AN ASSESSMENT OF THE TECHNIQUE FOR TRUE METABOLIZABLE ENERGY (TME) AND THE PREDICTION OF TME OF BARLEY FROM CHEMICAL COMPOSITION AND PHYSICAL MEASURES

BY

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A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANIMAL SCIENCE

UNIVERSITY OF MANITOBA

WINNIPEG, MANITOBA

JUNE, 1992

ISBN 0-315-77951-9

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A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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AN ASSESSMENT OF THE SIBBALD TECHNIQUE FOR TRUE METABOLIZABLE ENERGY (TME) AND THE PREDICTION OF TME OF BARLEY FROM CHEMICAL COMPOSITION AND PHYSICAL MEASURES

ABSTRACT

A series of experiments were conducted to assess the reliability of the true metabolizable energy (TME) bioassay as a technique for measuring bioavailable energy in poultry and to investigate the feasibility of predicting TME of barley from chemical composition and physical parameters. Length of starvation interval, provision of supplementary energy, excretion of endogenous energy loss, correction of excreted energy to zero nitrogen balance and bird numbers used in the assay were factors that were evaluated in relation to the precision and accuracy of the TME bioassay with adult SCWL cockerels. Ninety one samples representing three barley types (6-row malting, 6-row feed and 2-row feed) were used to develop prediction equations based on chemical composition and physical measures (i.e. test weight). Simple and multiple regression analyses including stepwise regression and the Mallows Cp value were used in the development of prediction equations for TME.

Lengthening of the starvation interval from 24 to 28 h significantly reduced FmE + UeE loss and improved the resulting TME estimate. Extension of the interval to 36

h showed no further improvement. Although the provision of supplementary energy (30 g granular sucrose) to unfed birds significantly reduced excreta weight and nitrogen loss relative to controls, the correction of energy excretion to zero nitrogen balance resulted in no influence on TME values.

There was a substantial variation in energy loss among birds. The gross energy content per g of excreta was observed to be negatively proportional to excreta weight, and the energy excretion was positively correlated with endogenous nitrogen loss for both fed and unfed birds. The standard deviation values of the means of energy and nitrogen excretions showed a trend to decrease with increasing bird numbers with the values reaching a relatively stable level at 24 and 8 birds for unfed and fed groups, respectively. The relative error between TME values was shown to be less than 1.0% when at least 8 birds per group of fed birds were used. There was little evidence to show a need for repeated measurement of endogenous energy in each trial.

Correction of energy excretion to zero nitrogen balance resulted in a substantial decrease in the standard deviation of the TME value and a slight decrease in TME. The magnitude of the difference between TME and nitrogen corrected TME values increased with increasing protein content of feedstuffs. The nitrogen correction factors derived from regression analyses were 41.42 and 41.28 (kJ/g nitrogen) for unfed and fed birds, respectively.

The TME contents of 91 barley samples indicated a range of 13.1 to 14.6 with a mean of 14.0 (MJ/kg. dry matter). Among individual variables relating to chemical composition, neutral detergent fibre (NDF) was found to be the most effective predictor

of TME and the physical measure, test weight, was found to be a less sensitive variable. Inclusion of NDF, gross energy (GE), fat, protein and starch terms in multiple regression equations resulted in a slight decrease in residual standard deviation (Rsd) and an increase in R^2 relative to the NDF prediction. The best combinations of the variables derived from stepwise regression analysis were, in decreasing order, NDF, GE and fat, and from the Mallows Cp value, NDF, fat and starch. With the exception of the equation in which test weight was used as sole predictor, the error estimated from the prediction equations approached that for the biological measure.

ACKNOWLEDGEMENTS

The author is deeply indebted to his supervisor, Dr. L. D. Campbell, for his invaluable guidance, encouragement and assistance during the course of this study.

Sincere gratitude is expressed to the members of the committee: Dr. W. Guenter, Dr. G. Blank, and Dr. G. Crow for their comments and interest in this work.

The technical assistance of H. Muc and the poultry barn staff was greatly appreciated.

The author wishes to dedicate this thesis to his family.

FOREWORD

This thesis was written in a manuscript format and four manuscripts will be submitted for publication. The authors of manuscripts 1, 2 and 3 will be W. Zhang and L. D. Campbell. The authors of manuscript 4 will be W. Zhang, L. D. Campbell and S. C. Stothers.

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GENERAL INTRODUCTION

Feed energy represents the largest and most expensive portion of the diets for all classes of animals and is an important nutrient as an essential constituent of animal metabolism. Energy also has an indirect effect on other nutrients through its influence on feed intake. In comparison to other nutrients, energy is more difficult to evaluate in feedstuffs and considerable research effort has been directed to the development of techniques for measuring bioavailable energy (BE) for different species of animals. In poultry, metabolizable energy (ME) systems (apparent metabolizable energy and true metabolizable energy) are used more extensively than digestible energy (DE) simply because birds excrete faeces and urine through a common vent. The determination of the BE content of a diet or a single feedstuff is, therefore, more conveniently carried out in terms of its ME than of its DE value. Currently there is little interest in the use of net energy (NE) as a basis for evaluating energy in feedstuff for poultry.

The bioassay for apparent metabolizable energy (AME) involves the use of a preliminary feeding period to establish equilibrium conditions and a prolonged collection period, consequently the method is costly and time consuming. In response to a need for a rapid test for BE, Sibbald (1976a) developed the bioassay for true metabolizable energy (TME). The TME assay is based on two assumptions: (1), there is a linear relationship

between energy input as feed and energy output as excreta; (2), the intercept of the regression equation relating the energy intake to energy excretion is a valid estimate of the faecal metabolic and urinary endogenous energy (FmE + UeE) losses. The TME value is thus obtained by calculating AME and discounting FmE + UeE from total excreta energy, and consequently the TME of a feedstuff is not affected by feed intake. In the routine assay FmE + UeE is usually estimated by measuring the energy loss of unfed birds rather than by the regression method as indicated above.

There have been several suggestions for modification to the original Sibbald method in an attempt to improve the accuracy and precision or reproducibility of the TME estimate. These modifications include correction of energy excretion to zero nitrogen balance, length of starvation and collection periods and attempts to control variation in endogenous energy excretions. In this latter regard there is considerable debate in the literature focused on the validity of the TME estimate as influenced by the variability in the FmE + UeE measurement.

The AME value is, when determined with growing birds, adjusted on the basis of nitrogen balance. This is based on the observation that a portion of the AME retained in the body as nitrogenous compounds is unavailable when it is catabolized for energy-yielding purpose. This concept has been questioned on the consideration that retained protein is characteristic of growth and/or egg production and, therefore, it is difficult to justify the discounting of a diet that results in nitrogen retention. In the TME system it is recognized that unfed birds tend to catabolize more body tissue for energy-yielding purpose than do fed birds. In this regard the correction of energy excretion to zero nitrogen balance reduces the variability in FmE + UeE among birds and improves the

accuracy and precision of the TME estimate. The magnitude of nitrogen correction may increase with increasing protein content of the feedstuff tested, which implies that the correction is not only related to the difference in FmE + UeE loss between fed and unfed birds but also to energy retained as nitrogenous compounds. As with the AME assay this raises the question whether or not the correction to zero nitrogen balance is of practical importance, especially with regard to high protein feedstuffs.

The measurement of either AME or TME on numerous samples is impractical due to labour and cost involved and consequently a faster and more economical method is required for estimating BE values of feedstuffs. Recently considerable research effort has been directed toward the development of prediction equations relating chemical composition or physical properties to BE values. However, these equations may be meaningful only for a narrow range of feedstuffs or diets depending upon the data set that is used in generating the equations.

The present studies were conducted to assess the reliability of the TME bioassay and to further investigate the feasibility of predicting energy value from chemical composition and physical properties.

REVIEW OF THE LITERATURE

Conception and Basic Methodology of the TME Bioassay

Biological Measures of Energy in Animals

Feed energy is an important nutrient as an essential component of animal metabolism and is the most expensive constituent in diet for all classes of animals. Considerable research effort has been directed to the development of the techniques for measuring BE of feedstuffs.

The gross energy (GE) is the combustible heat of a feedstuff. It represents a maximum value and is the basic value for investigating other forms of energy. However, this energy has no practical applicability because not all of GE is available to the animal.

Ingested GE is subjected to a digestive process before it can be absorbed in the digestive tract. Most of the undigested and unabsorbed GE is excreted as faecal energy (FE). DE is the difference between ingested GE and FE. Since FE includes both energy of feed origin and of metabolic origin, calculated DE is usually termed apparent DE. ME is derived in terms of the difference between ingested GE and faecal plus urinary energy (FE + UE). ME is also referred to as AME, sometimes referred to as classical ME, as the calculation does not account for the energy in faeces and urine that originates from

metabolic and endogenous sources. The portion of gas energy , if any, should be treated as a digestive rather than a metabolic loss since it is formed in the intestinal tract. In poultry gas energy is negligible in practice as it arises from microbial fermentation of feedstuffs occurring primarily in the ceca and to some extent in the crop. In this regard gas energy is generally not involved in the calculation of AME content. TME is an estimate of BE in which a correction is made for FmE + UeE. It is assumed that FmE + UeE is a energy cost for maintenance of life and should not be charged against feedstuffs. NE of a feedstuff can be obtained by determining its ME value and heat increment (HI) which includes the heat of fermentation, the heat of digestion and absorption, the heat of nutrient conversion and the heat of waste formation and excretion. NE can further be divided into two parts: the NE for maintenance (NEm) and the NE for production (NEp). The former is related to maintenance of life, and the latter is used for productive purposes. In general, the methods of choice for evaluating BE are DE for pigs, ME for poultry and NE for ruminants.

Premise and Basic Method of the TME Bioassay

The conventional technique for the measurement of AME involves feeding a group of birds for a preliminary feeding period to establish equilibrium conditions prior to a prolonged collection period. In addition it is necessary that the test feedstuff be mixed at at least one level with a basal diet and subsequently energy balance must be conducted for the basal diet and the test feedstuff-basal diet mixture to allow for the determination of the AME content of the test feedstuff. This method is time consuming

and costly. In addition, AME values measured with a variety of feedstuffs have been found to vary with age (Sibbald et al., 1960; Lockhart et al., 1963; Bayley et al., 1968; Zelenka, 1968; Lodhi et al., 1970; Rao and Clandinin, 1970; March et al., 1973; Fisher and Shannon, 1973), strain and breed (Sibbald and Slinger, 1963a; Slinger et al., 1964; Foster, 1968; March et al., 1973) and species of birds (Slinger et al., 1964; Bayley et al., 1968; Fisher and Shannon, 1973; Leeson et al., 1974; Charalambous and Daghir, 1976) and with the level of feed intake (Sibbald, 1975; Schang and Hamilton, 1982; Jonsson and McNab, 1983). The curvilinear relationship between feed intake and AME values reported by Guillaume and Summers (1970) and Sibbald (1975) may be explained by the fact that under standardized conditions the FmE + UeE loss is constant, and as the level of feed intake increases to a high level, FmE + UeE loss is small relative to the energy loss of feed origin and consequently has only a minor effect on the AME value. At low levels of feed intake, however, the Fme + UeE loss is proportionally large and results in a decrease in the AME value. In this regard, variability in AME values relative to age, breed and species of bird as well as environmental conditions may be associated with variation in the ratio of the FmE + UeE and feed energy loss in excreta.

Apart from the conventional measure of BE, there are two rapid methods in use currently for the determination of BE contents of feedstuffs for poultry. These are the AME technique of Farrell (1978) and the TME technique of Sibbald (1976a). Farrell (1978) recommended the use of adult cockerels trained to consume about 70 g feedstuff in 1 h period. The birds are starved prior to feeding and a 24 h collection period is used to determine the AME of a test ingredient mixed with a basal diet consisting of maize, fish meal and bone meal. The procedure is simpler, faster and less costly than the

conventional one. However, it is not always easy to train birds to consume their feed requirement during a short time period, especially when novel feeds are introduced into a diet (Schang and Hamilton, 1982; Jonsson and McNab, 1983; Mollah et al., 1983; Parson et al., 1984). Furthermore, since it is necessary that the test material be mixed with a basal diet, the level of inclusion of the test material could result in variable results through feed intake effects at high levels of inclusion or because of error magnification at a low level of inclusion.

In evaluating the AME technique, Guillaume and Summers (1970) observed that variation in AME was attributable to the influence of feed intake and concluded that a certain minimum daily feed intake was necessary for valid AME determination. It was demonstrated that a level close to or slightly less than the maintenance energy requirement resulted in a disproportionately high FmE + UeE loss relative to the total energy loss such that AME values were depressed. Sibbald in recognition of this developed a simple, rapid method for measuring BE content of feedstuffs. The method as originally described by Sibbald (1976a) is as follows:

Adult roosters housed in individual cages, with free access to water, are starved for about 21 h.

A bird is selected, weighed and force fed an accurately weighted amount (20 to 25 g) of the feedstuff under investigation.

The bird is returned to its cage, a plastic tray is placed under the cage, and the time is recorded.

A bird of similar body weight is selected and returned to its cage without feeding, a plastic tray is placed under the cage and the time is recorded.

Steps 2, 3 and 4 are repeated to provide the desired number of replications.

Exactly 24 h after placement, the plastic trays are removed, the excreta are collected quantitatively, frozen, freeze dried, equilibrated to room temperature and moisture, and weighed.

Samples of the feedstuff and excreta are ground to pass through a 20-mesh sieve and assayed for GE.

$$\text{TME (kcal/g. air dry)} = (GEf * x) - (yef - yec) / x$$

where: GEf is the GE of the feedstuff (kcal/g);

yef is the energy voided as excreta by the fed bird;

yec is the energy voided as excreta by the unfed bird;

x is the weight of feedstuff fed (g);

Adult roosters were suggested for use in the assay because young chickens have a limited feed intake, and laying hens tend to lay shell-less eggs when fasting, thus causing contamination of excreta. A feed intake level of 20 to 25 g was found to be the optimal input level for most feedstuffs for roosters weighing about 2.4 kg although this tended to vary with the nature of the feedstuff. Sibbald (1976a) indicated that at low levels of feed intake small errors in excreta quantity led to large influences on TME, whereas higher levels of feed intake increased chances of regurgitation.

Sibbald (1976a) indicated that by discounting the FmE + UeE loss from the total excreta energy, the resulting BE would be different from AME, and hence it was termed as "true metabolizable energy", that is TME = AME + FmE + UeE. Since the magnitude of FmE + UeE would vary with feed intake levels, TME was suggested to be independent of the variation in age, sex, breed and species of assay birds as well as

environmental conditions (season, temperature).

Modifications to the Original Sibbald Procedure

As testing proceeded and laboratories became more knowledgable concerning the TME bioassay, several suggestions of modifications to the original Sibbald technique for the TME bioassay were proposed. The modifications that have been generally adopted are: lengthening of the starvation period from 21 to 24 h; extension of excreta collection period from 24 to 48 h, and correction of energy excretion to zero nitrogen balance. Other modifications that have not been universally accepted include supplementary feeding of a highly absorbable nitrogen-free energy source and alterations in the collection methods including the use of colostomy bags with several types of attachment.

Preliminary Starvation Period

The interval of time required to ensure complete clearance of the feed residues from the GI tract of the test birds is an important consideration in the TME assay. In this regard, factors likely to be involved include the nature and intake level of the previous feed. Generally a time period greater than the 21 h period originally suggested by Sibbald (1976b) has been adopted for use by most investigators. There is evidence to show that the extension of the starvation period from 12 to 24 and even to 48 h was associated with increases of 1.5 and 3.0%, respectively, in the TME content of corn (Shires et al., 1979). Sibbald (1976b) and Sibbald and Morse (1983) indicated that

starvation for less than 24 h could influence the precision of the TME values particularly if the GI tract was not free from feed residues. Direct investigation of the residue present in the GI tract of test birds by Fisher and McNab (1987) indicated that starvation for 48 h reduced the amount of feed residues as opposed to that of starvation for 24 h. Consequently, Fisher and McNab (1987) recommended the use of a 48 h starvation period for the TME assay although intermediate time periods were not investigated. In contrast, Sibbald (1976b) and Sibbald (1979a) found no improvement in determined TME values by lengthening the starvation beyond 24 h. It is apparent that the original starvation interval as suggested by Sibbald may not be of sufficient time to empty the GI tract, and that the optimum length depends on the level of feed intake and the nature of feedstuffs. Furthermore, due to the small bird number for both fed and unfed groups usually used in the TME assay of Sibbald, a large amount of excreta occasionally observed in some birds could result in a large variation in TME estimate.

Extension of the starvation period has been found to increase stress and influence the subsequent estimate of FmE + UeE (McNab and Blair, 1988). A starvation interval of 72 h for a standard assay is prohibited in the United Kingdom (Sibbald and Morse, 1983). Indeed, starvation is not essential for the TME assay. An alternative approach of direct measurement of FmE + UeE is to feed several levels of the test material under investigation and to measure the regression of excreta energy on feed input and to calculate TME value in terms of FmE + UeE (intercept of regression line) and AME values (regression coefficient) (Sibbald and Morse, 1983). However, in view of the simplicity of the direct measurement, it is the method of choice and consequently it is important to include an optimal starvation period providing that humane limits are not

exceeded. The time requirement for this purpose is still a complicated and unresolved issue and further studies are needed to clarify this point.

Excreta Collection Period

The original time interval of 24 h for excreta collection as suggested by Sibbald (1976a) has been shown to be too short to ensure complete passage through the GI tract for some ingredients. Observations that extension of the excreta collection period resulted in a decrease in the TME values for high protein feedstuffs (soybean meal, rape seed meal, meat meal, fish meal and feather meal) and fibrous materials (wheat bran, oats and alfalfa meal) indicated that an excreta collection period of more than 24 h was required for complete clearance of feed residues from the GI tract (Sibbald, 1979b, 1980a; Chami et al., 1980; Kessler and Thomas, 1981; Muztar and Slinger, 1981c; Schang and Hamilton, 1982; Parson et al., 1984). In this regard, Salmon (1984) proposed a collection period of 29 h, and in a study of passage of feed residues through the GI tract of adult cockerels Sibbald (1979b) who observed that the interval of time for excreta collection depended upon the feedstuffs tested, also recommended that the interval be extended beyond 24 h. Consequently, Kesseler and Thomas (1981), Sibbald (1986) and Fisher and McNab (1987) proposed the use of 48 h as a standard collection period for all ingredients in the TME assay. Lengthening this period beyond 48 h was reported to show no further improvement in the accuracy and precision of TME values (Fisher and McNab, 1987; Sibbald and Wolynetz, 1988).

Correction of Energy Excretion to Zero Nitrogen Balance

Traditionally, in the conventional AME evaluation system a correction to zero nitrogen balance has been routinely used. This is based on the consideration that a portion of the AME retained by birds during the experimental period is unavailable when it is catabolized as an energy source, and that since the magnitude of nitrogen retention varies with age and genotype of birds, a correction of the AME value should be applied to allow for extrapolation among bird types. Such a correction would be of practical merit to decrease error in the AME estimate due to variation in the FmE + UeE relative to feed intake among bird types but it has been shown to be subject to several sources of error. Since protein retention is a characteristic of growth and/or egg production, no penalty should be exacted for nitrogen retention. Furthermore, not all nitrogen stored as nitrogenous compounds is catabolized for energy-yielding purpose (Proudman et al., 1970; Sibbald, 1975). Since the magnitude of nitrogen correction is proportional to the crude protein content of feedstuffs, nitrogen corrected AME tends to underestimate the energy content of protein-rich feedstuffs and to overestimate that of energy-rich sources (Sibbald and Slinger, 1962; Proudman et al., 1970; Shannon and Brown, 1970).

Diet has a direct effect on the distribution of nitrogenous products in the urine. The use of correction factors of either 8.22 kcal/g (Hill and Anderson, 1958) or 8.73 kcal/g (Titus et al., 1959) could result in a biased estimate of energy loss relative to nitrogen retention and hence introduce error into the AME estimate (Okumura et al., 1981; Askbrant and Khalili, 1990).

In the TME evaluation system, it is recognized that unfed birds depend completely on the catabolism of body tissue to meet the energy requirement for maintenance during the starvation and excreta collection periods. In fed birds, the FmE + UeE loss can be expected to be less than that by unfed birds due to a sparing influence of test material on the catabolism of body tissue. The use of unfed birds for estimating FmE + UeE loss for fed birds may not be completely accurate, and as such a difference in FmE + UeE loss between fed and unfed birds could result in an overestimate of the TME value (Dale and Fuller, 1982b; Parson et al.,1982, 1984; McNab and Blair, 1988; Askbrant and Khalili, 1990). It was reported that most of the FmE + UeE loss was due to the degradation of tissue protein (Parson et al., 1982; Sibbald, 1981a, 1981b; Sibbald and Morse, 1982). Correction of energy excretion to zero nitrogen balance could reduce the difference in FmE + UeE loss between fed and unfed birds and increase the accuracy and precision of the TME estimate (Shires et al., 1980; Dale and Fuller, 1984; Fisher and McNab, 1987). Sibbald and Morse (1983) indicated that application of nitrogen correction decreased error variance by more than 40%. Reasons for the change were not given but the resulting nitrogen corrected TME values (TMEn) were slightly lower. These researchers concluded, however, that TMEn was the most useful estimate of BE for practical purposes and Sibbald (1986) outlined the following equation for the routine application of the TMEn technique:

(1), Excreta energy voided by fed birds corrected to zero nitrogen basis is calculated as:

$$(FEn+UEn) = (FE+UE) + K\ (IN-FN-UN)$$

where: (FEn+UEn) is nitrogen corrected excreta energy voided by fed birds.

(FE+UE) is excreta energy voided by fed birds.

K is constant which represents GE of each g of nitrogen loss (8.22 or 8.73 kcal).

IN is the nitrogen input.

FN and UN are the faecal and urinary nitrogen output.

(2), Excreta energy voided by unfed birds corrected to zero nitrogen basis is calculated as:

$$(FmEn+UeEn) = (FmE+UeE) + K\ (IN-FN-UN)$$

where: (FmEn+UeEn) is nitrogen corrected excreta energy voided by unfed birds.

(FmE+UeE) is metabolic plus endogenous energy.

Under the conditions of the TME assay, IN for unfed birds is zero, and the term K (IN-FN-UN) is negative. TMEn is then calculated as:

$$TMEn = IE - (FEn+UEn) + (FmEn+UeEn)$$

where: IE is the amount of test material precision-fed to the fed birds.

Other studies also indicated that correction of energy excretion to zero nitrogen balance improved the accuracy and precision of TME estimate by reducing the difference in FmE + UeE between fed and unfed birds and the variation in FmE + UeE losses among unfed birds. These studies also showed the tendency for reduced TME values of feedstuffs when the nitrogen correction was applied (Shires et al., 1980; Muztar and Slinger, 1981a; Parson et al., 1982). Dale and Fuller (1984) observed that the magnitude of nitrogen correction was correlated with the protein content of feedstuffs, and that 60% difference observed between TME and TMEn was due to change in protein content.

Muztar and Slinger (1981c) reported a similar result but they failed to correct the nitrogen loss of unfed birds and in this case, the calculated TMEn values were higher than the TME values. Parson et al. (1984) indicated 3.7 and 12.3% differences between TME and TMEn values for corn fermentation solubles and menhaden fish meal, respectively. It may be concluded that differences between TME and TMEn values depend on both protein level and feed intake.

It is apparent that the importance of correction of energy excretion to zero nitrogen balance used in the TME assay is completely different from the concept for AME. Correction of TME to zero nitrogen balance reduces the difference in FmE + UeE loss between fed and unfed birds and the variability in FmE + UeE losses among unfed birds and hence improves accuracy and precision of the TMEn estimate. There is a trend in the difference between TME and TMEn values to increase with increasing protein levels of feedstuffs. In this regard, further work is required to study the relationship between TME and TMEn.

Feeding of Supplementary Energy

A prolonged starvation period and a lengthened excreta collection duration is stressful and body tissue is catabolized to meet maintenance energy requirements. However, since fed birds in the TME assay receive some energy, the difference in the FmE + UeE between fed and unfed birds is inflated. In this regard, the feeding of highly digestible nitrogen-free energy source to unfed birds was proposed (Dale and Fuller, 1982b; Sibbald and Morse, 1983). McNab and Blair (1988) precision fed 40 ml (25 g)

aqueous glucose to all birds during the starvation period and 50 g granular glucose to unfed birds at the same time as the fed birds received test materials. The results showed that the feeding of supplementary glucose reduced variation in the TME estimate as judged by standard deviation values. Data from similar experiments conducted by Sibbald and Wolynetz (1988) provided no evidence of such an improvement. In contrast, the procedure caused impacted crops and the regurgitation of glucose resulted in contamination of excreta and hence an underestimation of TME values. Another disadvantage cited for this procedure related to the unresolved problem of the difficulty of administering granular glucose to the birds. In addition, any apparent advantage of administration of supplementary energy to birds to reduce the difference in FmE + UeE loss between fed and unfed birds in the TME assay may be counteracted by the nitrogen correction modification to the original assay. Correction of TME to zero nitrogen balance may counteract the effect of supplementary energy, and hence TMEn values obtained from birds receiving supplementary energy would be similar to those from birds receiving no supplementary energy. Further work is needed to clarify these issues.

Methods for Excreta Collection

Excreta collection with trays is simple but is difficult to do well in the TME bioassay because of adherence of feathers, contamination with scales, microbial fermentation and physical losses in collection and transfer. Frequent collection, blowing on trays to remove scales and washing off of feathers have been recommended by Sibbald (1986) as part of the routine tasks in the TME assay. As an alternative to the use

of trays for excreta collection, Sibbald (1986) suggested the use of colostomy bags attached to the birds with a cement glue. Later as a modification Sibbald and Wolynetz (1987, 1988) used a harness recommended by Almeida and Baptista (1984) to equip the birds with the colostomy bags.

Excreta samples collected using bags were generally cleaner and were more easily freeze-dried. However, harness slippage and bag leakage frequently occurred preventing the accurate collection of excreta. In addition, excreta energy measured with fed birds and FmE + UeE determined for unfed birds were observed to be less when using bags as opposed to trays for collection (Sibbald and Wolynetz, 1986, 1988, 1989). Stress as a result of bag attachment was suggested as a possible cause of this latter effect as birds were observed to excrete compensatory excreta levels up to 24 h post-bag removal. Another potential problem with this technique is that excreta in bags remained moist during the experimental period producing conditions favourable for excessive microbial fermentation. Fisher and McNab (1987) and Sibbald and Wolynetz (1988) concluded that the harness and bag mechanism may introduce error into the assay and hence the procedure was in need of further study and improvement. In this regard, the original tray collection method is the preferred method.

Factors Affecting Precision of TME Estimate

From the viewpoint of methodology the TME bioassay is based on the assumption that the GI tract is completely free from previous feed residues prior to the beginning of the assay and empty at the end of the assay. A given amount of test material is fed and

the excreta energy voided by fed birds is corrected for the endogenous energy (FmE + UeE) measured with unfed birds. The factors contributing variation to the TME values of feedstuffs include age, body weight, genotype of birds and the level of feed intake and the nature of feedstuffs.

Factors Influencing FmE + UeE Loss

Considerable debate on the reliability of the TME bioassay has focused on the use of the correction for FmE + UeE, especially when unfed birds are used as estimators of FmE + UeE for fed birds. Since the initial development of the technique there has been a considerable amount of research done to study the variability in FmE + UeE and possible factors influencing FmE + UeE.

It was indicated that FmE + UeE varied widely from bird to bird within a population and that the variability was independent of time period (Sibbald, 1976a, Dale and Fuller, 1982b; Askbrant, 1988). Similarly, Sibbald and Price (1978), in a survey of 300 FmE + UeE values from 38 experiments carried out during a period of 3 years, found that the FmE + UeE loss measured with Leghorn roosters varied from bird to bird, ranging from 5.97 to 16.57 kcal/24 h and that there was no evidence to indicate any relationship between the year or the time of year and the FmE + UeE loss. Consequently it was concluded that the variation in FmE + UeE was largely characteristic of the birds, and it was suggested that each bird be used as its own estimator of FmE + UeE. In further studies Sibbald and Price (1980) indicated that there was a high level of variation in FmE + UeE among experiments and suggested the need to measure FmE + UeE in

each assay. Because of the variation in FmE + UeE loss being characteristic of the birds, the use of each bird as its own control has merit for reducing the variation, but this may be of little practical value as it involves extension of the duration of assay. The high variation between trials may have been related to a small number of birds for each trial. In this regard, increasing bird numbers would reduce the variation, and there may be no need for the repeated measurement of FmE + UeE for each assay.

Body weight of birds has been shown to have a minor influence on FmE + UeE loss (Farrell, 1978; Sibbald and Price, 1978, 1980; Arvat et al., 1980). In general, the rate of basal metabolism is assumed to be a function of body weight or more exactly metabolic weight ($W^{.75}$) and in this regard FmE + UeE loss would be expected to be a function of body weight. The absence of a relationship between FmE + UeE and body weight could be related to the difference in the body composition and the rate of basal metabolism as Miski and Quazi (1980) showed that the energy requirement for maintenance of birds with heavy lean body mass per unit of body weight was greater as compared to that for obese counterparts. Major body composition differences among groups of birds used in TME assay is unlikely, however, and consequently factors other than body composition most likely contribute to the marked bird to bird variation in FmE + UeE loss. Since this does not appear to be an effective way to control the variation in FmE + UeE, the use of sufficient bird numbers to reduce the variation among mean TME values should be considered in the routine assay. Further work is needed to exemplify this point.

Effect of the Nature of Feedstuffs on TME

The effect of the nature of feedstuffs on TME would be expected to be reflected in variation in FmE + UeE excretion by fed birds. Research has shown some variation in FmE + UeE as affected by the nature of feedstuffs (Farrell, 1981, Farrell et al., 1991; Tenesaca and Sell, 1978; Dale and Fuller, 1982b; Kussaibati et al., 1982b). Farrell (1981) calculated the regression of FmE + UeE on the content of neutral detergent fibre (NDF) for several feedstuffs to find that the intercept values varied from 12.4 for sorghum to 20.9 (kcal/bird) for barley. It was concluded that the composition of feedstuffs influenced FmE + UeE loss. A corresponding experiment made by Sibbald and Morse (1982) indicated no significant difference in the intercept values between oats and dehydrated alfalfa meal. The apparent departure for both sources may be due to the difference in excreta collection durations. The time interval of 32 h used in Farrell's study (1981) may have been insufficient, particularly for those feedstuffs which indicated high NDF contents. An additional source of error could have been related to the improper use of regression analysis in that the comparison of intercepts is invalid when the regression coefficients differ as was the case in the cited experiments. Intubation of indigestible silica gel, either alone or in combination with corn, was observed to result in an underestimate of TME value of corn (Tenesaca and Sell, 1979), but studies by Sibbald (1980b, 1981a), Sibbald and Wolynetz (1985) and Bourdillon et al. (1990) indicated no effects of inclusion of cellulose, sand or sawdust in feedstuffs on TME

values. In these studies the slopes of regression lines relating energy input to energy output were not significantly different, indicating that FmE + UeE was also independent of the nature of feedstuffs. Bilgili et al., (1982) reported that the exposure of assay birds to grit resulted in increasing endogenous dry matter output, but there was no effect on TME values.

The nature of feedstuffs may be a factor influencing FmE + UeE excretion by fed birds. The discounting of FmE + UeE from the total energy excretion and correction to zero nitrogen balance eliminate the effect and produce TMEn values that are not influenced to a great extent by the nature of feedstuffs.

Effect of Feed Intake on TME Value

A major advantage of the TME bioassay is that it accounts for the FmE + UeE loss which varies with the level of feed intake. Therefore, the resulting TME value is independent of feed intake. There are several reports to indicate a high repeatability of TME content of a single feedstuff measured at different levels of feed input (Sibbald, 1975, 1976a, 1977a, 1979c, 1979d; Muztar and Slinger, 1979; Tenesaca and Sell, 1979).

Examination of the literature, however, reveals certain contradictions concerning the reproducibility of TME values relative to feed intake. Data of Sibbald and Morse (1982), Flores and Castanon (1991), and Farrell et al., (1991) indicated that there was a trend for the TME value to increase with increasing level of feed intake, which could be attributed to a difference in the FmE + UeE between fed and unfed birds. Hartel (1986) fed birds continuously and found that the AME of a feedstuff was the same as the

TME. Consequently, he cast doubt whether there was any FmE + UeE for the birds receiving continuous feed supply.

The FmE + UeE excretion by fed birds tends to decrease with increasing feed intake due to the sparing effect of feed energy on the catabolism of tissue energy, which could introduce error into TME estimate. However, this curvilinear relationship between energy input and energy output may not always be evident because the difference makes a small contribution to the total variation in TME values. Correction of energy excretion to zero nitrogen balance reduces the variation between fed and unfed birds and the resulting TMEn is less affected by feed intake.

Effect of Age and Genotype of Birds on TME

Another important advantage of the TME assay agreed upon by various researchers is the fact that TME values observed for adult cockerels can be applied generally in the formulation of diets for all classes of poultry (Sibbald, 1978; Muztar and Slinger, 1979; Shires et al., 1980; Mollah et al., 1983; Dale and Fuller, 1980b). There are data, however, to show variation in TME values between ages or genotypes. Kussaibati et al. (1982a) demonstrated that for the same diet the TME values observed for adult cockerels were consistently lower than those for growing chickens. Hartel (1986), in a study concerning the influence of feed intake and procedures for ME with young and adult birds, did obtain similar TME values for broilers and cockerels although he concluded that the TME procedure of Sibbald delivered inaccurate results due to the use of unfed birds. In addition, Miski and Quazi (1980) reported a difference in the FmE

+ UeE observed for broiler chickens between 18 and 107 days of age. The average FmE + UeE loss per kg of body weight showed a progressively decreasing trend between 18 and 77 days of age and the value stabilized at a minimum value between 77 and 107 days of age. In this study it was also shown that White Leghorn cockerels at 122 days of age excreted a significantly higher FmE + UeE than broiler chickens of similar age. It was concluded that the differences in FmE + UeE observed for the same strain of different ages and between type of birds were due to differences in body composition and rates of basal metabolism, and that these differences would result in a biased estimate of TME.

The variation in FmE + UeE relative to age and genotype of birds may be of minor influence on TMEn as opposed to AME when the same age and genotype of birds are used for both fed and unfed groups. The discounting of FmE + UeE and correction of energy excretion to zero nitrogen balance counteracts, to a large extent, the variation and consequently the TMEn values observed for adult cockerels can be applied for a wide range of bird type. Furthermore, there has been little evidence to indicate the influence of the variation in the capacity to utilize feedstuff energy relative to age and genotype of birds on TMEn.

Reproducibility of TME Value

Limited information is available regarding the reproducibility of TME values of a single feedstuff measured with different groups of birds at the same time or over time. Dale and Fuller (1986) described a study in which the TME value of the same feedstuff was measured with 10 birds/treatment at different times. The data indicated that the

difference in TME between determinations ranged from 0 to 2.5 with a mean of 1.2% for yellow corn and 1.0 to 4.6% with a mean of 2.6% for soybean meal. The reason for the large error for soybean meal is not clear. Sibbald and Price (1975) mentioned that the error variance of TME values tended to decrease with increasing number of birds.

As discussed previously, since the reproducibility of TME is related to the variability in energy excretions by both fed and unfed birds, and there has been no appropriate method established to control the variation, increasing the number of birds in fed and unfed groups would appear to be of importance relative to the accuracy and precision of the TME estimate. Further research is needed to establish these relationships.

Additivity of TME Value

During the past 15 years, considerable interest has been directed towards the TME bioassay because of its relative simplicity. Research has been conducted to demonstrate the feasibility of the system based on its accuracy and precision as well as actual applicability.

Whether or not an energy evaluation system can be applied for general practical purposes depends to a large extent upon its ability to accurately predict the additive nature of the energy values of individual feedstuffs when provided to animals in combination. Sibbald (1976a) observed that the TME contents of soybean meal and fish meal assayed as single ingredients were similar to those calculated from mixtures of wheat with fish meal or of corn with soybean meal. In a latter study with 5 feedstuffs

and 10 diets it was shown that observed and calculated TME values were in good agreement with a mean difference of 0.02 kcal/g, which was attributed to technical error in mixing as shown by variation in GE values (Sibbald, 1977b). Similar observations were also reported by Tenesaca and Sell (1979) who found that the TME values of corn and oats were additive irrespective of level of feed input. Dale and Fuller (1980b, 1982a) indicated that, in seven of nine feedstuffs studied, the difference between calculated and observed TME values was less than or equal to 3.0%. Flores and Castanon (1991) precision-fed feed at different levels and found that the TME values indicated high additivity at 50 g of feed input but non-additivity of TME was observed at 25 g of feed input . This latter effect may be questioned, however, because of large experimental error as a consequence of low bird numbers for each treatment.

Among sources of energy, fat has been reported to be non-additive in terms of both AME and TME (Young, 1961; Young and Garrett, 1963; Jenson et al., 1970; Fuller and Rendon, 1977; Horani and Sell, 1977; Sibbald and Kramer, 1978, 1980; Sell et al.,1979; Dale and Fuller, 1980a; Meteos and Sell, 1981; Sell and Williams, 1981; Sell et al, 1986). Apart from improving feed efficiency, fats were found to improve the efficiency of energy utilization by birds. Jenson et al. (1970) demonstrated that inclusion of fat at the 3.0% level had 34% more adjusted ME value than that expected on the basis of the recognized contribution of fat to the energy of the diet. However, the effect of fat on precision of the TME estimate may be of minor importance since the fat content for most of the feedstuffs for poultry is low, and consequently its contribution to variation in TME would be small.

For general use the TME technique is acceptable since the majority of the

experiments indicate that TME is additive. The possibility of non-additivity should be considered, however, for feedstuffs that contain a relatively high content of fat.

Indirect Methods of Estimating BE

Feed comprises a major cost of poultry production. In this regard, energy represents the largest and most expensive portion of the diet. Since energy is an important nutrient from a cost perspective as well as being an essential component of animal metabolism and having an effect on other nutrients through feed intake regulation, the accurate assessment of its content in feedstuffs is critical to effective diet formulation. As opposed to other nutrients, energy is more difficult to assess in feedstuffs and average values are often used in diet formulation. Literature data indicates a substantial amount of variation among the BE (either AME or TME) of cereal grain (Sibbald and Slinger, 1962, 1963b; March and Biely, 1973). A compilation of data on Canadian feed grain by Sibbald (1986) showed a range in TME values of 12.4 to 14.3 MJ/kg for 29 samples of barley; 10.9 to 13.3 MJ/kg for 21 samples of oats; 14.4 to 15.6 MJ/kg for 32 samples of wheat. These data represent a variation of 14, 20 and 8%, respectively for barley, oats and wheat. The variability may be attributed to alteration in the chemical composition or physical properties as affected by variation in genetics, location and environmental conditions under which the grains were grown. Notwithstanding analytical error the use of an average value may result in a large error in diet formulation. In addition, the concept of discounting average values to avoid underestimation of feedstuff energy content only adds to the effect of energy in the overall cost of production. Considerable

interest has therefore been shown in methods of predicting BE values of feedstuffs based on the common principles of linearity or non-linearity for relationships established with one or a combination of chemical measurements and physical characteristics and BE. These predictive methods are used in place of the more time consuming and costly energy balance trial with animals.

Physical assays most commonly used are simple indices including test weight, bulk weight and kernel size. Chemical assays involve the determinations of fibre (crude fibre, acid detergent fibre, neutral detergent fibre, cellulose, hemicellulose, cell wall content and lignin), protein, fat, nitrogen-free extract, starch, sugar, GE and ash. Traditionally, bulk weight has been used as a criterion of assessing cereal quality and in this regard is thought to be a practical measure of the energy content of feed grains. The relationship between this physical property and BE has been confirmed for oats, but studies on barley and wheat have provided indefinite findings for poultry (March and Biely, 1973; Sibbald and Price, 1976a; Coates et al., 1977b). A highly significant correlation between TME and bulk density of barley was reported in one study by Sibbald and Price (1976b). In this study the correlation coefficient of 0.912 explained 80% of variation in TME values as being due to change in bulk density. It was suggested that the different result in that study relative to others could have been due to the fact that TME as opposed to AME is a more accurate estimate of feedstuff energy content as it is not influenced by variation in feed intake associated with differences in palatability. In a study with mice, Christison and Bell (1975) indicated that DE values increased with bulk density for oats but not for barley or wheat. These inconsistent results led Sibbald (1982) to conclude that the physical measure, bulk weight, may not be a sensitive

variable for predicting BE values of wheat and barley, and hence should be considered of limited practical merit.

Much effort has been made to find relationships between BE and single or multiple chemical measurements of a feedstuff or a group of feedstuffs. A large number of prediction equations based on chemical composition of feedstuffs are available, but in general have not been well accepted. A major reason for low acceptance is that the equations are affected by a number of factors and may be meaningful for only a narrow range of feedstuffs depending on the conditions under which the data were generated.

Fibre has been favoured over other constituents in many studies. The reason why fibre is often used in the prediction equations may be related to its small positive contribution to BE and large negative effect on the metabolism of other nutrients. For poultry, however, the positive contribution of fibre to BE is very small and could be negligible due to its low digestibility in the GI tract. In contrast, the negative effects of fibre are likely to be more significant. The negative influence of fibre on BE appears to depend upon the nature and amount of fibre in feedstuffs under investigation. Although the crude fibre has been shown to be less effective as a predictor of BE than other fibre fractions, there is no consistent evidence for the superiority of any one measure over others for predictive purpose. DE of feedstuffs for pigs was reported to correlate more closely to acid detergent fibre (ADF) than to crude fibre content of the feedstuff (Drennan and Maguir, 1970, quoted by King and Taverner, 1975). There is also evidence to demonstrate that neutral detergent fibre (NDF) is a valuable variable (King and Taverner, 1975; Alderman, 1985). In comparison to NDF, ADF, crude fibre and lignin, Carre et al. (1984) concluded that cell wall content was the best predictor of AME.

Morgan et al. (1975) and Just et al. (1984), however, suggested that crude fibre was a best predictor of ME content in feedstuffs. The reasons for these inconsistent findings are not clear but may be due somewhat to the inability of any fibre analysis to isolate nutritionally significant fractions. In addition, the source of fibres may be important. Farrell (1973) showed that the digestibility of cell wall constituents in diets high in fibre was both variable and dependent of other chemical constituents. Based on the information available, cell wall content could be considered to be one of the most useful measures mainly because of its negative effect on the metabolism of other nutrients. However, cell wall determination is a tedious and costly analysis, and consequently it is not best fitted to a rapid estimation of BE value. NDF would appear to be a valuable predictor based on its negative effects on other nutrients and ease and precision of determination.

Positive correlation between BE and soluble carbohydrates (nitrogen-free extract, starch and sugar) has been observed for oats, whereas studies on wheat and barley indicate a weak or no relationship (Bhatty et al., 1974; Sibbald and Price, 1976a, 1976b, 1977; Coates et al., 1977b; Carre et al., 1984). This latter effect is somewhat surprising as these carbohydrate components are major sources of energy in feedstuffs. In this regard the relatively consistent content of soluble carbohydrates for the same type of feedstuff may contribute primarily to the mean BE rather than variation from that mean.

Another important consideration in choosing predictor variables is the use of GE. However, the GE of feedstuffs is usually considered to be of minor significance for predictive purpose due to the fact that indigestible fibre contains a similar amount of GE to available carbohydrate. There is evidence, however, to indicate that GE of individual feedstuffs is correlated with BE content (Bhatty et al., 1974; Christison and Bell, 1975;

Sibbald and Price, 1977; Carre et al., 1984). In a study with pigs Wiseman and Cole (1985) indicated that an approach to prediction equations could be one where the intake of GE is considered. It was observed that the inclusion of a GE term in equations resulted in a decreased residual standard deviation (Rsd) and an increased determinant coefficient (R^2). In addition, GE content may be a valuable predictor variable as it indirectly reflects the amount of non-energy materials in feedstuffs. The effect of GE on BE, however, depends to a large extent on the range of values for a given feedstuff.

Protein, although it is an important source of BE, has not been observed to be a sensitive predictor of BE for poultry feedstuffs. A possible reason for this is that when corrected to zero nitrogen balance, variation in BE content of feedstuffs contributed by protein may be eliminated or substantially reduced. In this regard the effect of protein may be like that of soluble carbohydrates contributing to the mean BE rather than to variation in BE.

Fat and ash contents in cereal grains are relatively low and no obvious relationships between BE and these variables were reported in many of the earlier studies (March and Biely, 1973; King and Taverner, 1975; Sibbald and Price, 1976a). As opposed to carbohydrates, fat contribution to BE on a unit basis is greater since it contains more than twice as much energy. In this regard the use of a quadratic term in prediction equations to account for the curvilinear relationship between fat and BE has been suggested (Wiseman and Cole, 1985).

Other aspects relative to the selection of predictor variables include the use of interaction terms to explain negative effects of fibre on the availability of energy from other sources. The results from sheep experiments for compound feeds indicated

systematic changes in ME values due to variation in the level and source of fat and fibre added to the diets (Alderman, 1985). This interaction effect may not be apparent in poultry, however. In this regard the BE value of poultry feedstuffs has been observed to be additive.

In most cases R^2 is used as an important criterion for evaluating the validity of prediction equations. This may give a rather misleading impression of precision, since the importance of R^2 value depends to a large degree upon the total variability in the dependent and independent variables. For practical application, evaluation of the precision of prediction equations in terms of Rsd may be of greater importance than the use of R^2 as it reflects the accuracy and precision of the measurements of both parameters. However, it should be noted that in some cases the use of Rsd may be of limited significance as its magnitude relies heavily on sample number. To make prediction equations useful in a wide rage of situations, it would be of importance to include feedstuffs varying widely in chemical composition or physical properties. In this regard compound feeds show a large R^2 value as they span a wide range of dependent and independent variables. Prediction equations derived from compound feeds, however, are of limited value for application to single feedstuffs where there is a lack of appreciable variability in both dependent and independent variables.

The use of a single factor for predictive purpose is less accurate than a combination of factors (Campbell and Campbell, 1975; Coates et al., 1977b). Inclusion of more predictor variables in prediction equations should improve accuracy of prediction.

A re-evaluation of the use of a prediction equations for estimating BE content of

feedstuffs indicates that, to make prediction equations useful, improved standard analytical methods are needed to allow for more precise measurements of dependent and independent variables. In addition, the development of effective prediction equations is dependent on the samples showing maximum variation in chemical and physical parameters.

AN ASSESSMENT OF MODIFICATIONS TO THE SIBBALD TECHNIQUE FOR TRUE METABOLIZABLE ENERGY

ABSTRACT

Experiments were conducted with adult SCWL cockerels to determine if various modifications to the Sibbald technique would improve the precision of the TME bioassay. The modifications tested included length of starvation period (24, 28 and 36 h) and provision of supplementary energy (to all birds during the starvation period and to unfed birds during the collection period). Lengthening the starvation interval from 24 to 28 h significantly reduced the quantities of excreta weight and total energy excretion. Extension of the period to 36 h produced no further effect. Provision of supplementary energy to fed birds during the starvation period did not affect excreta weight, energy and nitrogen excretions but the feeding of supplementary energy (30 g granular sucrose) to unfed birds did significantly reduce excreta weight and nitrogen excretion. Correction to zero nitrogen balance using appropriate controls, however, resulted in similar TMEn values. These data indicated that the modification to the Sibbald technique involving a 28 h starvation interval was beneficial whereas the modification involving supplementary energy was of no apparent advantage.

INTRODUCTION

The Sibbald technique for measuring true metabolizable energy (TME) of feedstuffs using adult cockerels was first presented in 1976 (Sibbald, 1976a) and modifications that have been generally adopted since that time include the correction to zero nitrogen balance (TMEn) and lengthening of the collection interval from 24 to 48 h (Sibbald, 1986). Some additional modifications have been suggested but not generally accepted. One such modification is an extension of the time of starvation to ensure complete clearance of the digestive tract (Shires et al., 1979; Fisher and McNab, 1987). Lengthening either the collection interval or the starvation period increases stress on the birds and has been reported to increase endogenous energy and nitrogen losses (Dale and Fuller, 1982b; McNab and Blair, 1988). During the starvation period birds exhaust supplies of stored glycogen and must catabolize body tissue for energy-yielding purposes during the following excreta collection period. This is particularly true for the unfed birds which are used as indicators of endogenous energy and nitrogen losses for fed birds. In fed birds, however, feeding of test material undoubtedly spares tissue catabolism for basal energy requirement to some extent. In this regard the difference in endogenous energy and nitrogen losses between fed and unfed birds may affect the accuracy and precision of TMEn estimate. Provision of highly digestible nitrogen-free

supplementary energy has been reported to reduce these differences and to improve the precision of the TMEn estimate (Sibbald and Morse, 1983; McNab and Blair, 1988).

The experiments reported here were designed to test the effects of starvation time and supplementary energy on the precision of the TME bioassay.

MATERIALS AND METHODS

Two energy-balance experiments were conducted with adult SCWL cockerels during a 5 month period using the precision feeding technique described by Sibbald (1986). All the birds used in the trials were housed in individual metabolism cages (62.2 x 34.3 x 43.3 cm) in an environmentally controlled room (20 C $\pm$ 2, 14 h light beginning at 0600 daily). Water was available ad libitum during the entire experiment.

In Experiment 1, the effect of duration of the starvation interval on excreta weight and endogenous energy and nitrogen excretion was investigated. Forty eight cockerels were randomly assigned to three treatments, with 16 birds starved for 24 h, 16 birds starved for 28 h and the remaining birds starved for 36 h. Within each starvation group the birds were divided equally into a fed group and an unfed group except for 36 h interval which contained only on unfed group. The fed group received 30 g of wheat by tube feeding at the termination of the starvation period and the unfed group received no feed. Excreta were collected from all birds for the following 48 h.

The influence of providing supplementary energy to birds during the energy-balance trial on TMEn values was investigated in Experiment 2. One hundred and twenty

eight cockerels were utilized in 4 replicate trials to determine energy balance as described in Experiment 1 except that a 28 h starvation period was used. In each trial 32 cockerels were assigned at random to two treatments: one half of the birds received supplementary energy during the starvation period and one half of the birds received no supplementary energy. The supplementary energy (50 ml of a 60% sucrose solution) was provided via tube feeding 4 h after the initiation of the starvation period. In addition the unfed group received 30 g granular sucrose at the termination of the starvation period when the fed group received 30 g wheat via tube feeding in trials 1, 2 and 3 and 30 g soybean meal in trial 4. Excreta was collected on trays from all birds for the following 48 h.

In both Experiments 1 and 2, excreta collected from individual birds were frozen, freeze-dried, allowed to equilibrate to atmospheric humidity for 24 h prior to weighing, and ground to pass a 1-mm screen. Moisture and nitrogen analyses were conducted on excreta and feed samples according to the standard procedures (AOAC, 1984). Gross energy of excreta and feed samples was determined using a parr adiabatic oxygen bomb calorimeter (Parr Instrument Co. Illinois, U. S. A.). TMEn value was calculated according to the procedure described by Sibbald (1986). Experiment 1 was a completely randomized design with three treatments for unfed birds and two treatments for fed birds. Each replicate trial in Experiment 2 was a completely randomized design with two treatments. Treatment means of excreta energy, nitrogen and the TME values were compared according to Duncan's Multiple Range Test. Analysis of correlation was conducted using the procedure of the SAS Inc (1985).

RESULTS AND DISCUSSION

The data in Table 1 indicated that, in unfed birds, Lengthening the starvation interval from 24 to 28 h significantly ($p < 0.05$) reduced excreta weight and endogenous energy excretion. The birds starved for 28 h excreted less nitrogen than those starved for 24 h although the differences were not significant ($p > 0.05$). Extension of the starvation period to 36 h did not produce any further effect. In the fed birds, however, lengthening the starvation period from 24 to 28 h did not result in any change. The excreta weights and excretion of energy and nitrogen were similar for 24 and 28 h starvation groups (Table 1). The reason for this latter observation is not clear although it may be possible that the relatively large amount of excreta weight and energy loss of feed origin in the fed birds has a diluting effect on the endogenous products. These data indicated that starvation for 24 h may not be sufficient time to completely empty the digestive tract for all birds, and hence the occasionally increased endogenous energy and nitrogen losses could contribute to an increase in error for the TMEn estimate.

The provision of supplementary energy to unfed birds during the starvation and collection periods significantly ($p < 0.05$) reduced excreta weight in trials 1 and 3, and nitrogen losses in all trials (Table 2). The energy loss showed the same trend as for excreta weight but with differences being significant ($p < 0.05$) only for trials 1 and 3. The observed reduction in energy and nitrogen excretions can be attributed to the sparing action of supplementary energy on the catabolism of body tissue for basal energy

TABLE 1. Effect of starvation interval on excreta weight, energy and nitrogen losses measured with fed and unfed birds in the TME bioassay over the following 48 h collection in Experiment 1

Bird	Starvation interval (h)	Excreta weight[1] (g)	Energy[1] (kJ)	Nitrogen[1] (g)
Unfed	24	7.03 ± .27[a]	92.94 ± 3.96[a]	1.20 ± .04[a]
	28	5.86 ± .26[b]	74.63 ± 3.19[b]	1.10 ± .05[a]
	36	5.99 ± .25[b]	78.05 ± 2.84[b]	1.14 ± .05[a]
Fed	24	10.82 ± .22[a]	149.87 ± 3.60[a]	1.44 ± .04[a]
	28	11.05 ± .33[a]	152.26 ± 4.23[a]	1.58 ± .06[a]

[a-b] means within fed or unfed birds in a column with no common superscripts are significantly different ($p<0.05$).
[1] Mean ± SE.

requirement, a result in agreement with Dale and Fuller (1982b), Sibbald and Wolynetz (1988). The feeding of sucrose solution to the fed birds during the starvation period, however, did not produce a significant ($p>0.05$) influence (Table 2). Correction of energy excretion to zero nitrogen balance using respective controls resulted in similar nitrogen corrected energy losses (FEn + UEn) and the TMEn values for both treatments (Table 3). These data indicate that while the provision of supplementary energy influenced excreta weight, energy and nitrogen excretions, these responses were without effect on FEn + UEn and hence on the TMEn values. A similar result was reported by Sibbald and Wolynetz (1988), who found that feeding glucose had no improvement of precision of the TMEn estimate. Analysis of the endogenous energy excretion in relation to nitrogen loss indicated a highly significant correlation ($r = 0.826$, $p<0.01$). In this regard 65% of the variation in endogenous energy loss can be attributed to catabolism of the nitrogenous compounds of body tissue. The large energy loss exhibited by the unfed birds receiving no supplementary energy was, therefore, accounted for after correction for endogenous nitrogen loss (Table 3). The provision of supplementary energy did not improve the precision of the TMEn values as judged by the similar standard error values observed for the two treatments (Table 3).

The variability in the TMEn values observed for the wheat sample among replicate trials was evident in the current study (Table 3), and the effect was due primarily to variation in energy losses between replicate trials, which may be related to the small number of birds used in each trial. Further work is needed to investigate this effect.

TABLE 2. Effect of supplementary energy on the excreta weight, energy and nitrogen losses measured with both fed and unfed birds in Experiment 2

Trial	Suppl. energy[1]	Excreta weight (g)		Energy (kJ)		Nitrogen (g)	
		Fed	Unfed	Fed	Unfed	Fed	Unfed
1	none	11.47 ±.34[a]	7.05 ±.24[a]	161.3 ±3.0[a]	84.0 ±3.3[a]	1.61 ±.08[a]	1.46 ±.06[a]
	yes	11.82 ±.33[a]	5.87 ±.33[b]	165.9 ±4.0[b]	74.5 ±4.3[b]	1.60 ±.06[a]	1.02 ±.05[b]
2	none	11.22 ±.39[a]	7.41 ±.49[a]	155.6 ±5.3[a]	88.0 ±6.5[a]	1.62 ±.08[a]	1.49 ±.10[a]
	yes	11.00 ±.55[a]	6.64 ±.39[a]	157.9 ±6.2[a]	85.4 ±5.3[a]	1.45 ±.13[a]	1.17 ±.07[b]
3	none	10.68 ±.66[a]	6.70 ±.34[a]	145.0 ±6.4[a]	71.3 ±2.7[a]	1.49 ±.11[a]	1.24 ±.06[a]
	yes	10.89 ±.46[a]	5.35 ±.44[b]	139.7 ±4.9[a]	61.8 ±3.6[b]	1.35 ±.04[a]	0.89 ±.08[b]
4	none	15.78 ±.32[a]	6.26 ±.48[a]	236.0 ±7.1[a]	72.0 ±4.9[a]	2.05 ±.08[a]	1.36 ±.12[a]
	yes	16.54 ±.43[a]	5.47 ±.29[a]	265.5 ±10.7[a]	70.7 ±3.9[a]	2.30 ±.05[a]	1.00 .05[b]

[a - b] means within trial in a row with no common superscripts are significantly different ($p < 0.05$).

[1] Both fed and unfed birds received supplementary energy 4 h after the initiation of the starvation period, and unfed birds received supplementary energy at the termination of the starvation period concurrent when fed birds received test feed.

TABLE 3. Effect of supplementary energy on the FEn + UEn and TME values in Experiment 2

Trial	Supplementary energy[1]	FEn + UEn[2] (kJ)	TMEn[2] (MJ/kg. DM)
1	none	95.3 ± 2.4	15.72 ± .09
	yes	95.1 ± 2.9	15.73 ± .11
2	none	86.4 ± 3.7	16.06 ± .20
	yes	86.7 ± 3.2	16.04 ± .13
3	none	89.1 ± 2.9	15.95 ± .11
	yes	89.1 ± 4.4	15.95 ± .17
4	none	202.4 ± 7.1	15.98 ± .39
	yes	201.7 ± 10.4	15.97 ± .29

[1] Both fed and unfed birds received supplementary energy 4 h after the initiation of the starvation period, and unfed birds received supplementary energy at the termination of the starvation period concurrent when fed birds received test feed.
[2] Mean ± SE.

VARIABILITY IN RELATION TO THE BIOASSAY FOR TRUE METABOLIZABLE ENERGY (TME) OF POULTRY FEEDSTUFF

ABSTRACT

Two experiments with adult SCWL cockerels were conducted using the Sibbald technique with some modifications to determine the factors influencing precision of TME estimate.

The excreta weight measured with the unfed birds varied from bird to bird throughout the entire experiment and differences between replicate trials (time period) were apparent ($P<0.05$). The FmE + UeE varied from 50.29 to 125.56 kJ with an overall mean of 79.96 kJ. The differences between time periods and among random groups were not significant. The variability was primarily related to variation among birds while time periods and random groups were of minor importance. Correlation analysis indicated a negative relationship between FmE + UeE and excreta weight. The endogenous nitrogen loss and distribution of variation showed a similar trend to FmE + UeE although the differences were evident ($P<0.05$) between time periods. The nitrogen loss was found to be related to FmE + UeE ($r=0.859$), indicating that 65% of the variation in FmE + UeE was due to change in nitrogen loss. The standard deviation values of the means for FmE + UeE, nitrogen and FEn + UEn losses decreased with

increasing number of birds; the values reached a relatively stable level at 24 or more birds.

Differences among mean FE + UE, nitrogen and FEn + UEn losses by fed birds were apparent ($p<0.05$) between replicate trials but there were no significant effects attributable to random groups. A large portion of the variability was accounted for by variation among individuals. The FE + UE loss was observed to be related to excreta weight and nitrogen loss. Correction of nitrogen to zero basis reduced the variability in FE + UE between replicate trials. The standard deviation values of the means for FE + UE and FEn + UEn losses decreased with increasing bird number; stable values were reached with 8 or more birds. There were no significant differences in the TMEn values between time periods and random groups. The relative error values decreased with increasing bird numbers and the error was less than 1.0% at a group size of at least 8 fed birds. There is little evidence to support the need for measuring FmE + UeE and nitrogen losses for each bioassay.

INTRODUCTION

There have been several suggestions of modification to the original Sibbald method of bioavailable energy (BE) determination in an attempt to improve accuracy and precision or reproducibility of the true metabolizable energy (TME) estimate (Sibbald, 1976a). Extension of the excreta collection period from 24 to 48 h in order to allow complete clearance of feed residues from the digestive tract and correction of TME to zero nitrogen balance are two modifications that have received general acceptance (Sibbald, 1986; Fisher and McNab, 1987). Much of the debate on the reliability of the TME bioassay has focused on the use of the correction for faecal metabolic energy (FmE) and urinary endogenous energy (UeE), especially when unfed birds are used as estimators of FmE + UeE for fed birds (Farrell, 1981; Hartel, 1986).

In general, several methods have been employed for measuring FmE + UeE. The direct method recommended by Sibbald (1976a), which involves the use of unfed birds, has the advantage of relative ease of determination but may result in an overestimate of FmE + UeE for fed birds and has shown high variability (Sibbald, 1976a, 1982; Dale and Fuller, 1982b; Hartel, 1986; Askbrant, 1988). McNab and Blair (1988) have recommended feeding supplementary energy to unfed birds to reduce FmE + UeE and nitrogen losses and increase precision of the TMEn estimate but Sibbald and Wolynetz (1988) and Zhang and Campbell (1992b) have reported no apparent advantage of this

procedure. As an alternative procedure a regression technique involving the feeding of gradient levels of feedstuff and extrapolation of energy intake to zero point has been used to obtain the determination of FmE + UeE but this technique has been judged of limited practical value (Kussaibati et al., 1982a; Sibbald and Morse, 1982; Farrell et al., 1991).

Published information on the variability in relation to the TME bioassay has been confined generally to age, sex, genotype of birds, environmental condition and feed intake as well as bird physiological status (Sibbald and Morse, 1983; Hartel, 1986; McNab and Blair, 1988; Farrell et al., 1991). There is little information available concerning the variability in endogenous energy excretion of unfed birds or in the TMEn values of a single feedstuff measured at the same time or at different times under standard conditions. Consequently the purpose of this study was to obtain information regarding energy and nitrogen excretion by both unfed and fed birds under standard conditions and at different time periods to determine the factors influencing their variability and potential effects on TMEn values.

MATERIALS AND METHODS

Studies were conducted with adult SCWL cockerels to determine factors contributing to variability in the bioavailable measure of energy in feedstuffs. Balance experiments conducted according to the Sibbald method (Sibbald, 1986) with modifications made by Zhang and Campbell (1992a) were carried out over a one year period during which measurements were made using either unfed birds or fed birds precision-fed a sample of the same barley (6 - row cv. Bedford). The modifications to

the technique were a 28 h starvation interval and the collection of excreta on trays.

The birds used in the trials were chosen from a group of 98 cockerels maintained in individual cages in a temperature controlled room with 14 h light and supplied feed (wheat - based maintenance diet) and water ad libitum. During the balance trial the birds were moved to a separate environmentally controlled room (20 C $\pm$ 2, 14 h light beginning at 0600 daily) and housed in metabolism cages (62.2 x 34.3 x 43.3 cm.). Water was available ad libitum. An interval of at least 14 days elapsed between trials for any one bird and birds that did not maintain body weight were replaced in the group.

Experiment 1, consisting of 6 replicate trials conducted over a 1-year period, was designed to test the effects of time period (over time) and group size (bird number) on excreta weight and excretion of energy and nitrogen by replicate groups of unfed and fed birds. In this experiment balance data were collected for individual birds. In each replicate trial (except replicate 6 which involved the use of 24 fed birds) 48 birds were precision-fed 30 g of a sample of the same barley. In addition to the fed-bird data, balance data was collected from 48 unfed birds in each replicate trial at a time period similar to that for the fed birds. The TMEn values were calculated in each trial using the mean FmE + UeE and endogenous nitrogen values for all unfed birds in the 6 replicate trials.

Experiment 2, consisting of 24 trials, was conducted to measure the variability in the TMEn estimate and to determine whether the repeated measurement of FmE + UeE and endogenous nitrogen losses by unfed birds for each bioassay would improve the precision of TMEn estimate. The number of birds for each trial varied from 12 to 16 birds. One half of the birds were used as unfed birds while other one half received 30

g test material of the same barley as used for Experiment 1. The energy and nitrogen excretions by both fed and unfed birds and TMEn values were obtained from pooled data for each trial.

In both Experiments 1 and 2, data for some birds were lost because of regurgitation of feed or for mechanical reasons. Excreta collected were frozen, freeze-dried, equilibrated to atmospheric humidity for 24 h, weighed and ground to pass a 1-mm screen. Analyses for nitrogen on feed and excreta samples, and for dry matter on feed samples were conducted according to the procedures of AOAC (1984). GE content on feed and excreta samples was measured using a parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Illinois, U. S. A.).

TMEn value was calculated according to the procedure described by Sibbald (1986). Nitrogen correction was carried out using the correction factor 34.39 kJ/g N. The model for analysis of variance and subsequent partition of variance was,

$$Y = u + A + B + e$$

where u is overall mean. The parameters A, B and e are random variables. Specifically, A is 1, 2, 3, 4, 5 and 6 replicate trials, B is 1, 2, 3, 4, 5 and 6 random groups and e represents 1, 2.....n individuals.

Standard deviation values of means of excreta energy and nitrogen excretions by both fed and unfed birds were obtained by dividing 48 unfed birds and 44 fed birds into several groups varying in bird number (1, 4, 6, 8, 12, 16 and 24 or 22) chosen at random from the individual bird data observed for replicate trial 3 in Experiment 1. The relative errors of TMEn values for these fed groups were calculated in terms of the overall mean of TMEn. Treatment means of excreta weight, energy and nitrogen losses between replicate

trials were compared by the use of Duncan's Multiple Range Test (Snedecor and Cochran, 1980). Regression and correlation analyses were conducted to study relationships between excreta weight and excreta GE and nitrogen contents; excreta weight and energy and nitrogen losses; body weight and energy and nitrogen losses according to the procedures of the Statistical Analysis System Institute Inc. (1985).

RESULTS AND DISCUSSION

The data of average body weight for both the fed and unfed birds are presented in Table 4. The body weight ranged from 2.42 to 3.29 kg with an overall mean of 2.76 kg for the unfed birds and from 2.41 to 3.30 kg with a mean of 2.77 kg for the fed birds during the entire experimental period. The unfed birds were similar in weight to the fed birds, and neither the unfed birds nor the fed birds were significantly different in body weight between replicate trials. These data also indicated that the birds were uniform within replicate trial as judged by standard deviation values.

Average excreta weight, FmE + UeE, endogenous nitrogen losses and FEn + UEn measured with the unfed birds in Experiment 1 are shown in Table 5. There was considerable variation in excreta weight among birds treated alike throughout the entire experimental period, ranging from 4.13 to 11.19 with an overall mean of 6.67 g. The range and average excreta weight in the current study, however, were less than those reported by Sibbald (1980). The departure may be due to the fact that the data presented from this study represent excreta amount of adult cockerels with an average body weight of 2.76 $\pm$ 0.04 kg, while the values from Sibbald were calculated from several

TABLE 4. The mean body weight of birds during the experiment period (Experiment 1)

Reps.	Fed bird (kg)	Unfed bird (kg)
1	2.81 ± .21	2.77 ± .18
2	2.74 ± .18	2.77 ± .20
3	2.74 ± .19	2.74 ± .18
4	2.75 ± .20	2.74 ± .19
5	2.76 ± .19	2.73 ± .20
6	2.83 ± .20	2.81 ± .17

TABLE 5. Mean excreta weight, FmE + UeE, nitrogen and FEn + UEn loss measured with the unfed birds in the standard TME assay (Experiment 1)

Replicate trial	Excreta weight[1] (g)	FmE + UeE[1] (kJ)	Nitrogen[1] (g)	FEn + UEn (kJ)
1	6.49 ± 1.22^{ab}	79.17 ± 12.50^{a}	1.33 ± 0.27^{b}	33.06 ± 5.54^{ab}
2	6.69 ± 1.15^{ab}	79.81 ± 13.33^{a}	1.36 ± 0.26^{ab}	32.91 ± 6.87^{ab}
3	6.95 ± 1.43^{a}	80.80 ± 14.92^{a}	1.47 ± 0.34^{a}	30.27 ± 5.71^{b}
4	6.63 ± 0.78^{ab}	78.29 ± 9.41^{a}	1.34 ± 0.18^{b}	32.38 ± 6.26^{ab}
5	6.92 ± 1.25^{a}	83.14 ± 15.10^{a}	1.44 ± 0.28^{ab}	33.53 ± 9.23^{a}
6	6.32 ± 0.99^{b}	78.55 ± 12.04^{a}	1.34 ± 0.22^{b}	32.34 ± 7.10^{ab}
Overall mean	6.67 ± 1.14	79.96 ± 13.01	1.38 ± 0.27	32.41 ± 6.92

$^{a-b}$ means within the same column with no common superscripts are significantly different ($p < 0.05$).

[1] Mean ± SD.

genotypes and experiments during a period of 5 years. Significant differences ($P<0.05$) were observed between replicate trials. However, the greater excreta weights observed in replicates 3 and 5 in comparison with that in replicate 6 were not likely due to the effect of environmental conditions because all the birds were held in an environmentally controlled room. In addition to the individual difference in FmE + UeE loss which would contribute to varied excreta weights, the content of other non-energy materials in excreta may influence excreta weight. Sibbald (1980) reported that in TME trials, greater excreta weight observed for some birds was associated with excreta moisture content.

Based on 280 observations, the FmE + UeE losses varied from 50.29 to 125.56 with an overall mean of 79.96 kJ. The ranges for each of the replicate trials were not apparently different from the overall range. The smaller range observed with the birds in replicate 4 could be attributed to the smaller variability in excreta weight. Analysis of the relationship between FmE + UeE loss and excreta weight showed a highly significant ($p<0.01$) correlation (Table 6), suggesting that variation in FmE + UeE is due primarily to the variable excreta weight. Dale and Fuller (1982b), in a study in which completely absorbable materials were fed to unfed birds, noted that variation in endogenous energy loss was reflected mainly by differences in quantity of excreta. In addition, there were no significant differences in FmE + UeE losses between replicate trials and among random groups. Distribution of variation in FmE + UeE loss showed that 98% of the variability was attributed to variation among individuals while remaining the 2% of variation was due to change in replicate trials and random groups (Figure 1), indicating that the effect of time period and grouping were of minor importance as compared to the variability among birds. Regression analysis showed that GE content (kJ) per unit weight

TABLE 6. Relationships between excreta weight and energy excretion and GE content per g of excreta measured with fed and unfed birds (Experiment 1)

Bird	Regression equation	r value	p value
Unfed	(FmE+UeE) = 45.40 + 3.72 excreta weight	0.953	$p<0.001$
	GE[1] = 13092 - 159 excreta weight	- 0.309	$p<0.001$
Fed	(FE+UE) = 160.87 + 3.47 excreta weight	0.885	$p<0.001$
	GE[1] = 17644 - 213 excreta weight	0.449	$p<0.001$

[1] GE content per g excreta.

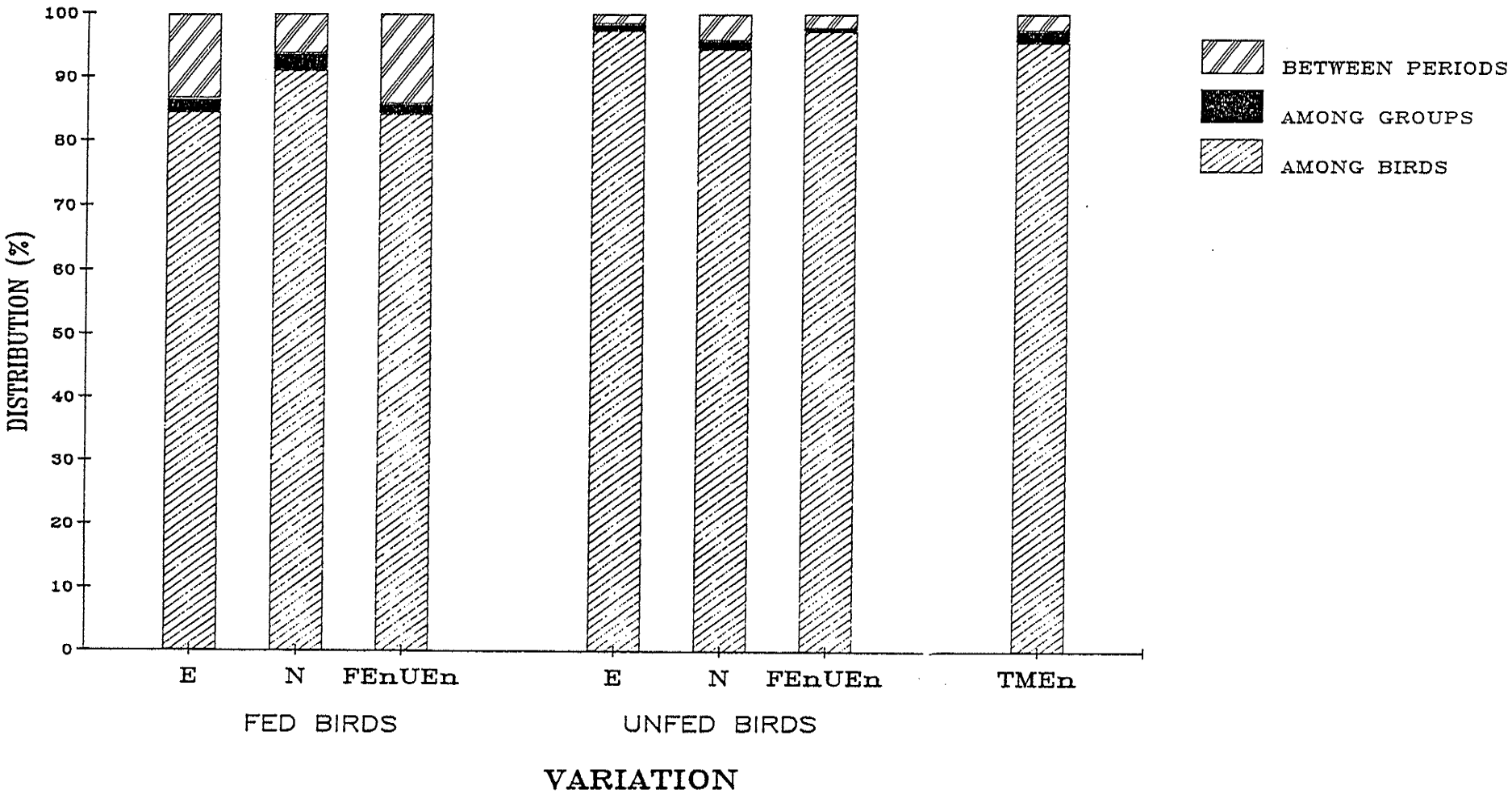

Fig 1. DISTRIBUTION OF VARIATION IN RELATION TO THE TMEn ESTIMATE

(g) of excreta was negatively related to excreta weight but the R^2 value indicated that only 10% of variation in GE content was accounted for by change in excreta weight (Table 6). Similarly, Sibbald (1980) illustrated that there was a minor trend for GE per g of excreta to decrease with increasing excreta weight in the TME assay.

Body weight had a minor effect on the FmE + UeE as analysis of FmE + UeE and body weight data generated no correlation ($r=0.113$, $P>0.05$). Theoretically, FmE + UeE which represents the by-product of energy metabolism for maintenance of life would be a function of metabolic body size. The low r value may be associated with the relatively narrow range of body weights of the birds involved in this study. Sibbald and Price (1978, 1980), Arvat et al. (1980), however, also reported no relationship between body weight and FmE + UeE loss.

The endogenous nitrogen losses measured with the unfed birds varied from 1.33 g for replicate 1 to 1.47 g for replicate 3 with an overall mean of 1.38 g (Table 5). There were no significant differences among random groups whereas the differences were observed to be apparent ($p<0.05$) between replicate trials, with the birds in replicate 3 showing greater nitrogen losses than those in replicates 1, 4 and 6 even though no differences were evident in FmE + UeE loss from these birds. This may be due, in part, to the difference in the source of FmE + UeE from bird to bird. Miski and Quazi (1980) documented that variation in FmE + UeE loss was mainly attributed to the difference in body composition and basal metabolic rate. As with FmE + UeE loss, 95% of variation in nitrogen excretion was attributed to individual change while time period and random group accounted for only a small portion (Figure 1). Analysis relating FmE + UeE loss to nitrogen loss yielded a highly significant correlation ($P<0.001$) with a r

value of 0.859 which indicated that 65% of the variation in FmE + UeE loss was due to changes in endogenous nitrogen loss. It can be suggested from these data that endogenous nitrogen loss would be a important factor influencing FmE + UeE loss, but other factors are also involved. The FEn + UEn values for replicates 1, 2, 4, 5 and 6 were not different from each other but a significantly ($P<0.05$) lower FEn + UEn was found for replicate 3 which may have been primarily related to a large endogenous nitrogen loss relative to the FmE + UeE loss for this group.

By dividing the 48 individual values for the unfed birds in replicate 3 into several groups varying in number, it was possible to calculate standard deviation values of means of the FmE + UeE, endogenous nitrogen and FEn + UEn for each group. These values decreased progressively with increasing number of birds and the values reached a relatively stable level with 24 or more birds (Figure 2). It can be suggested from the data that since there would appear to be no effective way to control the substantial variability in FmE + UeE and endogenous nitrogen losses from bird to bird, an increase in bird number (i.e. at least 24) used in the assay is necessary to realize an improvement in precision of FmE + UeE and nitrogen measurements and subsequently the TMEn estimate.

Average excreta weight, FE + UE, nitrogen and FEn + UEn excretions by the fed birds in Experiment 1 are shown in Table 7. For average excreta weight, the differences between replicates were significant ($p<0.05$), with the birds in replicate 5 showing a greater excreta amount than those in replicates 1, 2, 4 and 6, and lower value in replicate 4 than those in replicate 3. These data indicated a similar trend as was observed for the unfed birds. The average FE + UE loss showed a significant difference

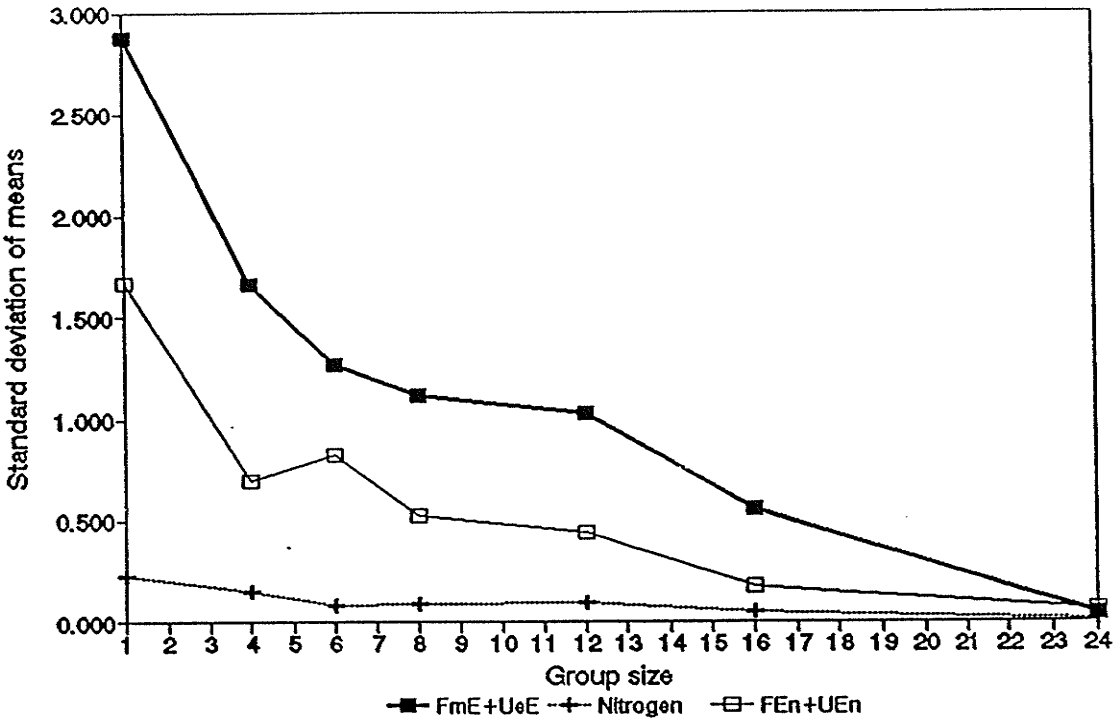

Fig. 2. Standard deviation of means of FmE+UeE, endogenous nitrogen and FEn+UEn versus group size measured with unfed birds

TABLE 7. Mean excreta weight, energy, nitrogen and FEn+UEn losses measured with the birds precision fed 30 g barley in the standard TME assay (Experiment 1)

Trial	Excreta weight[1] (g)	FE+UE[1] (kJ)	Nitrogen[1] (g)	FEn+UEn[1] (kJ)
1	12.59 ± 1.27^{bc}	192.39 ± 17.04^{b}	1.46 ± 0.27^{b}	142.08 ± 12.08^{a}
2	12.62 ± 0.87^{bc}	193.34 ± 12.52^{ab}	1.41 ± 0.18^{b}	144.74 ± 10.19^{a}
3	13.03 ± 0.96^{ab}	195.49 ± 11.81^{ab}	1.51 ± 0.22^{ab}	143.64 ± 9.44^{a}
4	12.30 ± 0.96^{c}	183.19 ± 12.70^{c}	1.43 ± 0.21^{b}	133.92 ± 9.44^{b}
5	13.15 ± 1.25^{a}	199.76 ± 14.74^{a}	1.59 ± 0.29^{a}	145.10 ± 8.25^{a}
6	12.48 ± 0.91^{bc}	191.92 ± 12.52^{b}	1.51 ± 0.23^{ab}	140.03 ± 11.91^{a}
Overall mean	12.71 ± 1.10	192.61 ± 14.66	1.48 ± 0.24	141.57 ± 10.83

$^{a-c}$ means within the same column with no common superscripts are significantly different ($p<0.05$).
[1] Mean ± SD.

($P<0.05$) between replicates but the differences among random groups were not significantly different. The significantly greater FE + UE in replicate 5 than in replicate 1, and as well as the lower value in replicate 4 than in other replicates represented a similar trend as was observed for excreta weight. These data indicate that the energy excretion by the fed birds as with unfed birds also depends upon excreta weight to a great extent, while other non-energy materials presumably existing in excreta are of minor influence. Variation in FE + UE relative to individuals indicated a large portion (84.6%) although the value was less than that for FmE + UeE observed for unfed birds (Fig 1). Regression equations relating FE + UE loss to excreta weight and GE content per unit weight (g) of excreta to excreta weight are shown in Table 6. The r value of FE + UE with excreta weight indicated that 78% of the variation in energy loss was related to changes in excreta weight while the r value of GE content of excreta with excreta weight showed only about 25% of difference in the GE content attributable to excreta weight. Although the standard deviation values for FE + UE were, on a relative basis, lower than those for FmE + UeE measured with the unfed birds, the variability among birds throughout the entire experiment was evident, varying from 142.51 to 230.08 KJ with a overall mean of 192.61 kJ. It may be possible that an unusually large energy excretion by some birds reflects individual differences in the capacity to metabolize feedstuff energy but other factors are undoubtedly involved as indicated by the high variability among unfed birds.

The nitrogen losses by the fed birds varied from 1.43 g in replicate 4 to 1.59 g in replicate 5 with a overall mean of 1.48 g. No significant differences were observed between replicates except that birds in replicates 2 and 4 showed relatively low values

which were in correspondence to a small amount of FE + UE, and a large portion of variation (91.3%) was due to variation among birds (Fig 1). Analysis of the relationship between FE + UE and nitrogen loss showed a significant correlation ($r = 0.684$, $n=224$, $P<0.001$), indicating that the nitrogen components are the important source of FE + UE although the FE + UE is of both endogenous and feed origins. By correcting nitrogen to zero equilibrium, calculated FEn + UEn values showed no significant differences for replicates 1, 2, 3, 5 and 6. The FEn + UEn value observed in replicate 4 was lower than others as were the excreta weight and FE + UE loss.

As was done for the unfed birds, the random selection of individual data for fed birds in replicate 3 in Experiment 1 into groups of varying bird number allowed for the calculation of variation as influenced by group size (Figure 3). The standard deviation values of means of FE + UE and FEn + UEn calculated for each group decreased with increasing bird number and indicated the same trend as was observed with the unfed birds. It can be suggested from the data that a group size of at least 8 birds is required to realize sufficient improvement in the precision of the TMEn estimate.

The data from Experiment 2 (Table 8) indicated that the mean FmE + UeE losses observed for each trial varied from 70.3 to 87.7 kJ with an overall mean of 77.7 kJ. The mean nitrogen losses for each trial showed a range of 1.09 to 1.45 g with an overall mean of 1.35 g. These represent 22.4 and 26.7% of the differences between low and high values for the FmE + UeE and endogenous nitrogen losses, respectively, although the overall means for these parameters are not different from those observed from Experiment 1. Correction of FmE + UeE to zero nitrogen balance resulted in the decreased FEn + UEn values, but the variation was still large.

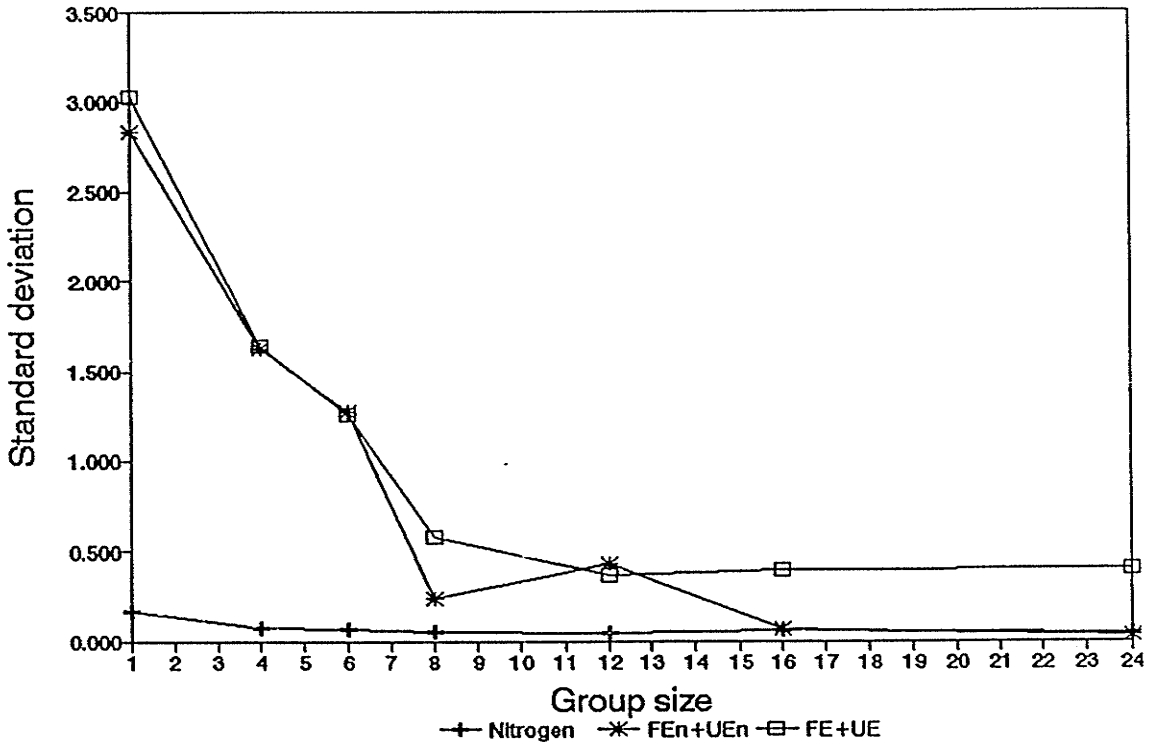

Fig. 3 . Standard deviation of means of FE+UE, nitrogen and FEn+UEn measured with fed birds.

TABLE 8. Mean energy, nitrogen and FEn + UEn excretions and TMEn values measured in Experiment 2

Trial	Fed			Unfed			TMEn (MJ)
	FE+UE (KJ)	N (g)	FEn+ UEn (KJ)	FmE+UeE (KJ)	N (g)	FEn+ UEn (KJ)	
1	201.33	1.34	155.12	70.31	1.09	32.94	13.51
2	196.60	1.42	147.69	79.61	1.36	32.75	13.74
3	194.56	1.60	139.58	75.50	1.24	32.72	13.80
4	210.51	1.70	151.89	86.17	1.42	37.49	13.66
5	195.28	1.58	140.85	87.68	1.41	33.24	13.85
6	196.72	1.54	143.74	76.10	1.34	30.11	13.60
7	197.92	1.64	141.64	71.63	1.27	27.92	13.51
8	197.08	1.52	144.84	78.55	1.39	30.65	13.62
9	186.29	1.46	136.21	74.10	1.33	28.41	13.82
10	195.76	1.49	144.62	76.29	1.38	28.70	13.54
11	193.95	1.54	141.02	77.62	1.38	30.20	13.73
12	190.51	1.48	139.47	80.45	1.45	30.75	13.79
13	197.24	1.43	148.02	78.41	1.37	31.17	13.51
14	181.50	1.44	132.11	75.55	1.31	30.65	14.04
15	184.54	1.44	134.94	82.57	1.38	32.96	14.06
16	192.93	1.39	145.28	74.64	1.23	32.28	13.51
17	171.85	1.30	127.02	72.24	1.26	29.04	14.09
18	185.03	1.44	135.63	82.54	1.29	33.14	13.96
19	188.38	1.44	138.89	71.80	1.32	26.35	13.57
20	187.53	1.45	137.63	82.62	1.43	33.39	13.94
21	174.87	1.49	123.79	78.59	1.30	33.90	14.49
22	190.69	1.47	140.25	74.55	1.22	32.74	13.87
23	184.37	1.41	135.97	76.75	1.39	28.80	13.87
24	189.90	1.46	139.53	80.26	1.39	32.48	13.83
Mean	191.05	1.48	140.24	77.69	1.35	31.37	13.79

The variability in mean FE + UE, nitrogen and FEn + UEn excretion measured with the fed birds for each trial in Experiment 2 (Table 8) was large between trials, ranging from 171.9 to 210.51 kJ with an overall mean of 191.1 kJ for FE + UE; from 1.30 to 1.70 g with an overall mean of 1.48 g for nitrogen; and from 123.8 to 155.1 kJ and an overall mean of 140.26 kJ for FEn + UEn. Correlation analyses indicated no relationships between FE + UE and FmE + UeE ($r = 0.171$, $p>0.05$); and between nitrogen voided by the fed birds and nitrogen voided by the unfed birds ($r = 0.338$, $p>0.05$). These results further indicate that the variation in excretion of energy and nitrogen by the fed bird could have been due to the small number of birds for each trial rather than the influence of FmE + UeE and endogenous nitrogen losses. Therefore, it is suggested that the use of unfed birds for each bioassay to decrease variation relative to time period may be of limited importance, but the number of birds for each fed group used in the TME bioassay is critical.

The average TMEn values for each replicate trial from Experiment 1 are shown in Table 9. There were no significant differences in TMEn values attributable to the replicate effect. The relative error values of the TMEn calculated according to the overall mean of 13.80 MJ/kg for replicates 1 to 6 were 0.1, 0.1, 0.8, 0.8, 0.3 and 0.2%, respectively. Analysis of the relationship between relative error value of TMEn and group size indicated that error of the TMEn values progressively decreased with increasing group size of the fed birds, and a stable level was observed with the groups of 8 or more birds (Figure 4), which is in good agreement with the result of standard deviation of means of FE + UE or FEn + UEn observed with the same birds. The overall mean of TMEn (13.79 MJ/kg,) measured in Experiment 2 (Table 8) was the same

TABLE 9. Mean TMEn values observed for replicate trials (Experiment 1)

Replicate trial	TMEn (MJ/kg)
1	13.81 ± 0.48[a]
2	13.81 ± 0.40[a]
3	13.71 ± 0.34[a]
4	13.88 ± 0.34[a]
5	13.74 ± 0.30[a]
6	13.83 ± 0.43[a]
Overall mean	13.80 ± 0.38

[a] means with no common superscripts are significantly different ($p < 0.05$).

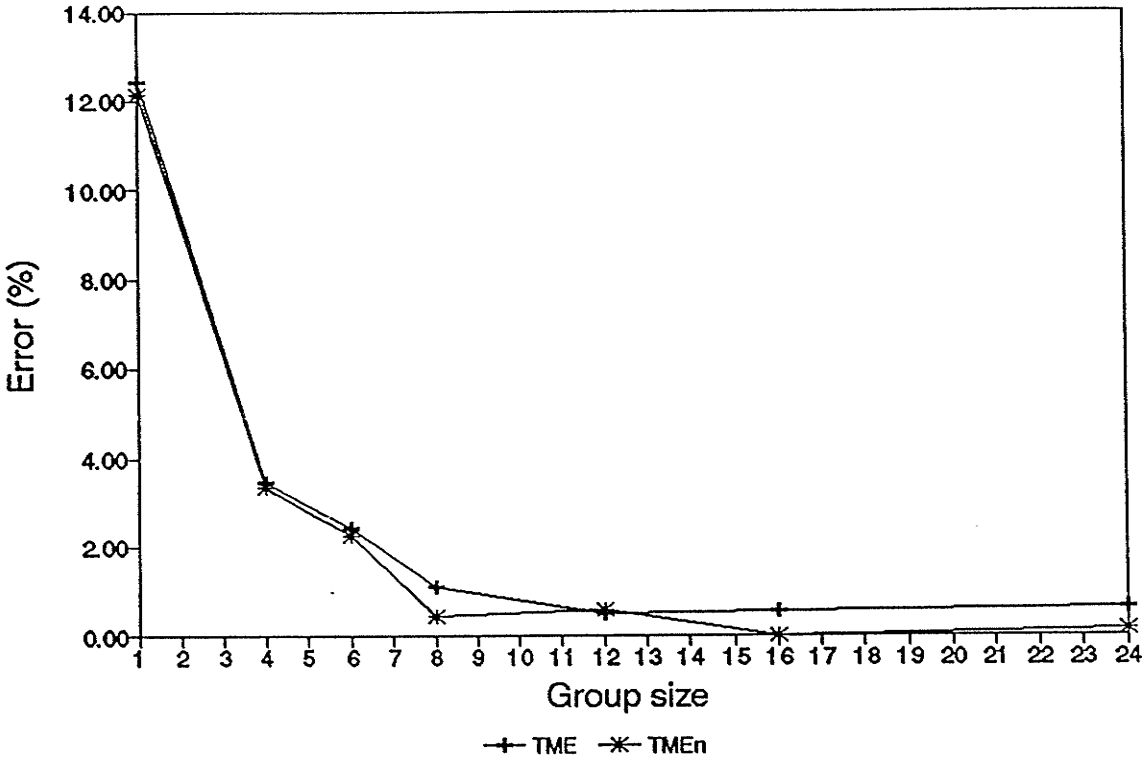

Fig. 4. Errors of TME and TMEn versus group size

as that in Experiment 1, but the variation from trial to trial was apparent, ranging from 13.51 to 14.49 MJ/kg. This represents a 7.1% difference between high and low values which further testified that, even though the FmE + UeE loss was measured for each assay, the TMEn value varied over time.

It is suggested that, due to large variability in the energy and nitrogen losses from bird to bird observed for both the fed and unfed birds, the use of 24 birds for estimating FmE + UeE loss and at least 8 birds for the fed group is necessary for improving the precision of the TMEn estimate. The cost involved in increasing group size is justifiable. There is, however, little evidence to support the need for measuring FmE + UeE and nitrogen losses for each bioassay.

EFFECT OF NITROGEN CORRECTION ON ACCURACY AND PRECISION OF THE TRUE METABOLIZABLE ENERGY BIOASSAY

ABSTRACT

Two experiments were conducted with adult SCWL cockerels to assess the effect of nitrogen correction on accuracy and precision of true metabolizable energy (TME) estimate.

Correction of TME to zero nitrogen equilibrium (TMEn) generated consistently lower values. The magnitude of the difference between TME and TMEn increased as protein level of feedstuffs increased. On the average, the differences between TME and TMEn values observed in Experiment 1 were 2.0, 4.7 and 10.4% for barley, wheat and protein feedstuffs (soybean meal and canola meal), respectively. In Experiment 2, correction of TME to zero nitrogen balance reduced the values for barley by 4.0 % and produced a 24.6% decline in standard deviation between TME and TMEn.

There was a significantly positive correlation ($r = 0.909$) between the protein level (X) and the difference between TME and TMEn values (Y). The resulting regression equation was $Y = 0.043 + 0.102X$ and the data indicate that 83% of variation in the difference between TME and TMEn values was due to change in the

protein content.

The energy loss showed a close relationship with nitrogen loss measured either with fed or unfed birds. Regression analysis generated similar regression coefficients, 41.30 and 41.42 (kJ/g N), for both fed and unfed birds, respectively, which represent the energy value per g of nitrogen loss.

INTRODUCTION

In the true metabolizable energy (TME) or nitrogen corrected TME (TMEn) systems as outlined by Sibbald (1976a, 1986) it is recognized that unfed birds depend completely on the degradation of body tissue to meet energy requirements for maintenance during the starvation and excreta collection periods. In fed birds, the catabolism of body tissue can be spared to some extent because of the feeding of a given amount of test material. It has been suggested, therefore, that the endogenous energy (FmE + UeE) loss of fed birds estimated from unfed birds may not be completely accurate, and could result in an overestimate of TME in feedstuffs (Shires et al., 1980; Dale and Fuller, 1984).

Since the FmE + UeE loss is due primarily to the degradation of tissue protein, a correction of energy loss to zero nitrogen balance has been used to reduce the variability in FmE + UeE loss between fed and unfed birds and to improve the accuracy and precision of the TME value (Parson et al., 1982; Sibbald and Morse, 1982; 1983; Wolynetz and Sibbald, 1984; Fisher and McNab, 1987). In contrast to this, data presented by Dale and Fuller (1984), and Muztar and Slinger (1981b) indicated that TMEn was not a constant function of TME, and that the relationship tended to be altered with the nature and protein level of feedstuffs. Thus, according to these authors there was

no advantage for correcting TME to zero nitrogen basis. Similarly, Parson et al. (1984), in a voluntary feed intake bioassay, found that the magnitude of the nitrogen correction varied with feed intake and protein level of feedstuffs. Other data by Sibbald (1980b, 1981a), however, showed that TMEn was constant regardless of the feed intake and the nature of feedstuffs.

Another source of variation in the TMEn value could be related to the nitrogen correction factor used in the assay. Two factors, 34.39 kJ (Hill and Aderson, 1958,) and 36.53 kJ (Titus et al., 1959) are generally quoted and the varied use of these could introduce error into the TME bioassay.

The current study was undertaken to further assess the effect of nitrogen correction on the accuracy and precision of the TME bioassay.

MATERIALS AND METHODS

Two experiments were carried out with adult SCWL cockerels according to the Sibbald procedure (Sibbald, 1986) with minor modifications. The modifications included a 28 h starvation interval, the collection of excreta on trays and the use of pooled as opposed to individual data. In the experiments, balance trials were conducted to test the effect of correction of TME to zero nitrogen basis on the accuracy and precision of TMEn estimate.

The birds used in the trials were randomly selected from a population of 98 birds maintained in individual cages and housed in a temperature controlled room with 14 h

light daily. A wheat-based maintenance diet and water were available ad libitum. During the balance trial the birds were moved to a separate environmentally controlled room (20 C $\pm$ 2, 14 h light, daily) and housed in individual metabolism cages (62.2 x 34.3 x 43.3 cm.).

Experiment 1 was conducted to measure the TME and TMEn values of 13 feedstuffs of varied protein content. The energy values for each of the feedstuffs were determined in duplicate or triplicate trials conducted at the different times in which 8 birds were each precision fed 30 g test material. In each trial FmE + UeE and endogenous nitrogen losses were obtained from pooled data for 8 unfed birds.

Experiment 2, consisting of 6 replicate trials conducted over a 1-year period, was designed to test the effects of time period and group size on excreta weight and excretion of energy and nitrogen by replicate groups of unfed and fed birds (Zhang and Campbell, 1992b). In this experiment balance data were collected for individual birds as opposed to pooled data for Experiment 1. In each replicate trial (except replicate 6 which involved the use of 24 fed birds) 48 birds were precision fed 30 g of a sample of the same barley and TME and TMEn values were calculated. In addition to the fed-bird data, balance data were collected from 48 unfed birds in each replicate trial at a time period similar to that for the fed birds. A period of 6 to 8 weeks elapsed between replicate trials. The TMEn values were calculated in each trial using the mean FmE + UeE and endogenous nitrogen values for all unfed birds($n = 280$) in the 6 replicate trials. In all trials data for some birds was lost because of regurgitation of feed or for mechanical reasons.

Excreta samples collected individually were frozen, freeze-dried, equilibrated to

atmospheric moisture for 24 h, weighed and ground to pass through a 1 mm screen. In Experiment 1 individual excreta samples were pooled for respective groups prior to analysis. To avoid an effect of moisture on feed and excreta weights, the feed samples for laboratory analysis were taken at the time when they were being prepared for precision feeding and excreta samples were taken at the same time as the total weight of excreta was measured. Analyses for nitrogen on excreta and feed, and for moisture on feed were conducted using AOAC procedures (1984). Caloric contents of excreta and feed were measured using a parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Illinois, U. S. A.).

Regression and correlation analyses were used to study the relationships between protein level and the nitrogen correction value; between energy excretion and nitrogen losses by both fed and unfed birds according to the procedure of the Statistical Analysis System Institute Inc. (1985).

RESULTS AND DISCUSSION

The protein content, TME, TMEn values and the differences between TME and TMEn of the 13 feedstuffs investigated in Experiment 1 are shown in Table 10. The protein levels ranged from 12.8 for barley to 35.7% for canola meal, with mean contents of 13.1, 16.8 and 35.7% for barley, wheat and protein-rich feedstuffs, respectively. The variability in protein level encompassed a relatively large range and consequently allowed an investigation of the dependence of the magnitude of nitrogen correction on protein

TABLE 10. Differential between TME and TMEn in feedstuffs.

Feed sample		Protein (%)	TME (MJ/kg)	TMEn (MJ/kg)	TME-TMEn (%)
Barley	1	12.78	13.98	13.77	1.5
	2	12.85	14.07	13.84	1.6
	3	12.98	14.68	14.31	2.5
	4	13.21	14.75	14.41	2.3
	5	13.48	14.01	13.74	1.9
Wheat	1	13.55	15.19	14.57	4.1
	2	14.06	15.82	15.14	4.3
	3	16.48	15.68	14.99	4.4
	4	18.68	15.30	14.57	4.8
	5	18.73	16.00	15.31	4.3
	6	19.21	15.42	14.56	5.6
Protein feedstuff	soybean meal	35.63	15.39	14.04	8.8
	canola meal	35.67	10.43	9.22	11.6

content of feedstuffs.

The TMEn value for each of the feedstuffs is a mean obtained from duplicate or triplicate observations. The relative error value estimated from these trials was less than 1.0%. The results obtained from these trials were treated as single values for correlation and regression analyses. The differences between TME and TMEn values increased as protein level increased. A large variation was observed for the protein-rich feedstuffs, 8.8 and 11.6% for the soybean meal and canola meal, respectively, while the differences for barley were small, ranging from 1.5 to 2.5% and those for wheat were intermediate, varying from 4.1 to 5.6%. A highly significant correlation was observed between protein level (X, as % crude protein on dry matter) and the difference between TME and TMEn values (Y, in MJ/kg on dry matter) ($r = .909$) and the resulting regression equation was, $Y = 0.04 + 0.102X$. The data indicate that 83% of the variation in nitrogen correction value is due to alteration in the protein level, although there was no apparent relationship between protein level and the TMEn value of a feedstuff.

Data on the difference between TME and TMEn values and the corresponding difference in standard deviation values observed in Experiment 2 are shown in Table 11. The correction of TME to zero nitrogen balance resulted in a marked decrease in standard deviation values and a slight decrease in TMEn values for the barley samples. This indicated that the decrease in standard deviation is not due entirely to the decreased TMEn values. The correction reduced variability in the FmE + UeE loss from bird to bird as observed by Zhang and Campbell (1992b) and hence improved precision of the TMEn estimate, which is in good agreement with the data of Sibbald and Morse (1983).

TABLE 11. Influence of correction to zero nitrogen balance on the TME estimate.

Replicate trial[1]	TME[2] (MJ/kg)	TMEn[2] (MJ/kg)	TME-TMEn/TME (%)	TME SD-TMEn SD/TME SD (%)
1	14.29 ± .63	13.81 ± .48	3.4	23.8
2	14.39 ± .45	13.81 ± .40	4.0	11.2
3	14.24 ± .42	13.71 ± .34	3.7	19.8
4	14.52 ± .44	13.88 ± .34	4.4	25.7
5	14.27 ± .53	13.74 ± .30	3.7	43.7
6	14.33 ± .46	13.83 ± .43	3.5	4.6
Mean	14.35 ± .51	13.80 ± .38	3.8	25.5

[1] Balance trials were conducted according to the method of Sibbald (1986) for the same barley sample.
[2] Mean ± SD.

Therefore, TMEn can be considered as a better measure of energy value for poultry feedstuffs than is TME. The question remains whether the correction to zero nitrogen balance gives a more accurate estimate than the non-corrected TME value.

The obvious advantages of both TME and TMEn are that they are independent of FmE + UeE loss and hence are not influenced by the level of feed intake as opposed to apparent metabolizable energy (AME). However, the use of unfed birds to estimate FmE + UeE for fed birds may introduce error into the bioassay. This error would tend to be inflated as feed intake increases since the energy voided by the fed birds would contain a reduced endogenous energy portion due to the sparing effect of feed energy on the catabolism of tissue protein. In this regard a slight overestimation of TME would result as feed intake increases. Since the determination of FmE + UeE loss by fed birds is difficult and impractical, there is no information available to provide a quantitative estimate of the difference in FmE + UeE between fed and unfed birds. Alternatively, nitrogen correction has been reported to reduce or eliminate the error (Parson et al., 1982; Sibbald and Morse, 1982, 1983; Wolynetz and Sibbald, 1984). The observation, however, that the difference between TME and TMEn values increased with increasing protein level indicates that the correction would appear to be useful for improving the accuracy of TME estimates for low protein feedstuffs such as cereal grains but may be inappropriate for protein-rich feedstuffs. The differences of 8.8 and 11.6% between TME and TMEn values observed for soybean meal and canola meal in Experiment 1 were large, and such variation might not be thought to be justifiable in the TME bioassay. Data presented by Dale and Fuller (1984) and by McNab and Blair (1988) indicated that

the TME values were reduced by 1.7 and 5.0% for corn and wheat feed, respectively, when supplementary energy was administered to unfed birds. Zhang and Campbell (1992c) observed a 1.8% difference in TME value of wheat by feeding 30 g granular sucrose to unfed birds at the termination of starvation period as opposed to the value observed for the birds receiving no supplementary energy. Therefore, the correction of energy loss to zero nitrogen basis for the protein feedstuffs seems not only to involve correcting the nitrogen components contributing to the difference in FmE + UeE loss between fed and unfed birds, but other sources of nitrogen as well. It should be noted that the importance of nitrogen correction used in the TME bioassay is completely different from the concept for AME. In the TME assay, the correction is employed to decrease the variability in FmE + UeE between fed and unfed birds. The nitrogen correction, if it deviates from this point, would bias the TMEn estimate. In this regard it appears to be of importance to determine at which protein level the nitrogen correction would correctly match the variability in FmE + UeE between fed and unfed birds and to develop an alternative technique for estimating TMEn values for those protein feedstuffs.

The nitrogen correction factor, another source of variability in TMEn value, was investigated on the basis of the assumption that , as tissue protein is the major source of FmE + UeE loss, the regression of FmE + UeE on endogenous nitrogen loss would be linear. When this assumption holds, the constant of the regression equation would be the energy loss from sources other than the degradation of tissue protein, and the regression coefficient would represent the energy loss per unit of nitrogen loss. The relationship

between FmE + UeE and endogenous nitrogen excretion for unfed birds is depicted in Figure 5 and the regression equations derived for both fed and unfed birds were,

$$Y1 = 131.35 + 41.28X1$$

$$r = .685 \qquad n = 224 \qquad p < .001$$

$$Y2 = 22.69 + 41.42X2$$

$$r = .895 \qquad n = 280 \qquad p < .001$$

where: Y1 and X1 are excreta energy (kJ) and nitrogen (g) voided by the fed birds, respectively.

Y2 and X2 are FmE + UeE (kJ) and endogenous nitrogen (g) voided by the unfed birds, respectively.

The constant term of the regression calculated for the fed birds was different from that for the unfed birds and the difference can be attributed to the feed energy source. The correlation coefficient for the fed birds was low and in this regard nitrogen loss accounted for only 47% of variation in excreta energy. The higher correlation coefficient and lower constant for the regression equation computed for the unfed birds confirms the finding that FmE + UeE loss is primarily as a consequence of the degradation of tissue protein. The extent to which this was observed to vary from bird to bird, as shown by the distribution of individual values in Figure 5, may explain why the standard deviation values were different among the replicate trials. The regression coefficients for both the fed and unfed were similar even though the excretions of energy and nitrogen by the fed birds are of endogenous and feed origins as opposed to only of endogenous origin for the unfed birds. These values which would be expected to represent the energy loss per unit

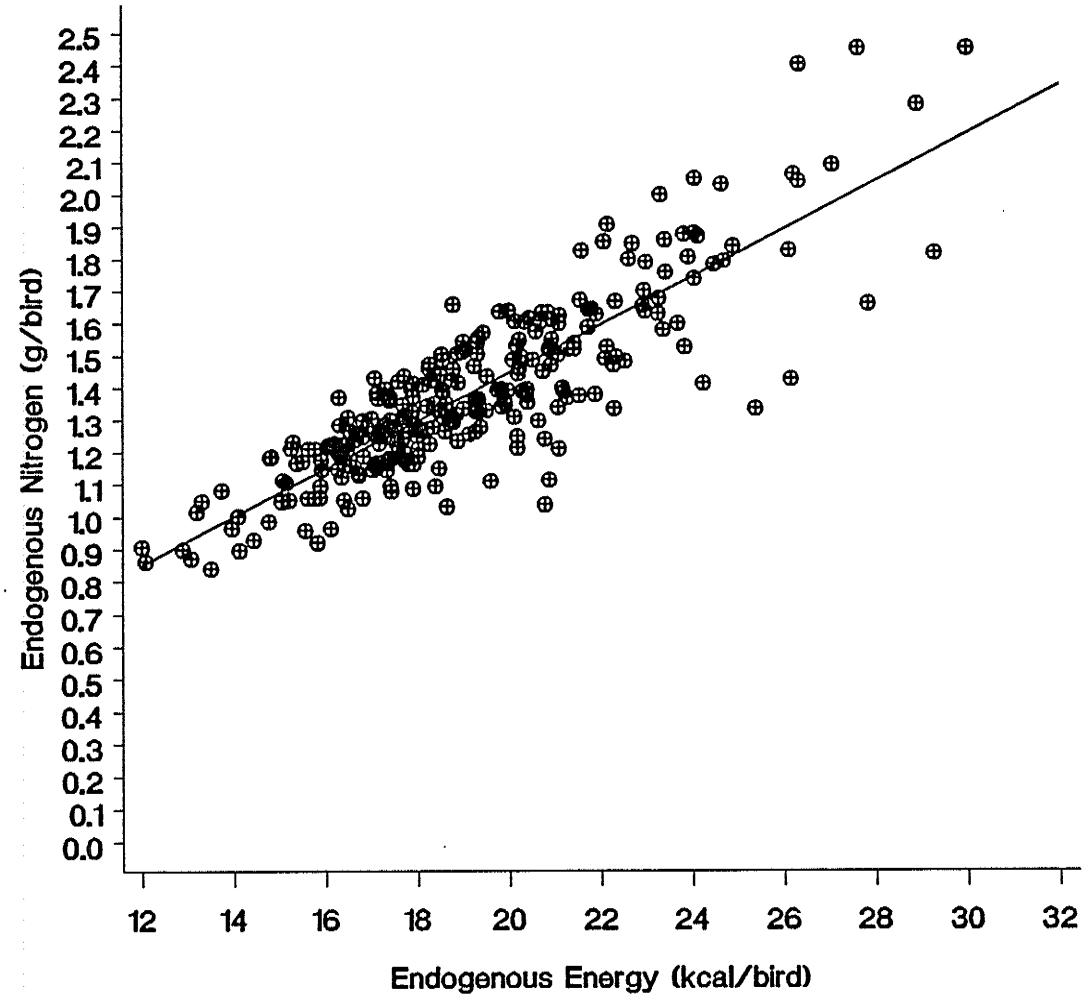

Figure 5. Relationship between endogenous nitrogen and energy

of nitrogen loss are slightly higher than those currently in use (34.39 or 36.53 kJ), suggesting that the nitrogen correction factor may not be a fixed value. The use of either 34.39 or 36.53 kJ could result in a biased TMEn value. In addition, similar regression coefficients for both the fed and unfed birds indicate that the energy losses relative to nitrogen losses are not different for fed and unfed birds although the nitrogen excretion by fed birds is of endogenous and feed origins. The reason for this is not clear and requires further study.

The results from the current study indicate that TMEn is preferred over TME for cereal grains but the use of the nitrogen correction in the energy bioassay of protein feedstuffs requires further study. The factors currently used to correct TME to zero nitrogen basis may tend to underestimate energy loss relative to nitrogen loss.

AN INVESTIGATION OF THE FEASIBILITY OF PREDICTING TMEn CONTENT IN BARLEY FROM CHEMICAL COMPOSITION AND PHYSICAL CHARACTERISTICS

ABSTRACT

To investigate the feasibility of predicting energy value in barley for poultry , a total of 91 barley samples were used in this study for determining chemical components and physical properties and nitrogen-corrected true metabolizable energy (TMEn). The samples included three barley types (6-row malting and feed and 2-row feed), grown at 12 locations in Manitoba from 1986 to 1988. Prediction equations for TMEn were developed from chemical composition and physical properties. Substantial variation in chemical and physical measurements was evident among the barley samples studied. Data of TMEn indicate a range of 13.10 to 14.59 with a mean content of 13.97 MJ/kg (dry matter basis).

Initial evaluation of prediction equations for estimating TMEn involved an examination of the simple correlation and regression between TMEn content and chemical or physical parameters. It was found that TMEn value was negatively correlated with NDF level ($r = -0.784$) and positively correlated with test weight ($r = 0.627$). The

relationships between TMEn value and starch and fat contents were also apparent whereas only 7 to 8% of the variation in TMEn value was due to changes in these variables. With the exception of NDF, no single variable was shown to be any more accurate than a combination of the variables for predictive purpose as adjudged by residual standard deviation (Rsd) values. Inclusion of NDF, GE, fat, protein and starch terms into a multiple regression equation resulted in a slightly decreased Rsd and an increased R^2, but no significant difference in R^2 values between the simple regression in which NDF was used as predictor and the multiple regression was apparent.

The best combinations of predictor variables were, in decreasing order, NDF, GE and fat, selected from stepwise regression, and NDF, fat and starch, from the Mallows Cp values, respectively.

In contrast to the equation using test weight as sole predictor, which showed a large prediction error of TMEn, the errors estimated from the simple, multiple, stepwise regression equations and the equation selected according to the Mallows Cp value approach that for the biological measure.

INTRODUCTION

Barley is one of the major cereal grains and a large portion of the barley grain in Canada is used for feed. There is evidence to show that barley varies widely in bioavailable energy (BE) values due to variation in chemical composition relative to genotype of barleys, and location and environmental conditions under which the grain is grown. Data of Coates et al. (1977a) on 16 Canadian barley samples indicated a range in apparent metabolizable energy (AME) values of 2.88 to 3.21 with a mean of 3.05 kcal/g for chicks. A similar observation was made by Sibbald (1986) who found a range in true metabolizable energy (TME) values of 12.4 to 14.3 MJ/kg for 29 barley samples. These observations indicate a 11 - 14% difference between high and low values for AME or TME, respectively, and consequently the use of the average values in diet formulation could result in substantial error. The need for information regarding the energy value of individual barley sample has become increasingly important for practical application, but the biological measurement of BE on numerous samples is impractical due to cost and time involved. In this regard, indirect methods have been developed for estimating the BE value of feedstuffs and although a large number of prediction equations are available, they may be meaningful for only a limited range of feedstuffs depending upon the condition under which the data sets were generated.

Prediction equations developed previously have generally been linear and based on relationships between chemical components or physical properties and BE values. The physical property, bulk weight, has traditionally been used as a criterion of assessing cereal quality and is thought to be a reliable and practical measure of BE content. The relationship between bulk weight and BE has been confirmed for oats, but studies on barley have failed to provide a clear-cut result (Lockhart et al., 1961; Sibbald and Slinger, 1963b; March and Biely, 1973; Bhatty et al., 1974; Sibbald and Price, 1976a, 1976b, 1977; Coates et al., 1977b). Fibre materials have been reported to be a sensitive predictor variable for BE value but other chemical parameters were indicated to have weak or no relationship (Bhatty et al., 1974; Campbell and Campbell, 1975; Coates et al., 1977b; Carre et al., 1984).

The current study was planned to measure the TMEn contents of several barley samples and to investigate the feasibility of predicting the energy value of barley samples from chemical measurements and physical properties.

MATERIALS AND METHODS

In the current experiments, a total of ninety one barley samples were investigated. These included three barley types: 6-row malting (cv. Argyle); 6-row feed (cv. Bedford); 2-row feed (cv. Deuce/Norbert) and were grown at 12 locations in the province of Manitoba from 1986 to 1988.

The TME bioassay was carried out with adult SCWL cockerels during a one year

period using the precision-feeding technique described by Sibbald (1986) except for some modifications. The modifications included a 28 h starvation interval, collection of excreta samples on trays and the use of pooled as opposed to individual data. In the experiments reported here, balance trials were conducted to measure the TMEn contents of the barley samples. All the birds used in the balance trials were housed in individual metabolism cages (62.2 x 34.3 x 43.3 cm.) in an environmentally controlled room (20 C $\pm$ 2) with 14 h light beginning at 0600 daily. Water was available ad libitum during the entire experiment. The TMEn value for each barley sample was measured in duplicate or triplicate trials conducted at the different times in which 6 to 12 birds were randomly chosen from a population of 98 birds and each precision fed 30 g test material. In each trial FmE + UeE and endogenous nitrogen losses were obtained from pooled data for 6 to 12 unfed birds. TMEn values were calculated for each trial using the mean FmE + UeE and endogenous nitrogen values for all unfed birds throughout the entire experiment. The repeatability of TMEn values for each sample measured for duplicate trials was determined in terms of the relative error, and the trial was repeated if error was greater than 1%.

Excreta collected from individual bird was frozen, freeze-dried, allowed to equilibrate to atmospheric humidity for 24 h prior to weighing, and ground to pass a 1 mm screen. Samples for each barley within a trial were pooled for analysis. Analyses for dry matter, fat on feed sample and for nitrogen on feed and excreta samples were conducted according to standard procedures (AOAC, 1984) and for GE using a parr adiabatic oxygen bomb calorimeter (Parr Instrument Co. Illinois, U. S. A.). NDF was

assayed by the method of Van Soest and Wine (1967). Starch content was measured according to the procedure described by Henry and Saini (1989). The determination of test weight was made using the standard Canadian Grain Commission procedure.

The results obtained from the TMEn trials were treated as single values for correlation and regression analyses. Correlation and regression (simple, multiple, stepwise and Mallows Cp value) analyses were carried out to investigate the relationships between TMEn and chemical composition or physical property in order to develop prediction equations, and interaction effects of NDF and other chemical measurements on the TMEn value were tested according to the procedure of Statistical Analysis System Institute Inc. (1985).

An alternative technique of selecting a regression equation in the current study was to use Cp statistics which relies on residual sum of squares (RSSP) and is associated with R^2.

$$Cp = RSSP / S^2 - (N - 2p)$$

where: p is the number of independent variables in equation including constant term.

S^2 is residual mean square from the largest equation including all the variables.

To examine the validity of the equations developed in the current study, all the barley samples were randomly divided into two groups. The regression were computed in terms of the TMEn values and chemical composition and test weight for 46 barley samples, and the comparison was made on the observed and predicted TMEn for the other 45 samples.

RESULTS AND DISCUSSION

Chemical composition, test weight and TMEn value

The chemical composition, test weight, GE and mean TMEn content for 91 barley samples are shown in Table 12. Among the chemical composition data, fat content indicated the largest and GE the smallest variation as shown by the coefficient of variance values. The content of fat was relatively low, ranging from 1.45 to 2.78 with a mean of 2.09% while GE values varied from 17.77 to 18.83 with a mean of 18.44 MJ/kg for the three types of barleys used in the study. Protein and NDF levels spanned similar ranges, 9.49 to 18.23% and 12.19 to 19.20%, respectively. Starch indicated a relatively narrow range of 55.71 to 69.85 with a mean of 64.93%. Test weight varied from 56.57 to 78.42 with a mean of 70.91 kg/hl.

The TMEn contents of the 91 barley samples ranged from 13.10 to 14.59 with a mean of 13.97 MJ/kg (dry matter basis). The range is relatively low as opposed to those for chemical composition, except for the GE content. This represents an 11% difference between high and low values which could be attributed to a change in chemical composition or physical properties as affected by genotype, location effects or environmental conditions under which the grains were grown. These data indicate that the use of mean values in diet formulation could result in over or underestimation of the

TABLE 12. Chemical composition, test weight, GE and TMEn value for 91 barley samples

Item	Mean	Minimum value	Maximum value	C.V.[1]
Fat (%)	2.09	1.45	2.78	13.49
Protein (%)	13.50	9.49	18.23	10.45
NDF (%)	15.12	12.19	19.20	10.44
Starch (%)	64.93	55.71	69.85	5.04
Test wt. (kg/Hl)	70.91	56.57	78.42	5.60
GE (MJ/kg)	18.44	17.77	18.83	0.95
TMEn (MJ/kg)	13.97	13.10	14.59	2.07

[1] coefficient of variation expressed as %.

energy content of individual barley samples.

Effects of chemical composition and test weight on TMEn value

The correlation matrix describing the relationships between TMEn value and chemical composition and test weight is shown in Table 13. It was observed that the TMEn value was negatively correlated with NDF and the r value indicated that 61.5% of variation in the TMEn value was due to alteration in NDF level. The positive correlation relating TMEn content to test weight was significant ($p<0.01$), but the r value was low and test weight accounted for only 39.5% of the variation in TMEn value. The correlation between TMEn value and starch level was statistically significant ($p<0.01$), but it was weak, indicating that even though starch represents a large portion of the composition of barley it accounted for only 8.2% of the variation in TMEn. In this regard the relatively narrow range of this variable may indicate that starch contributes mainly to the average TMEn value rather than to variation from that mean. On the other hand, the intercorrelation between starch and other chemical composition may be an important reason. Analysis of the relationships between protein and GE and TMEn indicated that the effects of protein and GE contents on TMEn value were similar to starch. Fat was found to be significantly ($p<0.05$) correlated with TMEn value but only 7.0% of the variation in the TMEn content was accounted for by this variable. From a theoretical perspective a curvilinear relationship between fat and TMEn would be expected since fat contains more than twice as much energy as carbohydrate. The

TABLE 13. Correlation matrix describing the relationships between TMEn, chemical composition, GE and test weight measured for 91 barley samples

	Fat	Protein	Starch	NDF	GE	Test wt.	TMEn
Fat	1.000						
Protein	0.070	1.000					
Starch	0.050	-0.401**	1.000				
NDF	0.167	-0.100	-0.460**	1.000			
GE	0.109	0.490**	-0.426**	0.314**	1.000		
Test wt.	0.307**	-0.073	0.290**	-0.515**	-0.273**	1.000	
TMEn	0.265*	0.135	0.287**	-0.784**	-0.113	0.627**	1.000

** $p < 0.01$.

correlation coefficient involving the use of a quadratic term in an attempt to explain this relationship was significant only at the 10.0% level. The weak correlation between TMEn and fat may be related to the low fat content in these samples.

The correlation matrix demonstrated that several variables which were correlated with TMEn were also related to each other. The increase in protein level was associated with a significant decrease in starch content and an increase in GE value. This result is in agreement with an earlier study by Sibbald and Price (1976a) who found that as protein content in wheat raised, GE increased. Starch content was negatively correlated with NDF and GE levels and positively related to test weight. In addition to the effect of starch content, NDF was observed to increase with increasing GE and decreasing test weight.

The results indicate that NDF content in the barley samples is an important factor affecting the TMEn content. Test weight accounts for a low fraction of variation in TMEn value, and other variables such as starch and fat are correlated with TMEn value but the r values are low. Several variables which were correlated with TMEn were also related to each other, which could affect the relationships of any one of the variables with TMEn.

Equations of predicting TMEn value of barley

Initial evaluation of prediction equations for estimating TMEn content involved an examination of the simple correlation and regression between TMEn and chemical

composition and test weight. It was found that NDF was the most accurate predictor of TMEn value, followed by the variable test weight as judged by Rsd and R^2 values (Table 14). The regression equation relating TMEn to NDF indicated a close relationship and since the Rsd is relatively low the equation is of practical merit. The regression coefficient of TMEn on test weight although statistically significant accounted for only 39.3% variation in TMEn value. Thus, this latter equation may be meaningful for only a limited range of feedstuffs depending upon variation in test weight.

In terms of the cost involved in obtaining information on a large number of predictor variables and subsequently monitoring them, it may be meaningful to include a single predictor variable for practical application. Therefore, NDF content would be the most useful measure based on its limited positive contribution to feeding value and large negative effect on other nutrient metabolism. The ease and precision with which its measurement may be made are additional considerations when choosing among effective predictor variables. Test weight may not be a sensitive variable for predictive purpose and hence is of limited practical importance. This confirms other findings with monogastric animals (Bhatty et al., 1974; Christison and Bell, 1975; Sibbald and Price, 1976b; Coates et al., 1977b).

Multiple regression analysis may provide a means of improving the precision of prediction by incorporating several predictor variables. NDF, starch, fat, protein and GE were used as predictor variables in the present study as these ingredients account for a major amount of variation in the TMEn in feedstuffs. The multiple regression equation is shown in Table 14. A strong relationship ($p < .01$) between chemical measurements

TABLE 14. Regression equations for predicting TMEn content of barley

Regression	Prediction equation	Rsd	R^2
Simple	TMEn = 10.7473 + 0.0455test wt.	0.226	0.393**
	TMEn = 16.1399 - 0.1433NDF	0.180	0.615**
Multiple	TMEn = 12.2623 + 0.1139fat + 0.0002GE - 0.1529NDF - 0.0091protein - 0.0046starch	0.176	0.649**
Stepwise	TMEn = 12.0703 + 0.1201fat + 0.0002GE - 0.1470NDF	0.174	0.648**
Cp value	TMEn = 16.3128 + 0.1319fat - 0.1454NDF - 0.0064starch	0.177	0.638**

** $p<0.01$.

and TMEn value was shown and the intercept value was significantly ($p < .01$) different from zero. The regression coefficients varied from predictor to predictor. This may be related to the fact that the effect of each variable on TMEn value depends on its contribution to TMEn and that it is influenced by the intercorrelations within the data. In comparison to the simple regression equation in which NDF was used as a predictor, inclusion of NDF, fat, protein, starch and GE terms into a multiple regression equation resulted in a slightly decreased Rsd and an increased R^2 value. The multiple regression equation because of its usefulness over a wide range of predictive purpose would be expected to be more valuable as compared to the simple equation based on NDF. The improvement in precision, however, may not be considered justifiable because of increased cost involved in chemical analysis.

There is evidence to show that fibre interferes with digestion and absorption of nutrients by squandering bile acids and increasing thickness of the unstirred water layer barrier at the intestinal surface (Vahouny, 1987). This was not confirmed in the current study as a significant interaction effect of NDF and other chemical measurements on TMEn value was not evident.

Stepwise regression equations are thought to make a compromise between the two extremes concerning precision of prediction and the cost involved in generating equations and for final practical application. The equation selected by stepwise regression analysis is shown in Table 14. It was found that NDF was the variable most correlated with TMEn which is in agreement with the examination of the correlation matrix (Table 13), and was accepted as the first-order. Fat was then chosen as the next variable showing a

high partial correlation coefficient to enter into the regression. The acceptance of GE as a predictor variable supports the finding of Wiseman and Cole (1985) who suggested that an approach to predictive equations could be one where the input of GE is considered. In addition, King and Taverner (1975) observed that inclusion of GE in regression equation for DE resulted in a considerable improvement on the accuracy of the equation in a study with pigs. The results for stepwise regression analysis were similar to the multiple regression equation. The stepwise regression equation would be expected to be more useful as opposed to the multiple regression equation because of decreased cost relative to chemical analysis.

Several of the possible subset regressions are shown in Table 15. The Cp value depressed with an increase in the number of independent variables and showed consistent tendency to an improvement in the R^2. As determinations were made in terms of the minimum Cp value, selected equations including 1 to 3 variables were similar to those derived from the simple and stepwise regression analyses, suggesting that for a given condition in which the dependent and independent variables are fixed, the R^2 depends heavily upon Rsd. Inclusion of more than three variables was found to have no advantage.

Based on the assumption that the S^2 from the largest equation including all the variables would be a reliable estimate of the error variance, it is possible to plot Cp versus p value in which the adequate model can be determined as the points fairly close to the Cp = p line. It was observed that the equation with the predictors NDF, fat and starch was close to the Cp = p line, suggesting that as was indicated by those equations

TABLE 15. Possible regression including varied predictor variables

n	R^2	Cp	Variable
1	0.615	6.41	NDF
2	0.618	7.62	protein, NDF
2	0.623	6.74	NDF, starch
2	0.634	3.89	fat, NDF
2	0.635	3.61	NDF, GE
3	0.622	8.66	protein, GE, starch
3	0.635	5.47	protein, NDF, GE
3	0.636	5.29	fat, protein, NDF
3	0.636	5.26	NDF, starch, GE
3	0.638	4.90	fat, NDF, starch
3	0.648	2.46	fat, NDF, GE
4	0.638	6.80	fat, protein, NDF, starch
4	0.638	6.79	protein, NDF, starch, GE
4	0.648	4.37	fat, protein, NDF, GE
4	0.648	4.27	fat, NDF, starch, GE
5	0.650	6.00	fat, protein, NDF, starch, GE

developed according to simple, multiple and stepwise regression analyses, the fitted equation including NDF, fat and starch according to the Cp value is of practical applicability.

Precision of prediction

The equations and relationships and mean differences between the observed and predicted TMEn values are shown in Table 16 and Figures 6, 7, 8, 9, and 10. The R^2 values between observed and predicted TMEn values varied from 0.34 to 0.70. A large mean difference was observed with the equation in which test weight was used as sole predictor. This further confirms that test weight is not a sensitive predictor variable for barley probably due to failure of inclusion of the latent variables. The mean differences estimated from other equations approached zero and the maximum error ranged from 2 to 3%. In comparison with the data from other sources (Bolton, 1962, quoted by Sibbald and Price, 1977; Carpenter and Clegg, 1956; Sibbald and Price, 1976a), the results from the current study suggest good agreement between observed and predicted TMEn values.

TABLE 16. Relationship and mean difference between observed and predicted TMEn values for 45 barley samples

Regression	Equation	R^2	Mean diff.
Simple	TMEn = 9.9225 + 0.0567test wt.	0.34**	0.069
	TMEn = 16.4675 - 0.1650NDF	0.70**	0.002
Multiple	TMEn = 11.1150 +0.1866fat + 0.0003GE - 0.0128protein - 0.1707NDF - 0.0044starch	0.69**	0.005
Stepwise	TMEn = 15.8353 + 0.2243fat - 0.1543NDF	0.68**	0.004
Mallows Cp	TMEn = 16.4273 +0.2243fat - 0.1603NDF - 0.0077starch	0.68**	-0.001

** $p < 0.01$.

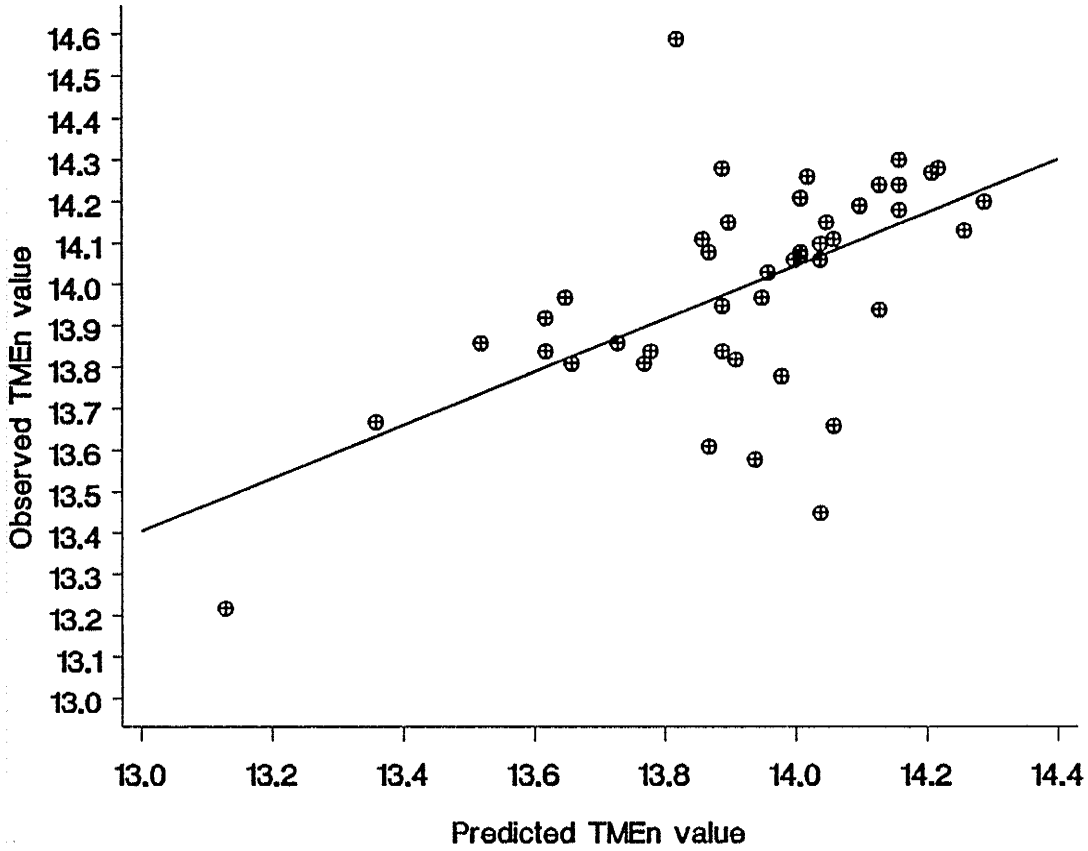

Figure 6. Relationship between observed and predicted TMEn
TMEn = 10.747 + 0.045test weight

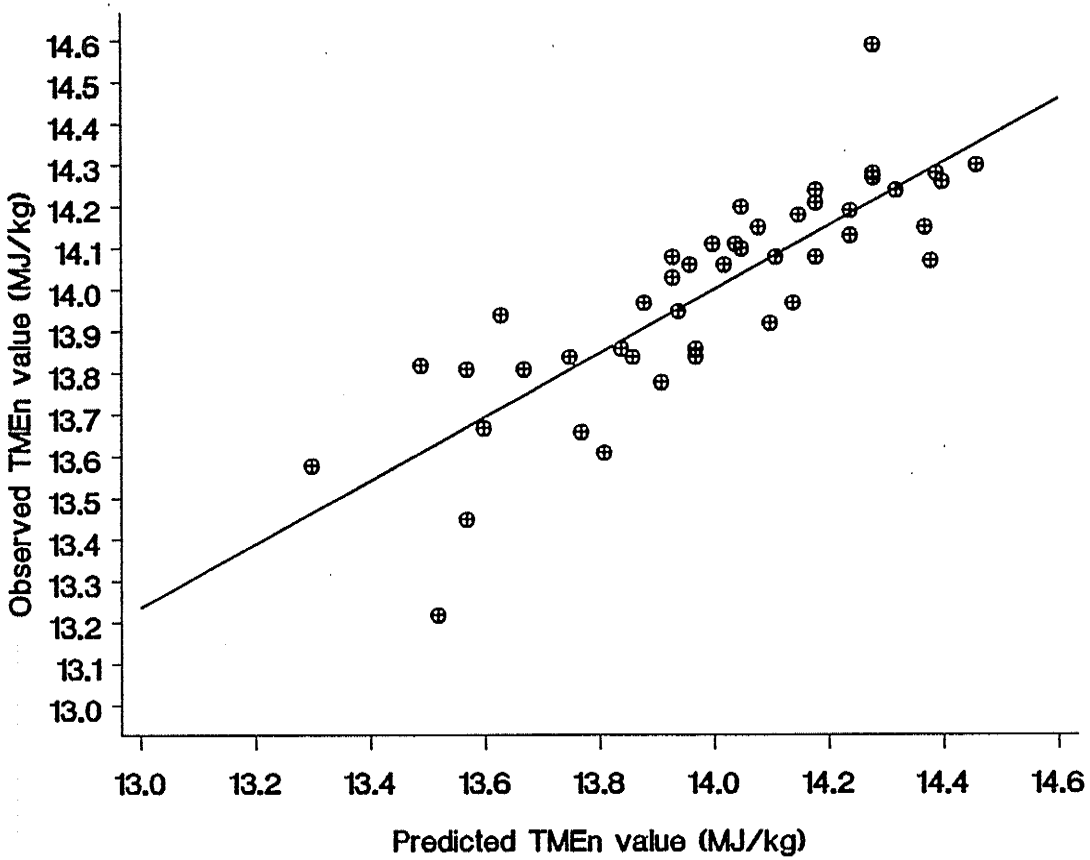

Figure 7. Relationship between observed and predicted TMEn (Simple regression based on NDF)

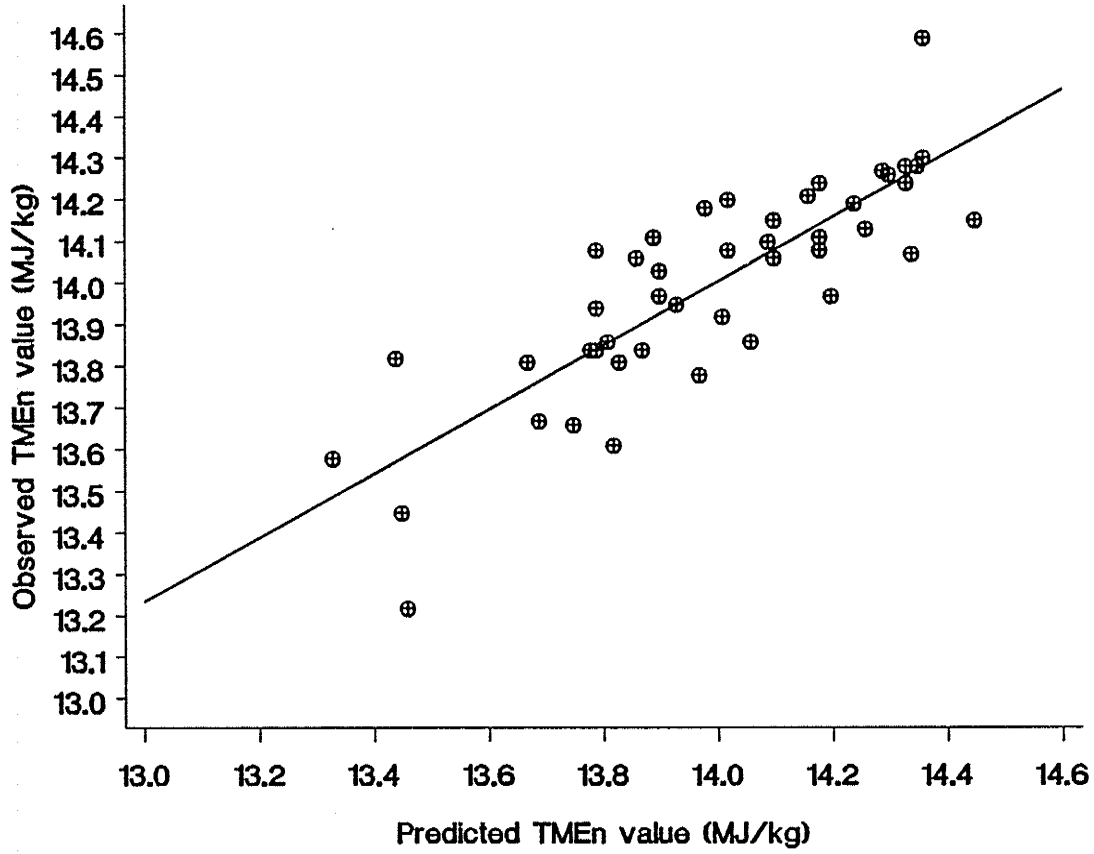

Figure 8. Relationship between observed and predicted TMEn (Multiple regression)

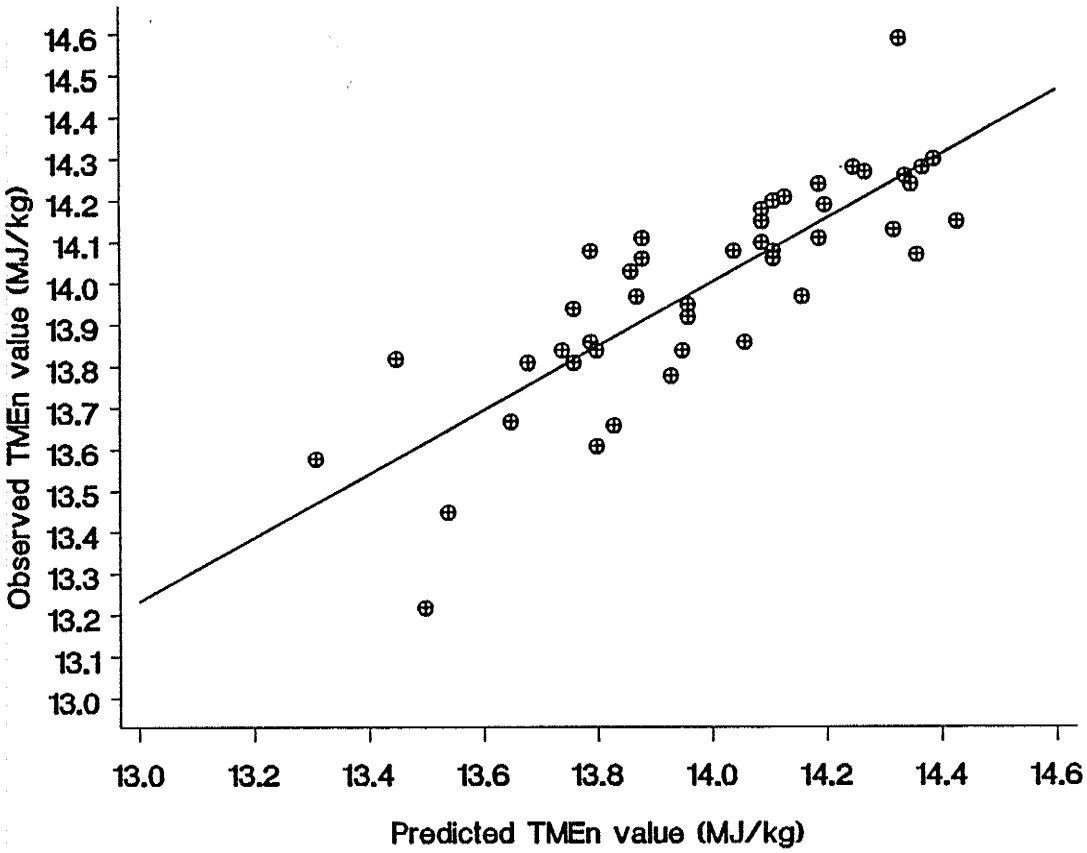

Figure 9. Relationship between observed and predicted TMEn (Stepwise regression)

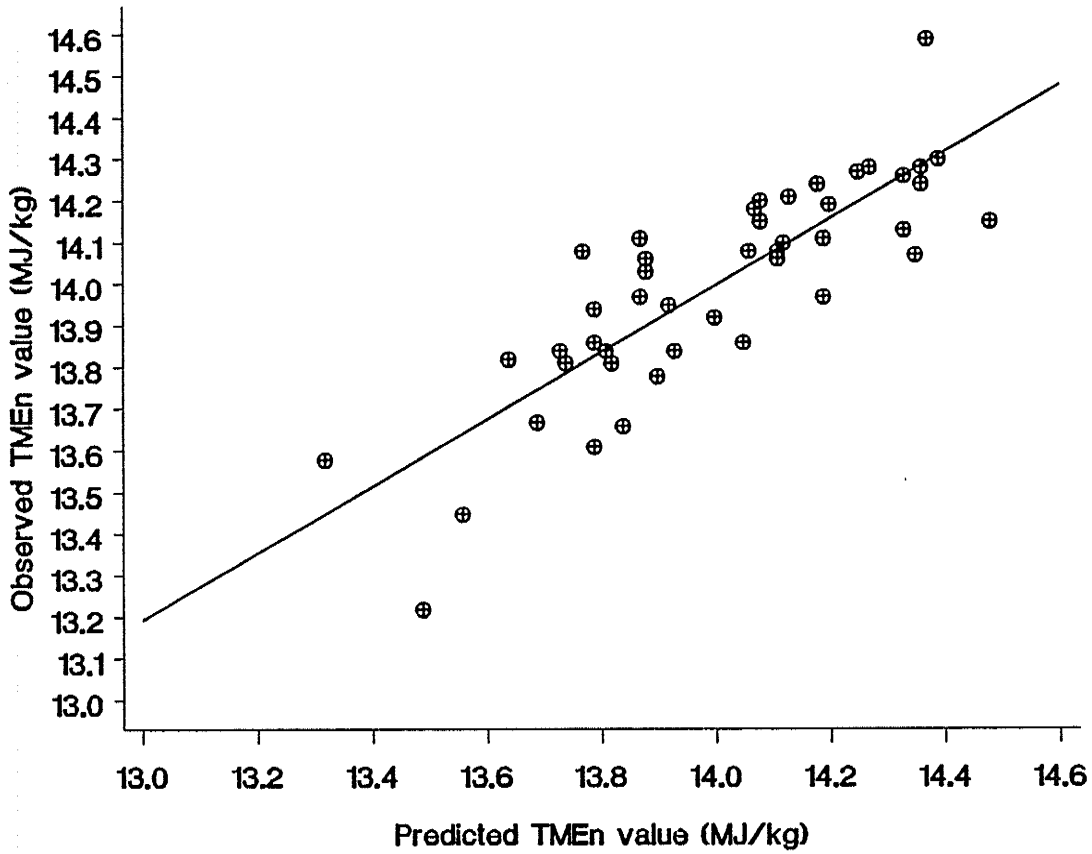

Figure 10. Relationship between observed and predicted TMEn (Mallows Cp value)

GENERAL DISCUSSION

An optimum time interval required to allow for complete clearance of the feed residues from the GI tract can be considered to be an important aspect for improving accuracy of the TME estimate. Observation that extension of the starvation interval from 24 to 28 h significantly reduced excreta weight and FmE + UeE and to a lesser extent endogenous nitrogen losses for the birds indicated that the interval of 24 h currently used in the TME bioassay may not be a sufficient time period for all birds. In this regard, variation as a consequence of the occasional increased FmE + UeE and endogenous nitrogen losses for some birds could result in a biased estimate of TME. However, the optimum time requirement depends on the level of feed intake and the nature of feedstuffs. In the current study for birds fed a wheat-based maintenance diet, lengthening of the period to 36 h showed no further improvement. Sibbald (1976b) reported that the TME values observed for the birds starved for 24 h were not significantly different as opposed to those for the birds starved for more than 24 h. This conclusion, however, is subject to question since the relatively small group size (i.e. 5 unfed birds) used in the cited experiments would influence the analysis of variance test. Based on the results of the current experiments a starvation period of at least 28 h is recommended.

Although an effect of the feeding of granular sucrose to unfed birds on excreta

weight and endogenous nitrogen loss was evident, the correction of energy excretion to zero nitrogen balance resulted in no influence in accuracy and precision of the TME estimate relative to controls. These results indicate that provision of supplementary energy to unfed birds is an important consideration for reducing the degradation of tissue compounds for energy-yielding purpose and consequently increasing accuracy of the TME estimate. However, since FmE + UeE loss is due primarily to the catabolism of tissue protein (Parson et al., 1982; Sibbald and Price, 1982), a correction of energy excretion to zero nitrogen balance counteracts the influence of supplementary energy on FmE + UeE loss. In contrast, the administration of supplementary energy is time consuming and results in a high chance of regurgitation of sucrose, and hence the procedure is considered to be of little practical importance.

The variability in TME bioassay is dependent, to a large extent, on the variation in FmE + UeE loss. Data from the current study showed a wide range among birds and the variation was independent of body weight. Furthermore, there were no significant differences between time periods and among random groups. The variation appears to be characteristic of the birds (Sibbald and Price, 1978). In this regard the use of each bird as its own control would be of advantage for reducing the variation between fed and unfed birds, but this may have limited practical value as it involves lengthening of the duration of assay. Sibbald and Price (1980) observed a substantial variation between experiments and suggested the need for measuring FmE + UeE for each assay. The inconsistent findings between the cited experiment and the current study may be related to the use of a small number of birds in the former assays. In this regard, since there is

no effective way to control the variation, the use of optimum bird numbers in the assay is necessary to reduce variation. In addition given the demonstrated nature of the variation, there is no evidence to show the need for repeated measurement of FmE + UeE for each assay. The number of fed birds used for each treatment also influences variation in the TME assay, presumably either due to the variation in FmE + UeE or to differences among birds in the capacity to metabolize feedstuff energy or a combination of these factors. Based on the results of the current experiments, it is necessary to increase bird numbers to 24 for the unfed group and at least 8 for fed groups.

The advantage of the TME system over the AME system is that the resulting TME value of feedstuffs is independent of the level of feed intake. However, the use of unfed birds as estimators of FmE + UeE for fed birds may not be completely accurate, The difference in FmE + UeE loss between fed and unfed birds could introduce bias into the TME assay and the magnitude of the variation depends on the level of feed intake (Dale and Fuller, 1982, 1984). The correction of energy excretion to zero nitrogen balance would reduce the variation such that the resulting TME is not influenced by the level of feed intake (Sibbald and Morse, 1983). It was observed that the nitrogen correction resulted in a substantial decrease in standard deviation values and a slight decrease in TME values, indicating that the nitrogen correction improves accuracy and precision of the TME values by reducing the difference in FmE + UeE loss between fed and unfed birds and the variation in FmE + UeE among birds. Observation that the magnitude of the difference between TME and TMEn values increased with increasing

protein level of feedstuffs suggests that the nitrogen correction may not be appropriate for the protein-rich feedstuffs as it tends to underestimate TME values for these feedstuffs (Parson et al., 1984).

The nitrogen correction factors 41.28 and 41.42 (kJ/g N) for fed and unfed birds derived from regression analysis were similar, indicating that the energy loss relative to nitrogen loss is not different for fed and unfed birds despite differences in source of nitrogen between the two groups of birds. These values are slightly higher than those currently in use (34.39 and 36.53), suggesting that the nitrogen correction factor may be not a fixed value and the use of either 34.39 or 36.53 (kJ/g N) could result in a biased TME value.

Although there are numerous prediction equations available, these equations may be valuable for only a limited range depending upon the conditions under which the data sets were generated. The most effective equations for predictive purpose are those that are based on both accurate and precise measurements of dependent and independent variables and are derived from the samples showing a maximum variation in both variables. In the current study an attempt was made to meet these criteria and it was found that NDF, among the variables measured, was most highly correlated with TME, which could be related to its small positive contribution to TME and large negative effect on the metabolism of other nutrients. The simple regression based on NDF was, TMEn = 16.1399 - 0.1433NDF, R^2 = 0.615. Test weight, although it was significantly correlated with TME, was not a sensitive predictor of TME as shown by the high error of prediction and hence is of less practical merit than the chemical measure NDF.

Inclusion of NDF, starch, fat, protein and GE terms in a multiple regression equation resulted in a slight decrease in Rsd and an increase in R^2 relative to simple regression based on NDF. It is apparent that the multiple equation would be meaningful for the use for a wide range of samples but the improvement in precision may be of limited importance because of increased cost. From the perspective of both practical applicability and prediction precision, the equations derived from stepwise regression analysis and the Mallows Cp value are considered to be valuable as judged by the relatively low Rsd and high R^2 values but, as with multiple regression analysis, cost and labour involvement are a significant consideration. The accuracy of the prediction equations which agree with those currently in use in Europe (Alderman, 1985) are encouraging but more research is needed to evaluate rapid and inexpensive techniques such as near infrared reflectance analysis.

GENERAL CONCLUSIONS

The starvation interval of 24 h may not be sufficient time to allow for complete clearance of the feed residues from the GI tract. Extension of the period to 28 h resulted in a decrease in excreta weight and FmE + UeE and to a lesser extent endogenous nitrogen losses with resulting improvement in the precision of the TME estimate. Extension of the period to 36 h showed no further improvement.

Provision of supplementary energy source to unfed birds reduced excreta weight, endogenous nitrogen and FmE + UeE losses but it had no influence on the nitrogen corrected TME values. This procedure is considered to be of limited practical value.

GE content per g of excreta was negatively related to excreta weight, and FmE + UeE was positively correlated with endogenous nitrogen and was independent of body weight. There was no relationship between FmE + UeE and time period. Variation in energy excretion was influenced by bird numbers used for both fed and unfed groups and it was demonstrated that at least 24 birds per unfed group and 8 birds per fed group were necessary to achieve adequate precision in the TME estimate. There was no evidence to indicate the need of repeated measurement of FmE + UeE for each TME bioassay.

The precision and accuracy of the TME estimate was shown to be influenced by the correction of energy excretion to zero nitrogen balance. This procedure is

recommended for low-protein feedstuffs (i.e. cereal grains) but may underestimate the TME value for protein-rich feedstuffs.

The nitrogen correction factors derived from regression analysis relating energy to nitrogen excretions were 41.42 and 41.28 (kJ/g N) for unfed and fed birds, respectively.

Effective prediction equations were developed from data observed for 91 barley samples. The simple regression equation based on NDF (TMEn = 16.1399 - 0.1433NDF, R^2 = 0.615) is of advantage due to decreased cost. The physical measure, test weight, was not a sensitive predictor and hence has less practical value than NDF. Multiple regression equations including stepwise regression and the Mallows Cp technique gave good predictive precision, but the analysis cost and labour involvement are a significant consideration when comparing these results with that for the simple regression equation based on NDF.

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