:16 before trypsin digestion of the casein was apparent. Other foods showed TI at dilutions of only 1:4. The soybean varieties

therefore have at least a 4-fold higher activity of TI than do the other foods.

Our initial endeavor was to demonstrate that TME and TME$_n$ assays yield accurate, reproducible ME values when using northern bobwhite. The TME and TME$_n$ values for Fayette soybeans and whole corn were determined in 2 different assay periods. As shown in Table 2, no significant differences ($P > 0.05$) were found between TME and TME$_n$ values determined in the first and second assays in which different bobwhite were fed the same test foods. Differences between ME values for assay 1 and 2 ranged from 3 to 5%, indicating that the TME and TME$_n$ bioassays result in accurate, reproducible data.

Because it was desirable to compare TME and TME$_n$ values of the different feedstuffs, we felt it was also necessary to verify that gelatin capsules used to administer the small tick-trefoil and lespedeza seeds would not alter ME values obtained. There were no differences ($P = 0.998$) in TME values (kcal/g dry matter) for corn fed as free grain ($\bar{x} = 4.72 \pm 0.12$, $n = 7$) or encapsulated grain ($\bar{x} = 4.72 \pm 0.08$, $n = 6$). The TME$_n$ values also did not differ ($P = 0.087$) for free grain ($\bar{x} = 4.34 \pm 0.05$, $n = 7$) and encapsulated grain ($\bar{x} = 4.47 \pm 0.05$, $n = 6$). Encapsulation therefore seems to be a prac-

Table 1. Composition of foods (% air dry basis).

	Fayette soybeans	WT soybeans	Corn	Korean lespedeza	Marion lespedeza	Tick-trefoil
Moisture	9.4	11.7	9.8	8.7	8.0	7.9
Crude protein	43.2	48.1	8.4	41.3	45.5	32.8
Crude fat	21.3	14.3	4.2	6.7	6.3	14.2
Crude fiber	5.6	8.2	1.4	13.1	14.0	24.5
Ash	5.7	6.1	1.3	4.2	4.5	4.3
NFE[a]	14.8	11.6	74.9	26.0	21.7	16.3
Gross energy (Kcal/g)[b]	5.38	5.03	4.83	5.14	5.13	5.54

[a]Nitrogen-free extract.

[b]Dry matter basis.

Table 2. Repeatability of metabolizable energy estimates for bobwhite foods.

Food	Assay[a]	n	Metabolizable energy values[b,c]	
			TME	TME$_n$
Fayette soybeans	1	11	4.26 ± 0.07	3.89 ± 0.09
	2	7	4.44 ± 0.05	3.99 ± 0.04
		$P =$	0.098	0.357
Whole corn	1	12	4.48 ± 0.12	4.35 ± 0.07
	2	14	4.71 ± 0.06	4.39 ± 0.04
		$P =$	0.087	0.629

[a]Assay 1 and 2 means were not different for either food ($P > 0.05$).

[b]Kcal/g dry matter.

[c]Means ±SE.

Table 3. True metablizable energy (TME) and nitrogen-corrected true metabolizable energy (TME$_n$).

Food	n	TME[a]	TME$_n$
		Kcal/g dry matter	
Whole corn	26	4.60 ± 0.07A	4.37 ± 0.04A
Fayette soybeans	18	4.33 ± 0.05B	3.93 ± 0.06B
Marion lespedeza	8	4.07 ± 0.18BC	3.71 ± 0.09BC
Korean lespedeza	9	3.89 ± 0.14CD	3.73 ± 0.17BC
Tick-trefoil	10	3.71 ± 0.19CD	3.51 ± 0.20C
WT soybeans	15	3.51 ± 0.07E	3.24 ± 0.04D

[a]Values (means ± SE) in the same column with different letters differ significantly ($P < 0.05$).

tical means of precision-feeding those test materials which tend to be regurgitated or are difficult to handle during the precision-feeding process.

Whole corn contained the most TME$_n$ (Table 3), followed by Fayette soybeans, the lespedezas, tick-trefoil, and WT soybeans. The high digestible carbohydrate content (NFE-Table 1) of corn and lack of any appreciable TI activity result in most of the GE being available to the bobwhite. Metabolic efficiency of corn was 95 and 90% when based on TME and TME$_n$, respectively. These values are slightly higher than the 86% obtained by Robel et al. (1979) but are derived after correcting for EE energy and nitrogen elimination.

DISCUSSION

Protein, carbohydrate, and fat fractions of a feedstuff all contribute to its ME content, while fiber is generally inversely related to ME, particularly in monogastric species. These energy-yielding fractions and fiber are of most concern during prolonged periods of harsh winter conditions.

As in the case of most legumes, the nutritional value of the soybean and lespedeza varieties and tick-trefoil is compromised by the presence of trypsin and other proteinase inhibitors (Borchers 1966, Garlich and Nesheim 1966, Rackis 1966). Robel and Arruda (1986) also found that despite the high fat content of soybeans, bobwhite were able to assimilate only a fraction of the GE consumed. Although we found the lespedeza varieties to have TI activity, they contained considerably less than the soybean varieties. The fact that the lespedezas contained less TME and TME$_n$ than Fayette soybeans is probably more the result of their high fiber and lower fat content than impaired protein digestion.

The TI activity of the desmodium was much less than in WT soybeans. This suggests that the high fiber content of desmodium was responsible for its lower ME values. Fiber is largely indigestible in avian species and also accelerates the passage rate of the digesta, thereby decreasing energy and nutrient utilization (Miles et al. 1981).

Correcting the EE of fed and fasted bobwhite for nitrogen elimination (TME$_n$ assay) reduced the TME estimate of every feedstuff. This is because the quantity of the EE which is charged against caloric intake is increased when the negative nitrogen balance of fed and fasted bobwhite is adjusted to zero. The EE is partitioned into that of food origin and endogenous origin. In addition, the endogenous fraction is further partitioned so that the quantity resulting from an elevated rate of tissue catabolism, induced by a limited caloric intake, is identified and subtracted from the endogenous component. The TME$_n$ assay therefore yields the most accurate estimate of the available energy content of a feedstuff because of the more stringent partitioning of the EE.

Since nitrogen correction requires only that food and excreta samples be analyzed for nitrogen, the length of time required for the TME$_n$ assay is not greatly increased over that required for the TME assay. Although the absolute amount by which nitrogen correction changes the total EE is usually small, its importance is magnified by the limited caloric intake. Nitrogen correction generally reduced the mean ME values of the feedstuffs by 5-10% which is similar to results obtained by Sibbald and Morse (1983).

Our studies indicate that the TME$_n$ bioassay is a quick, easily conducted alternative to the traditional AME bioassay. It yields accurate, reproducible estimates of the biologically available energy content of bobwhite foods.

RESEARCH AND MANAGEMENT IMPLICATIONS

As shown by Errington (1936), the diet of the bobwhite in its natural habitat is largely determined by availability and abundance of various food items. Only a small proportion of the many foods available to them is eaten in quantity, and a still smaller proportion qualifies as a winter staple. As discussed earlier, no significant correlation between the volume of food consumed and its energy value was observed in studies conducted by Robel et al. (1974). They concluded that consumption of a particular food by bobwhite is primarily related to its availability. Based on our studies, it is suggested that the TME_n procedure be used in future studies of bobwhite foods and that prior research on this aspect of bobwhite habitat management be reevaluated.

Since food plots for bobwhite are rarely single species plantings, TME_n values also need to be investigated with varying mixtures of major energy sources. The effect of grit on the TME_n value of whole seeds has not been established. Nestler (1946) reported that bobwhite receiving no grit and a diet of whole seeds from hatching through 20 weeks of age performed as well as bobwhite fed a similar diet plus grit for the entire period. He concluded that seeds such as wheat, millet, milo, soybeans, field peas, and vetch can be successfully macerated and digested without the aid of grit.

A comparison of the ME content of WT soybeans with that present in other foods consumed in quantity by bobwhite during winter was the primary objective of this study. Based on our TME_n assays shown in Table 3, the available energy content of WT soybeans was approximately 25% less than we found in corn and 5-15% less than found in the other foods tested. These data on TME_n content of foods analyzed will allow wildlife habitat managers to more accurately evaluate the relative benefits of including WT soybeans in food plot plantings for bobwhite. Among desirable attributes of WT soybeans other than their energy content are overwintering of viable seed and compatibility with other plants which provide both food and protection from aerial predators.

Lower seed yields are the primary reason for elevated costs associated with production of WT soybean seed for food plot plantings. It would seem that agronomic research similar to that which has resulted in significant increases in yields of domestic soybean varieties might be considered for WT soybeans if cost of their seed is the primary limitation in their use for food plots.

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