Standardization of Analytical Methodology for Feeds

Proceedings of a workshop held in Ottawa, Canada, 12–14 March 1979

Editors: W.J. Pigden, C.C. Balch, and Michael Graham

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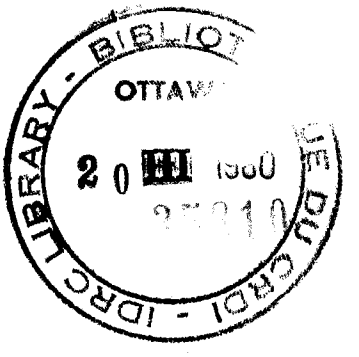

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Contents

A New Technique for Estimating the ME Content of Feeds For Poultry

I.R. Sibbald[1]

The terminology related to the available energy in feedstuffs is defined and described. A bioassay for true metabolizable energy (TME) is described in detail and its advantages over more conventional bioassays for apparent metabolizable energy are outlined. A plea is made for the adoption of a single, standardized, bioassay for available energy in poultry feedstuffs. A brief comment is made on the limitations of indirect assay procedures.

Knowledge of the available energy content of feedstuffs is essential if the most economical poultry diets are to be formulated. Birds tend to eat to satisfy their energy requirements; consequently, nutrients should be included in diets in proportion to available energy. Failure to do this can result in wasted nutrients and impaired productivity.

Although much has been written about energy in poultry nutrition, there are gaps in our knowledge and problems to solve. There are many feedstuffs of regional interest that require evaluation. Such feedstuffs will be better utilized when their available energy values are known. More information is needed about variability among different lots of feedstuffs and about the control of such variability through processing. The energy requirements of birds in the tropics need further study. However, the most important problem is the lack of standardization in the methodology for measuring available energy.

This paper describes a new method for measuring the available energy content of poultry feedstuffs. The assay is simple, rapid, and relatively inexpensive, and it yields data that are additive, reproducible, and more accurate than those obtained by more conventional procedures.

Terminology and Definitions

Poultry nutritionists have adopted metabolizable energy as the measure of available energy in feedstuffs. Unfortunately, metabolizable energy is a generic rather than a specific term. To avoid confusion it is appropriate to present some definitions of terminology.

GE_f is the gross energy of the feed.

FE is the gross energy of the feces.

FE_f is the fecal energy of feed origin, derived from unabsorbed feed residues.

FE_m is the fecal energy of metabolic origin, derived from abraded intestinal mucosa, bile, digestive fluids, etc.

UE is the gross energy of the urine.

UE_f is the urinary energy of feed origin, derived from absorbed, nonmetabolized feed.

UE_e is the urinary energy of endogenous origin, derived from the products of tissue catabolism.

$FE = FE_f + FE_m$

$UE = UE_f + UE_e$

AME (apparent metabolizable energy) is the difference between the gross energy of the feed and the gross energy of the feces + urine. In poultry the energy lost as gases of fermentation is negligible and ignored.

$$AME = GE_f - (FE + UE)$$

AME_n (apparent metabolizable energy corrected to nitrogen equilibrium) is similar to AME but a correction is made because nitrogen retained (NR) in the body, if catabolized, would yield energy containing compounds. The constant (K) in the following equation is usually 8.22 or 8.73 kcal/g. AME_n is the most widely used form of metabolizable energy.

$$AME_n = AME - (NR \times K)$$

TME (true metabolizable energy) is the difference between the gross energy of the feed and the gross energy of feed origin in the feces + urine. It differs from AME inasmuch as the metabolic and endogenous energy losses, which are a body

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maintenance cost, are not charged against the feed.

$$TME = GE_f - (FE_f + UE_f)$$

or

$$TME = GE_f - (FE - FE_m) - (UE - UE_e)$$

TME_n (true metabolizable energy corrected to nitrogen equilibrium) bears the same relationship to TME as AME_n does to AME.

$$TME_n = TME - (NR \times K)$$

Historical

During the course of an experiment designed to measure variation in AME values it was observed that the AME value of a feed, measured with an individual bird, varied from day to day in a saw-tooth manner. That is, one day the value was high and the next day low. The most likely explanation of this phenomenon was that the feed intake of the bird fluctuated from day to day. However, apart from a theoretical paper by Guillaume and Summers (1970) there was no evidence of a relationship between feed intake and AME.

An experiment was conducted in which starved, adult cockerels were allowed to eat various amounts of wheat. The excreta voided during the subsequent 24 h was collected quantitatively, frozen, freeze-dried, weighed, ground, and, together with a sample of the wheat, assayed for gross energy content. As wheat intake increased there was a linear increase in the energy voided as excreta (Fig. 1). The intercept of the regression line (8.5 kcal) was an estimate of the $FE_m + UE_e$ per bird. The slope of the line (0.709 kcal/g) was an estimate of the $FE_f + UE_f$ voided per gram of wheat consumed. The gross energy value of the wheat was 3.88 kcal/g and the TME value 3.17 kcal/g (3.88 - 0.709). The TME value was independent of variations in wheat intake but when the AME values were calculated they were found to increase in a curvilinear manner with intake (Fig. 2). The cause of the curvilinearity was that the $FE_m + UE_e$ was charged against an increasing energy input. Subsequent experiments with other feedstuffs have confirmed these relationships.

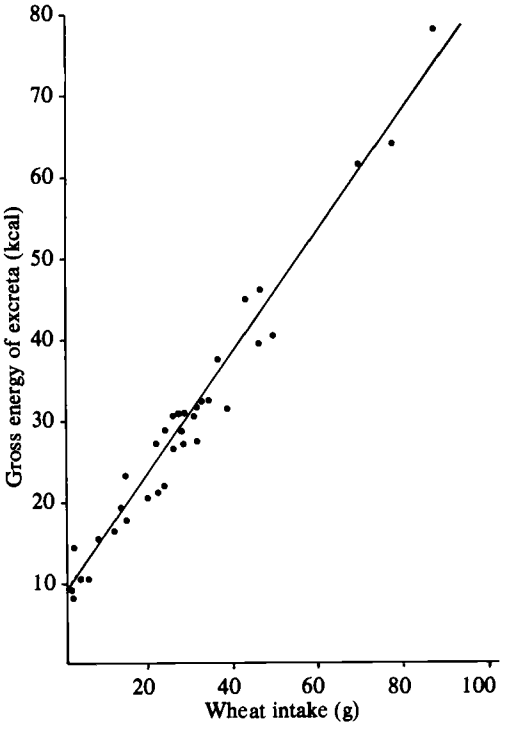

Fig. 1. The relationship between wheat consumption and the gross energy voided as excreta (y_e = 8.5 + 0.709 x; r = 0.991 at 46 degrees of freedom) (from Sibbald 1975).

The TME Bioassay

By assuming that there is a linear relationship between feed intake and excreta energy output it was possible to formulate a simple, rapid bioassay for TME (Sibbald 1976a). The assay involves the following steps:

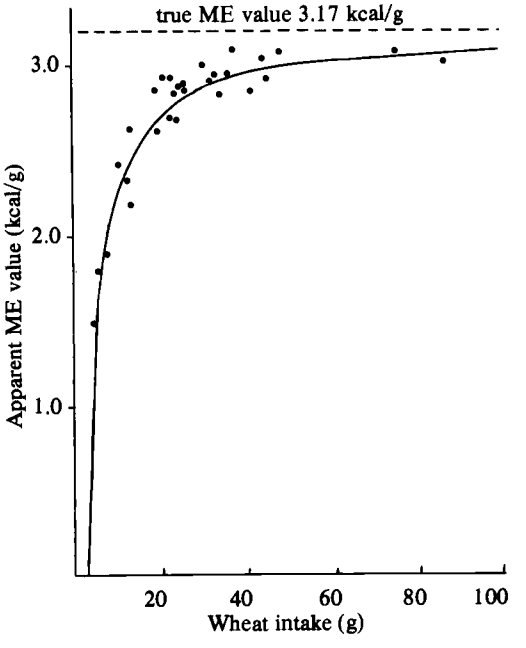

Fig. 2. The effect of level of intake on the apparent ME value of wheat (y=3.17x-8.5)/x) (from Sibbald 1975).

(1) Birds are starved to empty their alimentary canals of feed residues.

(2) A bird is selected, force-fed a known weight of the feedstuff under test, placed in a wire cage over an excreta collection tray, and the time is recorded.

(3) A similar bird is selected and placed in a cage over a tray at a known time but is not fed.

(4) Exactly 24 h after putting the birds in the cages their excreta is collected quantitatively, frozen, dried, and weighed.

(5) Samples of the feedstuff and excreta are ground and assayed for gross energy content.

(6) TME is calculated using the formula.

$$\text{TME (kcal/g)} = \frac{(F_i \times GE_f) - (Y_f - Ye)}{F_i}$$

where: F_i is the feed input (g); GE_f is the gross energy of the feedstuff (kcal/g); Y_f is the energy excreted by the fed bird; and Y_e is the energy excreted by the unfed bird.

The assay has been performed with adult cockerels, laying hens, meat-type hens, turkeys and ducks, and with egg- and meat-type chicks of several ages. However, for routine assay work the adult, single comb, White Leghorn cockerel is preferred. It tends to maintain a steady state, has good livability, and has sufficient feed capacity to minimize experimental errors. Meat-type males become heavy and obese and have higher mortality. Laying birds are not satisfactory because the starvation period, followed by suboptimal feed input, often causes the production of soft-shelled eggs that break and contaminate the excreta. Chicks and growing birds have less feed capacity than mature birds and must be replaced after each assay if several experiments are to be made with birds in a uniform physiological state. In addition, young chicks lose down, which contaminates the excreta

Birds should be housed in individual cages so that they can be handled with a minimum of disturbance. It is desirable to locate the cages in a windowless room so that day-length can be controlled and wide fluctuations in temperature can be avoided. Between assays the birds are fed a maintenance diet ad libitum and fresh water is available at all times, including the starvation and excreta collection periods. Adult cockerels have been used for as many as 30 assays, spaced 14 days apart, without any adverse effects. After an assay the birds are returned to the maintenance regime for a minimum of one day; a longer rest period is preferred to permit return to normal body weight (Sibbald 1978a).

The first step in the assay involves starving the birds to empty their alimentary canals of feed residues. A starvation period of 24 h is adequate but it may be extended to 96 h without altering the data obtained (Sibbald 1976b).

Birds selected for the assay must be healthy and clean. Any excreta adhering to the feet or to feathers around the vent must be removed. The birds must not be in a heavy moult because the feathers and scale make quantitative excreta collection difficult. Body weights should fall within a narrow range because it is assumed that the $FE_m + UE_e$ excretion of negative control birds is the same as that of fed birds. Only a small amount of the variability of $FE_m + UE_e$ can be explained by differences in body weight but it is desirable to minimize experimental variation.

The usual procedure is to assay several feedstuffs simultaneously. This minimizes the amount of work because only one negative control bird is required in each replication. Thus, in planning an assay the number of birds required is: (number of test materials + 1) times the number of replications.

The feed is prepared in advance of the assay. Proper sampling is very important because of the small amount of feed used. The feed is weighed to within 0.01 g and is stored in plastic containers until used. Gross energy and dry matter measurements are made at the time the feed containers are prepared. This ensures accurate knowledge of the dry matter and gross energy inputs and avoids errors that could occur due to fluctuations in moisture content between feed weighing and analysis. Pellets and crumbles are easier to feed than is ground feed. The latter may adhere to the outside of the force-feeding device unless particular care is taken during the feeding operation. Very finely ground feedstuffs may form a lump in the crop, which is slow to disintegrate and which causes delayed passage through the alimentary canal.

Many feedstuffs are assayed as single ingredients but a reference diet and a mixture of the reference diet and test material may be used. The latter introduces an extra treatment but it simplifies the feeding of dusty or greasy materials. It is usual to assay fats in conjunction with a basal diet.

Birds are force-fed because this ensures that the amount and time of the feed entering the bird is controlled. A funnel, which has a stem 40 cm long and 1.3 cm in external diameter, is pushed down the esophagus until it enters the crop. The previously weighed feed is poured from its plastic container into the funnel and pushed into the crop with a plunger. It is important that the feed is placed in the crop and not in the esophagus, as the

latter increases the incidence of regurgitation. If the end of the funnel is in the esophagus the funnel will be pushed out as feed is pushed in. After feeding, the funnel is removed with a rotary motion and pressure is applied to the wall of the esophagus to remove any adhering feed particles. With a 3-man team force-feeding takes less than 1 min per bird: one man catches and delivers the bird to be fed and places the fed bird in the appropriate cage; a second receives the bird inserts the funnel and pours the feed into the funnel; and a third pushes the feed down the funnel and maintains records of bird, feed type and weight, and time of completion.

The amount of feed input depends upon the size of the bird and the form, availability, and nature of the feedstuff being assayed. The greater the input, the smaller the effect of experimental errors; however, as feed input rises the incidence of regurgitation increases. For adult Leghorn cockerels weighing 2–3 kg, the optimum input is 30–40 g of pellets or 25–30 g of ground feed (Sibbald 1977a).

The excreta collection trays must have smooth surfaces and should extend beyond the cage in all directions. Plastic cafeteria trays are satisfactory and inexpensive.

The usual excreta collection period is 24 h. But, whatever time is selected it is essential that it be constant for all birds in an assay; hence, the importance of recording the times when birds are fed. Recently, it was found that the residues of some feedstuffs did not clear the alimentary canal within 24 h. Materials with slow rates of passage include dehydrated alfalfa, peanut skins, and some meat and fish meals (Sibbald 1979a). The problem can be overcome by extending the excreta collection period. If TME values obtained with a 24-h collection period are erratic, and if there is no evidence of regurgitation, it is possible that an extension of the excreta collection period will provide more uniform data.

The collection and processing of excreta are relatively simple but they must be done carefully to minimize experimental errors. Holding birds for force feeding can loosen feathers and scale, which fall onto the excreta collection trays. By blowing them off the trays about an hour after feeding a considerable amount of contamination is avoided. When collecting excreta, care must be taken to include any which adheres to the cage floor, and feathers, mixed with excreta, must be washed before being discarded. It is very important that each tray be examined for regurgitated feed. If regurgitation occurs, data from the bird must be discarded. Freeze-drying is the preferred method of removing water from excreta, but it involves a large capital cost. In a recent experiment it was found that oven-drying at 65, 80, or 95°C was just as satisfactory as freeze-drying (Table 1). The dry excreta is weighed, ground, and assayed for gross energy. Dry excreta tends to pick up atmospheric moisture; therefore, if time permits, the excreta should be equilibrated with the atmosphere for 2–3 days before further processing. Excreta can be ground with a mortar and pestle, which is easier to clean than most mills.

The calculation of TME values is straightforward. It is common practice to use a mean value for the $FE_m + UE_e$ to reduce data variability. If a TME value is large, relative to replicate determinations, it is probable that there was an incomplete collection of feed residues. There are three possible reasons: regurgitation beyond the collection tray; excretion beyond the tray; and incomplete passage of residues during the collection period. An extremely low TME value is usually due to regurgitated feed being mixed with the excreta. Regurgitation should not be a major problem once the art of force feeding is mastered.

Table 1. The gross energy output of negative control birds and the true metabolizable energy values of two feedstuffs (from Sibbald 1978d).

Drying method	Energy output of negative controls (kcal/bird)	TME (kcal/g)	
		Wheat	Laying diet
Freeze dried	11.1±0.8	3.71±0.06	3.37±0.05
Oven (65 °C)	10.0±1.1	3.82±0.04	3.45±0.06
Oven (80 °C)	10.3±0.6	3.71±0.06	3.50±0.11
Oven (95 °C)	11.1±0.6	3.70±0.03	3.46±0.05

Advantages of the Assay

The TME bioassay, with adult Leghorn cockerels, has several distinct advantages over the conventional chick assays developed for the measurement of AME and AME_n. The birds can be used for a large number of assays before being replaced and can be maintained in simple wire cages. By maintaining a flock of birds it is possible to initiate and complete an assay in a short period of time. The labour requirement is relatively small. One technician can make 6 replicate determinations on each of 15 feedstuffs in 10 working days provided a little supplementary help is available for force feeding. In an emergency, a sample can be assayed within 35 h provided 24-h notice of the time of arrival is given to permit starvation of the birds prior to sample receipt.

The small amount of test material required for a TME assay is an attractive feature. A 200-g sample permits six replicate determinations plus associated chemical and physical analyses. The small sample size places greater emphasis on the need for a proper sampling procedure but it has the advantage that materials in limited supply, such as new cultivars of grains, can be assayed. Of greater importance is the feasibility of shipping samples over relatively long distances to a central quality control laboratory.

Although the TME assay is simpler, faster, and less expensive than AME assays, its main advantage resides in the quality of the data it yields. The correction for $FE_m + UE_e$ makes TME values independent of variations in feed intake (Fig. 2). It may be argued that the $FE_m + UE_e$ excretion of a negative control bird differs from that of a fed bird. There is some validity to the argument because it was shown that $FE_m + UE_e$ excretion decreased with the duration of starvation (Sibbald 1976b); however, the error is probably small and is outweighed by the benefits resulting from the correction.

In AME bioassays a high level of test material is included in the assay diet to minimize the effects of experimental errors. If the test material is unpalatable, voluntary feed intake decreases and a low incorrect AME value is obtained. MacAuliffe and McGinnis (1971) found a marked decrease in the AME value of rye as the amount in the assay diet increased. The palatability problem can be overcome by feeding the test material at a practical level in combination with other ingredients that mask its low acceptability. However, this is self-defeating because data obtained with low levels of inclusion are highly variable.

It is known that AME_n values vary according to the type of bird used in their derivation. Work with the TME assay is less extensive but it appears that values obtained with adult cockerels can be used in the formulation of diets for laying hens, broiler hens, turkeys, and chicks (Sibbald 1976a, c; 1978b). Although additional work is required, it seems probable that differences in feed intake relative to $FE_m + UE_e$ output contribute to between bird-type variation in AME data.

There are several examples of AME values of feedstuffs varying according to the compositions of the diets with which they are fed. The TME values of five feedstuffs and of 10 diets prepared from them were measured. The observed values of the diets did not differ from those calculated using the values for the component parts (Sibbald 1977b). This evidence of additivity is important in selecting an available energy assay system. It should be noted that the TME values of fats are not additive because of interactions with other dietary components (Sibbald 1978c; Sibbald and Kramer 1977, 1978), but this is a problem that also affects AME data. There is no apparent solution to the problem.

An important feature of the TME assay is the reproducibility of data between laboratories. In a collaborative study conducted by the Animal Nutrition Research Council, 17 mean AME_n values for a sample of yellow corn ranged from 3.08 to 4.03 kcal/g DM; whereas, 9 mean TME values ranged from 3.98 to 4.15 kcal/g DM (Table 2). By definition, TME values should be greater than AME_n values. The lower variability associated with the TME assay is persuasive to its adoption particularly when it is noted that five of the laboratories had no prior experience with the TME assay.

Although not directly relevant it is of interest that the basic methodology of the TME bioassay has been successfully applied to the measurement of bioavailable amino acids (Likuski and Dorrell

Table 2. A comparative study of the AME_n and TME bioassays — energy values are expressed as kcal/g DM (from Sibbald et al. 1979).

	Corn		Alfalfa	
	AME_n	TME	AME_n	TME
n	17	9	17	7
Mean	3.53±0.07	4.10±0.02	1.52±0.09	1.67±0.05
Low	3.08	3.98	1.06	1.45
High	4.03	4.15	2.63	1.94

1979; Sibbald 1979c,d). It is in fact possible to measure both TME and true available amino acids using a single set of feed and excreta samples. This additional flexibility of the assay should be of interest to those establishing quality control laboratories, particularly where operating funds are limited.

The major objection to the adoption of TME as the available energy standard in poultry nutrition is that most energy requirement data are expressed in terms of AME_n. This should not be a major stumbling block and indeed some feed manufacturers have already adopted the TME system. A temporary solution is the use of a factor to convert AME requirement data to TME values. This has been discussed at length by Sibbald (1977c).

The feed industry and regulatory bodies will decide which measurement of metabolizable energy will be adopted. There is need for additional work on the TME system, but it appears to be more attractive than conventional systems in terms of cost, data quality, reproducibility, and flexibility.

Indirect Assays for Metabolizable Energy

There have been several attempts to predict the metabolizable energy values of poultry feeds from physical and chemical data. Few of the published prediction equations can explain more than 80% of the variability in ME values when tested on independent data. This is not surprising because although chemical techniques can yield accurate quantitative data on absolute amounts of nutrients in feedstuffs they are, as yet, unable to measure those portions that can be digested and absorbed. In addition, they fail to take account of interactions between nutrients and between nutrients and other dietary components.

Major attractions of the indirect assay were the speed with which it could be executed and the relatively low cost. The TME bioassay has reduced the time and cost differentials between bio- and indirect-assays. A simple, rapid, accurate, indirect assay would be extremely valuable, but its development seems unlikely because of the complexity of feedstuffs and the variation in nutrient availabilities.