A STUDY OF FACTORS AFFECTING NITROGEN BALANCE TRIALS AND THE EFFECT ON THE NITROGEN CORRECTED TRUE METABOLIZABLE ENERGY ASSAY

By

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ABSTRACT

Seven experiments were designed to investigate some of the errors typically associated with nitrogen balance trials which may affect the TMEn assay. The incidence and magnitude of these errors in a standard TMEn assay were evaluated. The effect of these errors on the TME and TMEn value of feedstuffs were determined. In three of the seven experiments, chemical preservatives were added to excreta samples to prevent any possible errors.

In experiment 1, the TME and TMEn values of several different cultivars of corn and barley were determined using SCWL cockerels. Only the TME values of corn were similar to literature values. This data illustrates the potential variability of results obtained in a standard TME assay due primarily to the errors associated with nitrogen balance trials.

In experiment 1, the contribution of uric acid nitrogen to total excreta nitrogen was determined. Over 80% of the total nitrogen was in the form of uric acid nitrogen, however, uric acid was not the sole excretory nitrogen product. As a result, the uric acid based nitrogen correction factor proposed by Hill and Anderson (1958) may be inappropriate for the TMEn assay.

In experiment 2, the proportion of the excreta nitrogen containing compounds was determined at various feed intake levels. Data of experiment 2 illustrated that the proportion of nitrogen containing compounds, which make up the nitrogen correction factor, vary with the magnitude of negative nitrogen balance of the assay birds. These results suggest that the nitrogen correction factor developed by Titus (1956), using full fed

birds, may be inappropriate for the force fed and unfed birds of the TMEn assay.

Experiments 3 and 4 investigated the effect of duration of excreta collection period and freeze drying procedure on excreta nitrogen containing compounds. Extension of the duration of the excreta collection period produced significant losses of urea nitrogen (up to 87.5%) and significant increases in ammonia nitrogen (up to 194.4%) presumably due to bacterial activity. Freeze drying of excreta produced significant losses of ammonia nitrogen (up to 65.7%) due to volitilization. These errors caused major shifts in the proportion of excreta nitrogen compounds which in turn altered the TMEn nitrogen correction factor and nitrogen balance.

Two chemical preservatives were investigated as potential agents to prevent the errors of nitrogen balance trials. Mercuric chloride (0.3 per cent w/v) in glacial acetic acid proved ineffective (experiment 5). Boric acid powder plus (0.3 per cent w/w) mercuric chloride was shown to prevent the errors of nitrogen balance trials (experiment 6). Therefore, the boric acid plus (0.3 per cent w/w) mercuric chloride preservative was incorporated into a standard TMEn assay (experiment 7).

Experimental design of experiment 2 and 7 were identical except for the addition of a preservative to excreta samples in experiment 7. Adding preservatives to excreta of a TMEn assay prevented the errors associated with duration of collection period and freeze drying. The nitrogen correction factors determined in experiment 7 for the force fed and unfed birds were 35.10 kJ/g RN and 37.10 kJ/g RN, respectively. Use of these correction factors resulted in an 8.9% increase in the TMEn value of cracked wheat determined in the assay with added preservative (experiment 7) over TMEn values subject to the errors of nitrogen balance trials (experiment 2).

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Completion of this work would not have been possible without the help of so very many friends. I am indebted to you all.

I extend a special acknowledgement to my major advisor, Dr. L. D. Campbell, for his advice and guidance throughout the course of this study.

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This thesis is dedicated to my parents, Gerald and Dora Humphreys, without their love and encouragement I could not achieve.

This thesis is also dedicated to my wife, Barb. It is only with her love, help and moral support that I am able to complete this work.

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LIST OF ABBREVIATIONS

ADE	apparent digestible energy
AME	apparent metabolizable energy
AMEn	nitrogen corrected apparent metabolizable energy
BE	bioavailable energy
°C	degree celsius
cm	centimeter
DE	digestible energy
E	gross energy
FE	fecal energy
FiE	feed energy of indigestible feed residues
FmE	feed energy of metabolic by products
FN	fecal nitrogen
g	gram
GE	gaseous energy
HE	heat increment
HdE	heat of digestion and absorption
HfE	heat of fermentation
HiE	heat produced by food consumption
HrE	heat of product formation
HwE	heat of waste formation and excretion
HPLC	high performance liquid chromatography
I	feed intake
ICU	international chick unit
IE	intake energy

IU	international unit
k	nitrogen correction factor
kg	kilogram
kJ	kilojoule
ME	metabolizable energy
MEn	nitrogen corrected metabolizable energy
Ml	milliliter
MJ	megajoule
N	normal
Ni	nitrogen intake
Ne	nitrogen excretion
NE	net energy
NEm	net energy for maintenance
NEp	net energy for production
PE	productive energy
RN	retained nitrogen
RPM	revolutions for minute
SCWL	single comb white leghorn
SI	international system of units
TAAA	true available amino acids
TAL	true available liquids
TAM	true available minerals
TDE	true digestible energy
TME	true metabolizable energy
TMEn	nitrogen corrected true metabolizable energy
UE	urinary energy
UeE	urinary energy composed of endogenous energy
UiE	urinary energy composed of indigestible feed residue

UN	urinary nitrogen
w/w	weight per weight
w/v	weight per volume

INTRODUCTION

Efficient commercial poultry production demands control of input costs. Presently, feed accounts for 55 - 60% of the total cost of production. Bioavailable energy (BE) represents about 70% of the total feed cost, or in other words, BE accounts for approximately 40% of the total cost of poultry production. It has therefore been suggested that any reduction of BE input costs, through more accurate prediction of BE values, will have a large effect on increasing production efficiency. Increasing the accuracy of BE values will also improve diet formulation. In practical poultry diet formulation, the nutritionist must consider all nutrient requirements at specific levels in order to achieve maximal production. Many of these nutrients are related to, or regulated by, the BE of the diet due to a direct effect of BE on feed consumption (Sibbald 1982a).

The correlation of feed intake to energy balance is well documented (Hervey 1969; Anderson 1979). Generally birds will eat to satisfy energy requirements when fed free choice, depending on rate and type of production, environmental temperature, body size and activity (Sibbald 1980a). As a result, intake of all other nutrients (except water) is dependent upon specific ratio of nutrient to energy intake. When eating to satisfy energy requirements, birds will eat more of a low energy diet and less of a high energy diet (NRC 1977). Situations where this does not occur have been reported. Sibbald et al. (1960) showed that low energy feeds may not be consumed at a high enough level due to restricted physical capacity of the crop and digestive tract. Davidson et al. (1961) also suggested that unpalatible feeds will not be consumed regardless of energy content.

Nevertheless, poultry eat to satisfy their energy requirement. Therefore it is preferable to express nutrient requirement on a per unit of energy basis, as compared to a per unit weight basis to ensure proper nutrient intake. This illustrates the need for proper evaluation of the birds energy requirement. Energy requirement is defined by the National Academy of Science as, "That amount of available energy that will provide for growth or egg production at a high enough level to permit maximal economic return for the production unit."[1] However, the problem at hand is to determine available energy.

In 1976, Sibbald developed a direct bioassay to determine true metabolizable energy. The assay, however, has been subject to extensive criticism. Recently, a nitrogen correction factor has been added to the procedure to increase the accuracy of the bioassay. The objective of this thesis is to evaluate the nitrogen corrected true metabolizable energy assay.

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[1] NRC 1977. Nutrient requirements of Poultry, 7th ed., National Academy of Science, Washington, D.C.

LITERATURE REVIEW

I. Distribution of Ingested Energy

Not all of the gross energy contained in a feedstuff is available to the bird. As a result, a bioassay to determine the bioavailability of energy is necessary. Several bioassay procedures have been developed, all of which are based on the concept of partitioned ingested feed energy as illustrated in Figure 1. Discrepancies exist between assays regarding nomenclature, thereby making data interpretation and comparisons difficult (Pesti and Edwards 1983). The National Academy of Science realized this problem and set up a subcommittee under the Committee on Animal Nutrition to standardize terminology for the description of energy utilization by animals. The system that was developed describes in detail the distribution of ingested energy and has become adpoted internationally (NRC 1981).

A. Terminology

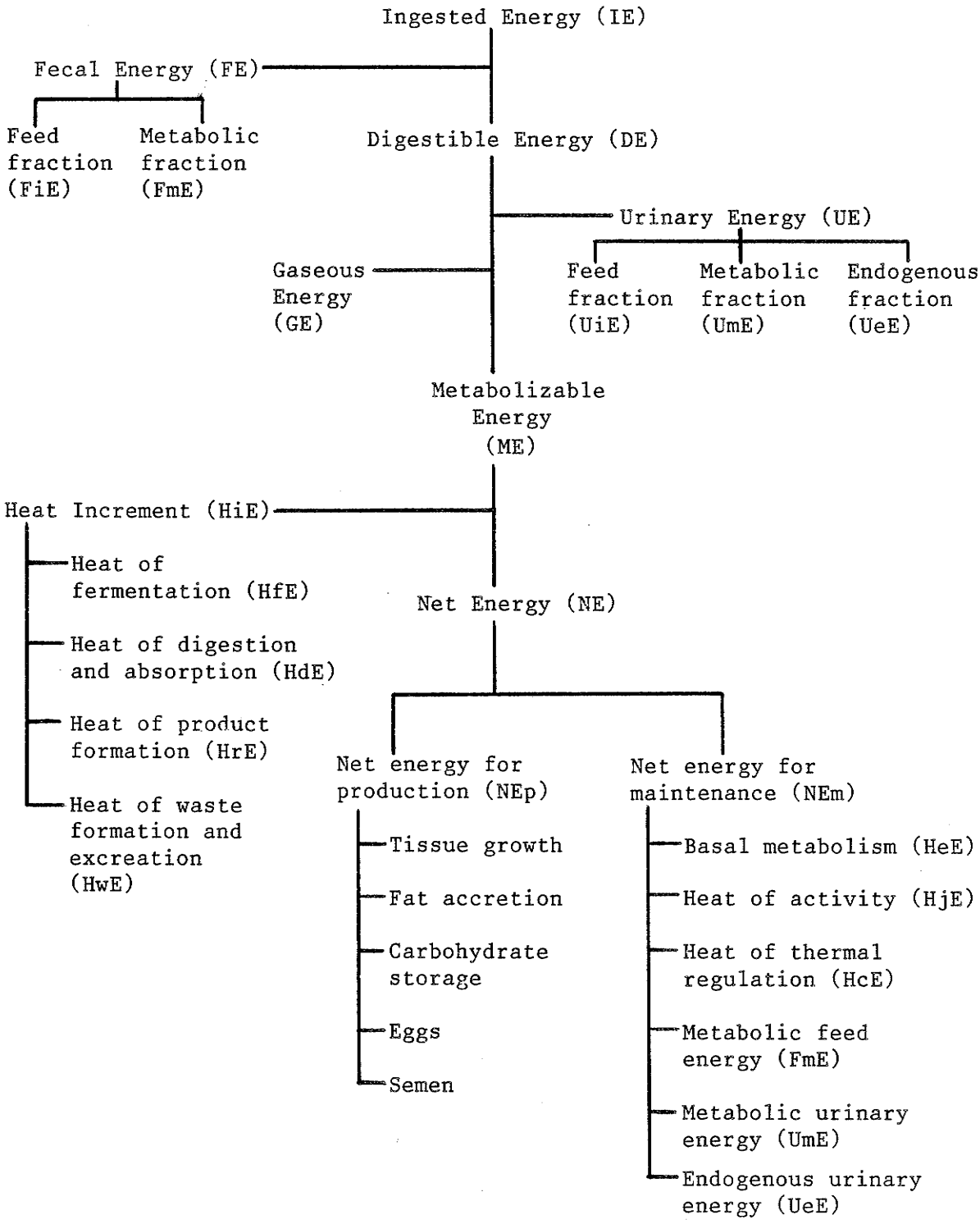
GROSS ENERGY (E) is the heat of combustion of a material as determined by oxygen bomb calorimetry (NRC 1981).

FEED INTAKE (I) is the weight of feed consumed (NRC 1981).

INTAKE OF FEED ENERGY (IE) is the gross energy of the feed times weight of feed consumed (NRC 1981).

FECAL ENERGY (FE) is the gross energy of the feces times the weight of the feces (NRC 1981). FE can be divided into two compounds; a feed fraction (FiE) composed of indigestible feed residues, and a metabolic fraction (FmE) composed of bile, digestive fluids and sloughed intestinal mucosal cells. The metabolic fraction (FmE) may be classified as fecal

Figure 1. Distribution of Ingested Energy



(adapted from Sibbald 1982a)

endogenous energy (Sibbald 1980a).

DIGESTIBLE ENERGY (DE) is characterized by two different classifications. Apparent digestible energy (ADE) is the energy of the feed consumed minus the energy in the feces: ADE = IE - FE (NRC 1981). The term apparent is used because the fecal energy contains both the feed fraction (FiE) and the metabolic fraction (FmE), ie: ADE = IE - (FiE + FmE) (Sibbald 1982a). True digestible energy (TDE) is the energy of the feed consumed minus the fecal energy feed fraction: TDE = IE - FiE. FmE is considered a body maintenance cost (see Figure 1) and therefore should not be charged against the feed. By definition, ADE will always be less than TDE or IE except when completely digestible feedstuffs such as dextrose or corn oil are fed. In this case TDE = IE because FiE of dextrose or corn oil is zero (Sibbald 1980a).

URINARY ENERGY (UE) is the gross energy of the urine (NRC 1981). UE can be divided into three energy fractions: a feed fraction (UiE) made up from the nonutilized feed compounds absorbed from the alimentary tract and excreted in the urine, a metabolic fraction (UmE) composed of the nitrogenous end products of nutrient utilization, and an endogenous fraction (UeE) consisting of the nitrogenous end products of tissue catabolism (Sibbald 1980a).

GASEOUS ENERGY (GE) is the energy lost in the form of combustible gases produced by microbial fermentation in the intestinal tract (NRC 1981). In the ruminant animal, GE losses are significant and are considered as a major metabolic loss. In the avian species, GE is formed in the lumen of the gut and is considered as a digestive loss. As such, GE losses in poultry are assumed to be negligable and are ignored (Sibbald 1982a).

METABOLIZABLE ENERGY (ME), like DE is characterized by two classifications. Apparent metabolizable energy (AME) is the energy of feed con-

sumed minus the energy lost in the feces and urine: AME = IE - FE - UE (NRC 1981). True metabolizable energy (TME) is the energy of the feed consumed minus the energy of food origin: TME = IE - FiE - UiE (Sibbald 1980a).

HEAT INCREMENT (HiE) is energy in the form of heat which is produced following the consumption of food by an animal. HiE is made up by the following heat sources: Heat of fermentation (HfE), the heat produced in the digestive tract as a result of microbial fermentation; Heat of digestion and absorption (HdE), the heat produced by digestive enzymes and the heat produced by digesta moving along the digestive tract; Heat of product formation (HrE), the heat produced in the metabolic pathways of the absorbed metabolites; Heat of waste formation and excretion (HwE), the heat produced during the synthesis and excretion of waste products. Heat increment is only useful as an energy form to maintain body temperature during cold stress (NRC 1981).

NET ENERGY (NE) is the remaining intake energy fraction which is available to the bird for maintenance (NEm) and production (NEp): NE = TME - HI (Sibbald 1980a).

RETAINED NITROGEN (RN) is the weight of nitrogen consumed as feed minus the weight of nitrogen excreted in the feces and urine. The RN value will be positive for birds using dietary nitrogen to synthesize new tissue, and negative for starved and feed restricted birds which must catabolize body tissue to meet energy requirements (Sibbald and Wolynetz 1984a). The RN value allows for a direct comparison between different birds by correcting to zero nitrogen balance. A nitrogen correction factor adjusts for total nitrogen gained or lost from body tissue: MEn = ME - (k x RN)(NRC 1981). The value of the correction factor (k) is controversial. Hill and Anderson (1958) initially proposed a value of 34.39 kJ per g of RN, which

represents the energy equivalent of uric acid per gram of nitrogen excreted. Titus (1956) later proposed a revised value of 36.53 kJ per g of RN. This value is based on the fact that catabolism of retained nitrogen produces urea, ammonia, creatinine and other nitrogen compounds in addition to uric acid, whereas the value proposed by Hill and Anderson (1958) assumes uric acid is the only end product of tissue catabolism.

B. Energy Units

Recently Canada has adopted the International System of Units (SI). In doing so, the Joule has become the energy unit in Canada (Sibbald and Wolynetz 1984a). This shift from calories to Joules has been highly criticised (Halloran 1983) because the Joule is a measure of mechanical work, the calorie is a measure of biological work. As a result, the SI system is attempting to relate kinetic energy to metabolism, which is argued as erroneous. Nevertheless, the Joule has become the accepted metric measure of energy (NRC 1981). Halloran (1983) suggests that energy units should be reported in both Joules and calories to prevent confusion and aid comparisons. Energy units can be converted as follows: 1 Joule = 0.239 calories; 1 calorie = 4.184 Joules. (Sibbald and Wolynetz 1984a).

II. Bioavailable Energy Assays

In an attempt to determine bioavailable energy, several assay procedures have been developed. In order for these assays to be useful to the industry, speed and ease of assay procedure are essential. However, before an assay can be accepted as a valid measure of bioavailable energy it must be shown to be reliable.

As a means to evaluate any particular energy assay, three conditions

must be observed. The assay must be accurately reproducable, the energy values of individual feedstuffs analyzed must be additive, and the assigned energetic value of diets must correspond to production requirements (Dale and Fuller 1981). Only when these conditions are met can an assay be considered as an acceptable measure of bioavailable energy. Using the above constraints, it is possible to critically evaluate the various energy assays which have been developed and determine the validity of such assays as an accurate measure of bioavailable energy for poultry.

A. Digestible Energy

By definition, digestible energy is the energy of the feed consumed, minus the energy in the feces alone (ADE = IE - FE)(NRC 1981). Poultry excrete both feces and urine as a single excretum via a common cloaca, yet, various attempts have been made to measure ADE and TDE values of feedstuffs. The assay procedures which have been developed are dificult as they require the separation of feces and urine. This problem has been approached in several ways.

The preferred method is surgical modification of the mature chicken by exteriorization of the colon, or colostomy (Paulson 1969). The use of colostomized birds is questionable as there is no proof that surgically modified birds behave in the same manner as normal birds. Emmanuel and Howard (1978) suggest that metabolism of colostomized birds is different from that of normal birds. Van Kampen (1981) also observed differences in water and food intake between colostimized and normal birds. A nonsurgical technique for collecting urine has been reported (Coulson and Hughes 1930), however, the method has proven to be unsatisfactory, particularly for extended collection periods.

These problems have led to the development of techniques for seper-

ating feces and urine after excretion. The urine content of a mixed excreta is determined by chemical measurements of uric acid. This procedure assumes that there is a direct relationship between uric acid and urinary energy, and that all fecal nitrogen is in the form of precipitable proteins (Sibbald 1982). These assumptions have been shown to be incorrect (Warring and Shannon 1969) and as a result the assay procedure is considered invalid.

Another technique was developed by Sibbald et al. (1962) which involves feeding a test ingredient with an indicator such as chromium sesquioxide (Cr_2O3). Birds are then killed and intestinal contents are collected proximal to the cloaca and distal to the ceca. A major criticism of the technique is that retro peristalsis of urine from the cloaca can contaminate intestinal and cecal (ie. fecal) samples (Sibbald 1982a).

Failure to seperate the urine and feces in the mixed excreta of poultry, by either chemical or physical methods, invalidates the digestible energy assay as a measure of bioavailable energy for poultry.

B. Productive Energy

Prior to 1960, the poultry industry used the productive energy system (PE) as developed by Fraps (1946) to determine the available energy in feedstuffs. The PE system is a comparative slaughter technique used to estimate NE by measuring the energy stored as fat and protein in growing or fattening birds. The assay procedure is difficult because it involves precise measurement of feed intake, weight change and changes in carcass composition. PE values have been proven to be unreliable and not necessarily additive (Davidson et al.1957; Hill and Anderson 1958), and therefore a poor measure of BE.

C. Apparent Metabolizable Energy

Hill and Anderson (1958) compared metabolizable and productive energy determinations using chicks, and showed metabolizable energy to be a more reliable measure of available energy. As a result, since 1960 poultry nutritionists have accepted AME as the preferred measure of available energy. However, there is much disagreement as to how AME should be measured. Over the years, several assay procedures have been proposed. AME assays that have been developed can be classified into two categories, indirect and direct assays.

i) Indirect Assay

Indirect assays use equations to predict ME from physical and chemical parameters such as gross energy, crude protein, crude fiber, starch, ether extract, ash, soluble sugars, tannins and bulk density (Sibbald 1980a). Indirect assays are particularly attractive because BE values can be obtained quickly and at low cost with only limited laboratory facilities.

Historically the first work to determine ME by indirect assay was reported by Fraps et al. (1940). They showed that the heat of combustion of poultry excreta could be calculated by proximate analysis of the excreta. More importantly, they also showed that the AME value of a feedstuff could be determined by proximate analysis of the feed. Titus (1955) later refined this work and calculated AME based upon digestibility coefficients and protein, fat, crude fiber, ash, water and nitrogen-free extract content of feed and excreta (Mehring 1983, personal communication).

After Titus (1955), several authors have modified and developed equations to predict BE. The subject was reviewed by Sibbald (1982a). The equations developed use coefficients to predict BE values from proximate analysis of the feedstuff. The coefficients are determined by esti-

mates of BE values of specific components, or by regression analysis. Many of the equations proposed, do not yield satisfactory results when tested with independant data. Part of the reason may be variability among analytical techniques (Sibbald 1982a). More importantly, prediction equations assume that all proteins, carbohydrates or fats are equally digestible, thereby yielding erroreous results (Sibbald 1980a).

Another method of indirect assay is based on the measurement of comparative growth. In this procedure, growing chicks are fed a basal diet supplemented with a test material. Observed growth responses are compared to the growth of chicks fed the same basal diet supplem nted with gra ed levels of a test material of known BE content (Sibbald 1980a). A modification of this procedure is described by Squibb (1971). The results obtained from these growth assays are highly variable and therefore a poor measure of AME. On the other hand, these assays have proven to be of use to identify toxic and unpalitable test materials (Sibbald 1982a).

The nutritional value of cereal grains is thought to increase with density. As a result, attempts have been made to predict the BE of cereal from bulk density. Lochart et al. (1961) showed the AME value of oats to increase with bulk density. This was later confirmed by Sibbald and Price 1977). For barley, AME values did not increase with density (Sibbald and Price 1976a) but TME values did (Sibbald and Price 1976b). For wheat, no significant relationship between energy and density could be found (Sibbald and Price 1976a). It is concluded that bulk density is not a valid prediction of BE.

ii) Direct Assays

Direct assays are balance experiments which measure the difference between energy intake as feed, and energy output as excreta (Sibbald 1982a). The general assay procedure involves feeding a diet to birds for a speci-

fied acclimatization period, after which feed intake and excreta output are measured for a period of three or four days. The AME value is calculated by measuring the difference between the gross energy of feed consumed and the gross energy of excreta output. If nitrogen retention (RN) is measured, the AME value can be corrected to zero nitrogen balance (AMEn)(Sibbald 1980a). In order to determine AME by direct assay, precise measurement of feed intake and excreta output is essential. This is achieved by total collection method or by inclusion of an indicator to the diet (Vohra 1972).

The total collection method requires measurement of feed intake, excreta output and energy per unit weight of sample. AME values are calculated according to Fraps et al. (1940): AME = IE - (FE - UE). The total collection method assumes that total excreta output during a specific time period corresponds to total feed intake over that same time period. This assumption may not be valid due to the diurnal variations in rates of feed intake and excretion, but it is generally assumed that for collection periods of three or more days, these errors will not be significant (Sibbald 1982a). The measurement of total feed consumption and total excreta output is difficult. Feed is often spilled and excreta is often contaminated with feathers, scales and down, thereby complicating quantitative measurements (Sibbald 1982a). To prevent changes of moisture content over time, samples have been freeze dried or over dried at a variety of temperatures. The lack of standardization of assay procedure has contributed to the variability of AME data.

To aviod the problems of total collection, inert indicators may be added to the feed. To determine AME, the energy and amount of feed and excreta is measured. AME is then calculated according to Hill and Anderson (1958):

$$\text{AME/g of feed} = \frac{\text{E/g of feed} - \text{indicator/g of feed}}{\text{indicator/g of excreta}} \times \text{E/g of excreta}$$

The use of indicators presents various problems. The indicator must be analyzed in both feed and excreta. The need for additional analytical work increases the possibility of error in the BE value. Also, indicators are assumed to be distributed uniformly throughout the feed and excreta, to have the same rate of passage as other feed ingredients and to be unabsorbed or unaltered along the alimentary tract. These assumptions have been shown to be incorrect (Sibbald 1982a).

A large variety of indicators have been used in AME studies. These include iron oxide (Berdheim 1929); silica (Gallup 1929); barium sulphate (Whiteson et al. 1943); lignin (Kane et al. 1950); crude fibre (Almquist and Halloron 1971); polyethylene (Roudybush et al. 1974) and acid insoluble ash (Vogtmann et al. 1975). The most common indicator used in AME studies is chromium sesquioxide (Cr_2O_3)(Sibbald 1982a), but Cr_2O_3 is not problem free. Vohra and Kratzen (1967) showed that Cr_2O_3 is not completely inert and may be absorbed along the digestive tract. Vohra (1972) suggests other problems of Cr_2O_3 are difficulties in chemical analysis of the indicator in the feed. Cr_2O_3 has also been shown to separate out of excreta samples during grinding.

Total collection and indicator methods have both been used extensively to determine AME values. Total collection is relatively simple and easily duplicated between laboratories. The use of indicators solves the sample collection problems of total collection, but the need for additional analytical work increases the possibility of error. In general, the preference for the use of total collection or indicator method is dependent upon individual laboratories (Sibbald 1982a)

There are numerous variations of the basic AME assay procedure. The test material may be fed as the sole diet (McIntosh et al. 1962; Lochart

et al. 1967). This is acceptable only if the test material is palitable, non toxic and as a sole diet will not cause severe nutritional deficiencies (Sibbald 1980a). Sibbald et al. (1962) prevented deficiencies by adding a small quantity of a vitamin:mineral supplement to the test material. As an alternative assay procedure, the test material may be substituted for an ingredient of known AME value in a reference diet which is then assayed for BE. Hill et al. (1960) used glucose as the ingredient for substitution by the test material, whereas Potter et al. (1960) used alphacellulose. Hill and Anderson (1958) used a semipurified reference diet which contained 44.1% glucose. The above assay procedures were criticised because of the possibility of nutrient interactions and deficiencies. This led to the use of a reference diet composed of practical ingredients (Sibbald et al. 1960). This assay was further modified (Sibbald and Slinger 1963) by preparing graded levels of basal plus vitamin: mineral supplements as a reference diet to accommodate various levels of substitutions with a test material. In this manner, regardless of the substitution level, a constant level of basal and vitamin:mineral ingredients were consumed by the birds. March and Biely (1973) proposed an assay which incorporated a 24 hour starvation period before and after feeding a reference diet or reference diet:test material mixture. This assay tended to underestimate AME since FmE and UeE were measured and changed against the feed (Sibbald 1982a). More recently, Farrell (1978) developed an AME assay based on the TME assay developed by Sibbald (1976a). In the assay adult roosters are trained to eat their daily feed intake in 1 hour. A basal diet or basal diet:test material (50:50) is offered in the form of pellets. Feed intake is measured and excreta is collected quantitatively for a subsequent period of 24 hours. This period was later extended to 32 hours (Farrell 1980).

Several variations of the AME bioassay exist. Assays that have been developed have produced useful data, but the AME and AMEn values are open to criticism because there is a lack of standardization among assay procedures. Assay variables which have been shown to affect BE values are species, breed, strain, sex and age of assay birds. Basal diet composition, physical form, inclusion rate of test material and period of diet acclimatization are also important variables. The choice of total collection or indicator methods adds additional variation between assay procedures (Sibbald 1982a). This lack of standardization has caused considerable variation in AME data (Sibbald 1976a). As a result, validity of the AME assay has been questioned.

D. True Metabolizable Energy

i) Development

Guillaume and Summers (1970) first hypothesized that the variability of AME values was the result of differences in feed intake. This was later confirmed by Sibbald (1975) who established that a curvilinear relationship existed between the AME value of wheat and feed intake (Figure 2). It was postulated that the reason for this is that the fecal metabolic energy (FmE) and urinary endogenous energy (UeE), which are assumed to be constant among birds in the same assay, are subtracted from IE by definition of AME. As feed intake energy decreases, FmE and UeE losses become proportionally greater than IE and result in negative AME values. Sibbald (1975) further established that a linear relationship exists between feed intake and excreta gross energy (Figure 3). The slope of the line gives an estimate of the feed fraction of excreta energy (FiE + UiE). The Y intercept is always positive and gives an estimate of (FmE + UiE). This work led to the development of a bioassay to determine true metabolizable

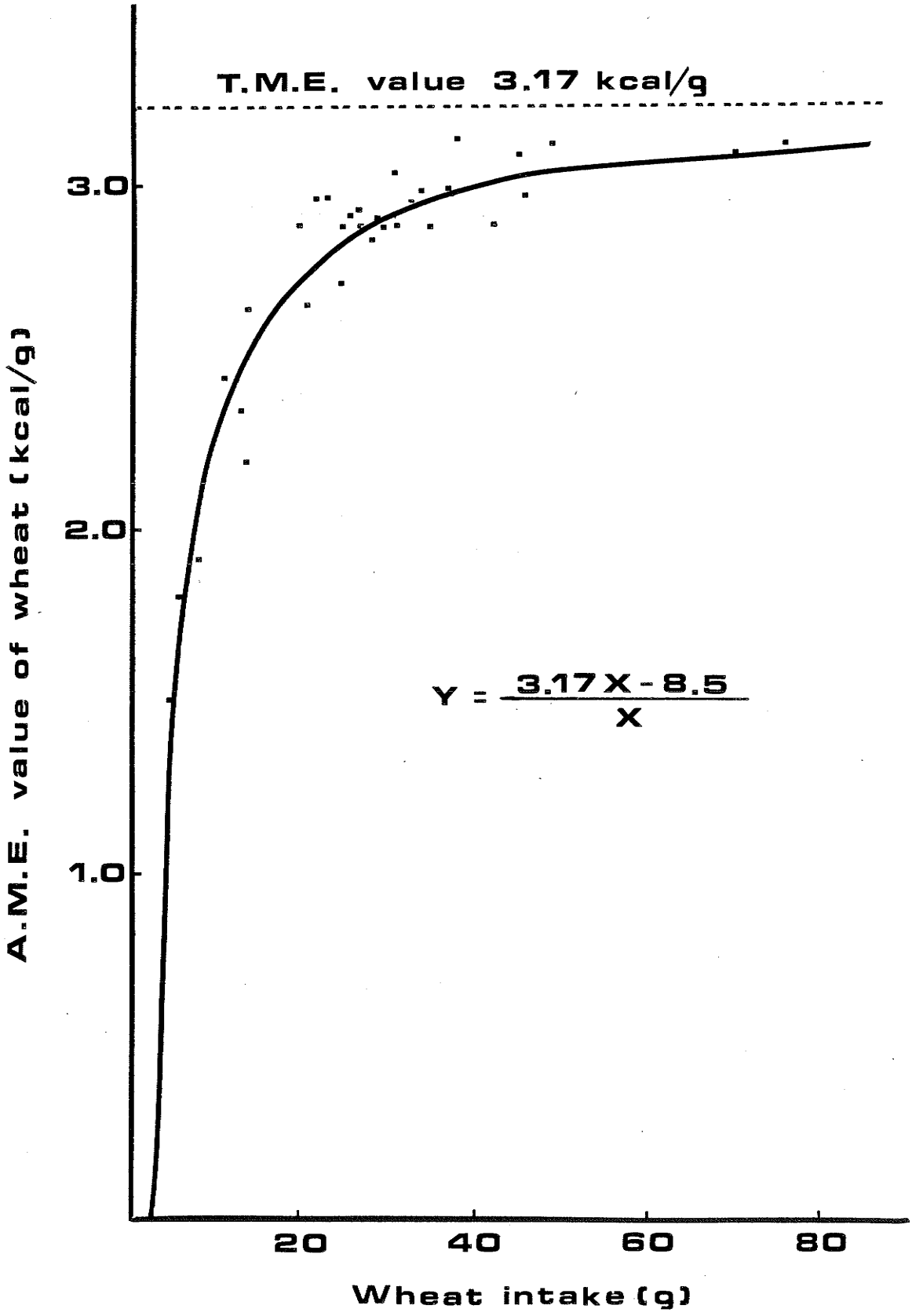


Figure 2. The effect of level of intake on the AME value of wheat.

(from Sibbald 1980a).

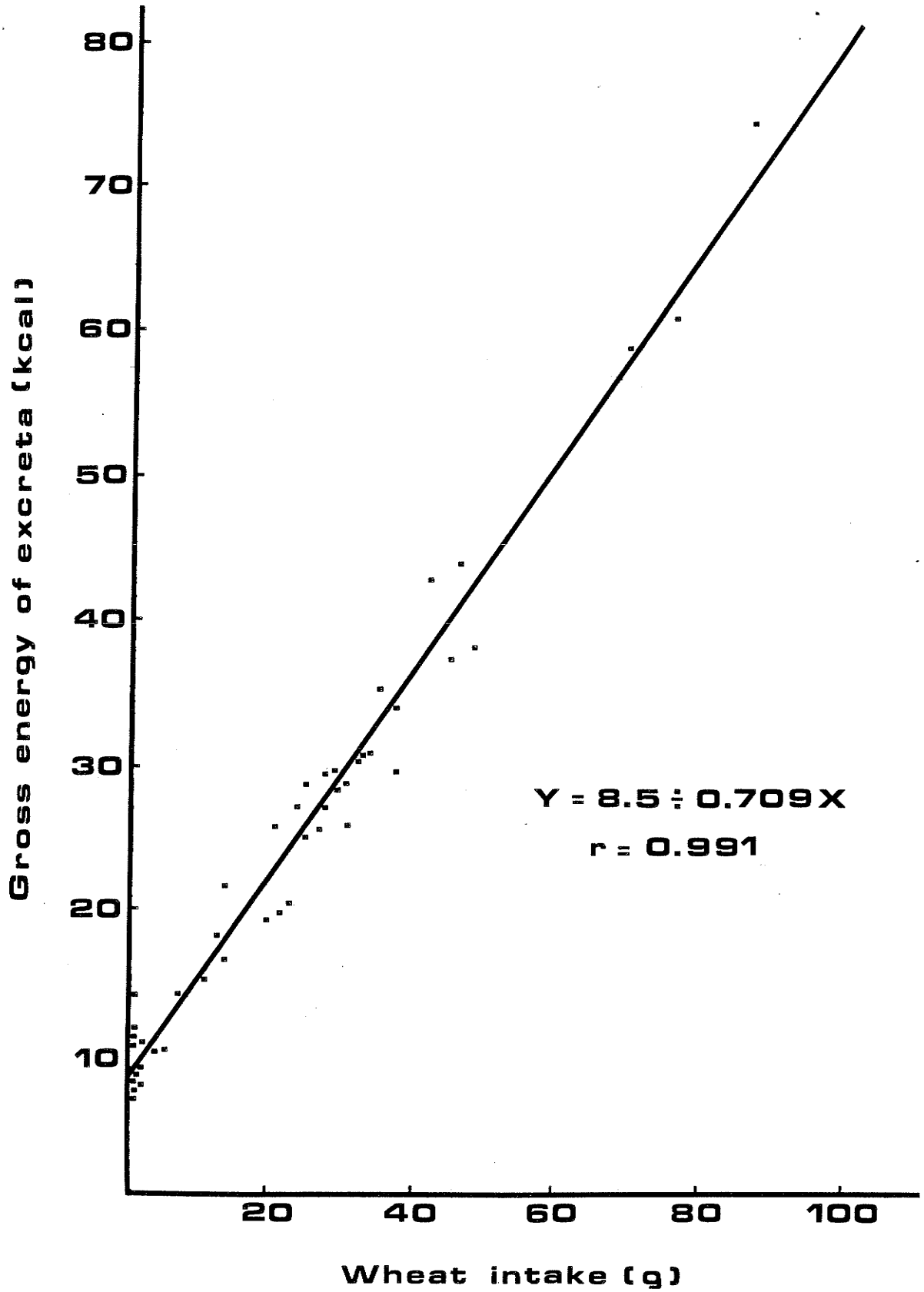


Figure 3. The relationship between wheat consumption and gross energy voided as excreta.

(from Sibbald 1980a).

energy (TME)(Sibbald 1976a).

Harris (1966) first proposed the true metabolizable energy system. He suggested that it is incorrect to include energy losses from the body (FmE + UeE) as part of the energy lost as feed origin (FE + UE). In order to estimate BE, a correction for endogenous losses must be made. Sibbald (1976a) developed Harris' TME system into a working model.

ii) Assumptions

The bioassay for TME is based on two assumptions: 1) in birds which have been previously fasted, there is a linear relationship between the energy placed in the crop as feed (IE) and the energy lost as excreta (FE + UE); 2) the Y intercept of the regression line gives a valid estimate of endogenous energy loss (FmE + UeE)(Sibbald 1981b). It is important to note that Sibbald (1982a) admits neither assumption can be proven, but must be accepted to validate the TME assay. Several authors (Sibbald 1975, 1976a; Tenesaca and Sell 1979; Shires et al. 1980) have shown that a linear relationship between IE and FE + UE does exist. Sibbald (1981a) demonstrated that a nonlinear relationship will occur if the excreta collection period is not adequate. The second assumption has become a major point of criticism of the TME assay, and as such will be covered in greater detail later. For the present, the assumption will be considered to be correct. The assumption implies that for the regression lines of all test materials, there is a single Y intercept at zero feed intake, which is a measure of FmE + UeE. Therefore, to measure FmE + UeE directly, the FE + UE of unfed birds is equivalant to the FmE + UeE of birds fed the test material.

iii) Assay Procedure

The experimental procedure of the TME bioassay is described in detail by Sibbald (1983). In general, adult single comb white leghorn (SCWL) cockerels are fasted 24 hours prior to feeding a test material to ensure that all feed residues have been cleared from the alimentary tract. A bird is then selected, weighed and a precise amount of feed is placed in the crop by a force feeding procedure. After feeding, the bird is returned to a wire cage, an excreta collection tray is placed under the cage and the time is recorded. Water is available ad libitum. The process is repeated until the required number of similar birds for each test material has been fed. Within each replication, one bird remains unfed to give an estimate of FmE + UeE. After a fixed period of time, excreta is collected quantitatively, frozen, freeze dried or oven dried, equilibrated with atmospheric moisture, weighed, ground, mixed and assayed for gross energy and total nitrogen. The TME and TMEn values are calculated:

$$TME = IE - (FE + UE) - (FmE + UeE)$$

$$TMEn = IE - ((FE + UE) + (RN)k) - ((FmE + UeE) + (RN)k)$$

or

$$= TME - (k \times RN)$$

iv) Assay Bird

The preferred TME assay bird is a dubbed adult SCWL cockerel, but other types of birds have been used. Chicks are not recommended since they have a limited feed capacity (Sibbald 1983). Sibbald (1976c) compared the TME values of several test materials between adult roosters, laying hens, broiler hens and turkey hens. TME values obtained were not affected by the type of assay bird. However, turkey hens were found to obtain more TME from soybean meal than did adult roosters, laying hens

or broiler hens. The reason for this was thought to be that the cecal microflora of the turkey are able to ferment the indigestible carbohydrates of soybean meal, unlike the microflora of the chicken. This was later confirmed by Parsons and Potter (1980) and Dale and Fuller (1980). Laying hens are difficult to use in the TME assays because the fasting period prior to force feeding results in the production of shell-less eggs which break and contaminate the excreta (Sibbald 1983).

Muztar et al. (1977) compared the TME value of freshwater plants between ducks and roosters, and found differences in TME values were due to incomplete clearance of feed residues during the collection period. Shen and Dean (1982) determined the TME value of corn and soybean meal using ducks and could find no difference to values obtained with roosters. Storey and Allen (1981a, 1981b, 1982) determined the TME values of feedstuffs using Embden Geese, but made no comparisons with adult roosters.

Genotypic comparisons of TME are limited. Sibbald (1976c) found no difference between meat-type and egg-type hens. Dale and Fuller (1980) compared leghorn cockerels and 6-week broilers and showed no difference in TME values.

A study to determine the influence of age on TME values showed no effect (Sibbald 1976c). This was later confirmed by Sibbald (1978b), Muztar and Slinger (1979a), Dale and Fuller (1980) and Shires et al. (1980).

v) Maintenance Diet

Birds used in a TME assay must be maintained on the same diet. Composition of the diet is not important, provided the nutrient requirements of the bird are satisfied (Sibbald 1983). Shires et al. (1979) confirmed this by showing no effect of previous diet on the TME value of corn.

Generally, the maintenance diet is a 15% protein laying hen ration

(Sibbald 1983) but may be a 17% protein laying hen ration (Dale and Fuller 1983, personal communication). Guillaume and Summers (1970) determined maintenance energy requirement for adult roosters to be 489.5 kJ ME per Kg body weight per day. The maintenance diet is fed ad libitum and birds eat according to their energy balance.

The 24 hour fasting period prior to force feeding should be adequate to clear the alimentary tract of maintenance diet feed residues, but a longer period may be required depending upon the amount of indigestible material present (Sibbald 1983).

vi) Force Feeding Technique

Force feeding (or precision feeding) is necessary to ensure a known amount of feed is consumed by the bird at a specific time. The procedure prevents the problems associated with ad libitum feed intake such as, feed spillage, selective feeding and variations in feed intake among birds (Sibbald 1983). These problems represent some of the fundamental drawbacks of the AME assay.

Force feeding is accomplished by inserting a tube from the beak, via the esophagus into the crop. A funnel is attached to the end of the tube to facilitate pouring or placing feed into the tube. The feed is then pushed into the crop with a rod and the tube is subsequently removed. Control of the bird is essential to a successful feeding (Sibbald 1976a). Force feeding equipment is generally constructed of glass or stainless steel. Plastic may be used, but the electrostatic properties of plastic cause adherence of feed to the equipment (Sibbald 1983). Recently, Teeter et al. (1984) developed a force feeding gun to decrease the time required to force feed birds. This procedure has produced good results, but for for proper gun operation, all test materials must be fed in combination

with water. The optimal ratio for test material to water is achieved when the dry matter content of the test material is less than 50%.

Animal welfare groups have developed strong opposition to the TME force feeding technique (Farrell 1981). Wehner and Harrold (1982a) studied the effect of force feeding stress on adult cockerels. The stressful condition tended to be higher when feeding dry test materials as compared to slurry or paste-feeding. Fraser and Sibbald (1983) also studied the effect of force feeding. They concluded that stress was the result of an extended time period required to administer the feed, often associated with inexperience. Wehner and Harrold (1982b) suggested that post feeding stress is the result of feeding birds at an input level beyond the physical capacity of the crop.

vii) Feed Input

Sibbald (1975) established that AME values are directly affected by feed intake. As feed intake energy decreases, the constant endogenous energy loss becomes proportionally greater, and therefore depresses the AME value. To offset the effect of endogenous energy, Sibbald (1975) suggested that a feed intake of 60g or more is required. This level is in agreement with that proposed by Muztar and Slinger (1980a). Guillaume and Summers (1970) found the necessary level of feed intake to be only 45g.

Sibbald (1975) further established that the TME value of feedstuffs are not affected by feed intake. Sibbald (1976a) states that the level of feed input is of no importance to the TME value, but an optimal level of feed input does exist. This level varies with the nature of the feedstuff and size of the bird. In general, the optimal input level for most test materials is about 20 to 25 g, or about 1% of total body weight. This was supported by Sibbald (1977b) who evaluated feed input levels ranging

from 10 to 100g per bird. An input level of 25g was found to be satisfactory. Larger quantities may be fed, but the possibility of regurgitation increases. In addition, feeding large quantities of test material has proven to be stressful to the bird (Wehner and Harrold 1982a). Wehner and Harrold (1982b) suggested that the amount of test material fed to birds should be based on the crop volume per unit body weight of the lightest bird in the experimentsl group. This would prevent overfeeding of birds and therefore prevent any stressful conditions. More recently, Sibbald (1983) recommended the feeding of 30 to 40g of a test material to be satisfactory. It is important to note that the rate of passage of feed is directly affected by the feed input level. Therefore, length of the excreta collection period must be adjusted to feed input level to ensure complete clearance of all feed residues (Muztar and Slinger 1976b).

The TME assay has been heavily criticized for force feeding adult cockerels 20 to 40g of feed only once in a 72 hour assay period, which is far below minimum nutrient requirements (Farrell 1981). This criticism prompted development of a rapid method for AME determination (Farrell 1978) based on the TME assay (cited previously). To reduce the nutritional stress of a single force feeding, birds are trained to eat about 100g of a test material:basal diet mixture in 1 hour. Excreta is then collected quantitatively 24 hours later. The collection period was later extended to 32 hours (Farrell 1980). The assay is of limited use when evaluating a poorly accepted or unpalatable test materials. Also, as an AME assay, there is no correction for endogenous energy (Sibbald 1980a). An investigation by Muztar and Slinger (1980e) showed that when fasted roosters were offered a palatable, pelleted feed, the highest intake was 54.8g achieved in 1.5 hours. Birds could not consume intakes of 70 to 100g in 1 hour as suggested by Farrell (1980). As shown by Sibbald (1975), vari-

ations in voluntary feed intake produce highly variable AME data, and therefore the assay is not reliable (Schang and Hamilton 1982). Schang et al. (1982b) proposed a method of double force feeding to reduce the nutritional stress of a single force feeding but the method has several errors.

Physical form of the test material has proven to be of little significance to the TME value. Initially, pelleting of the feed was recommended (Sibbald 1976a), however, cold pelleting caused small increases in the TME value of some cereal grains (Sibbald 1976b). Steam pelleting was shown to have no effect on TME value (Sibbald 1977c). Presently, feeds are force fed as a mash type diet (Sibbald 1983) but may be fed as a slurry or paste (Wehner and Harrold 1982a). Grinding of the feed does not affect the TME value (Sibbald 1982b).

The test material is usually fed alone, but may be fed in combination with other test materials. As such, the additivity of TME values has been established (Sibbald 1977a, Dale and Fuller 1980). Evaluation of poorly accepted feeds such as fats and oils is facilitated by this procedure (Farrell 1981).

viii) Collection Method

Quantitative collection of excreta is an important aspect of the TME assay. Excreta is collected on trays which are placed under the cages of assay birds for a specific collection period. Trays are usually made of plastic and are larger than the bottom of the cage to prevent loss of excreta (Sibbald 1983). Terpstra and Janssen (1975) prevented the loss of excreta by restraining birds using neck-tethers, which caused excreta to drop only in a small area. Blakely (1963) developed a method to collect excreta quantitatively by surgically attaching a plastic bag to the cloaca

of turkeys. This procedure is similar to the method of collecting urine in colostomized birds (Paulson 1969). Hayes and Austic (1982) published a method to collect feces in adult roosters which is identical to the method developed by Blakely (1963).

Recently, Sibbald (1983) developed a nonsurgical method of attaching plastic bags to the cloaca for the collection of excreta in adult roosters. Birds are prepared prior to the assay by trimming the feathers surrounding the cloaca. Immediately after force feeding, plastic human colostomy bags are attached to the bird with a strong adhesive. The colostomy bag is securely centered over the cloaca and birds are returned to their cages. At the end of the collection period, the colostomy bag is removed, sealed, frozen and stored ready for processing. The use of colostomy bags ensures precise total collection of excreta which is free of scales, feathers and other contaminants.

ix) Duration of Collection Period

The TME assay uses the total collection method of excreta collection. The object of the collection period is to obtain a representative sample of excreta produced from the test material fed. Therefore, all feed residues from previous feed must have cleared the alimentary tract and the collection period must be sufficient to allow complete passage of test material feed residues (Sibbald 1980a). Length of the collection period for test materials is determined by the various factors which affect the passage of feed through the gut.

The rate of passage of digesta through the gut of birds is well documented (as reviewed by Warner 1981). Several factors influence the rate of passage, but diet has been shown to ahve the most profound effect. Diets with a small aprticle size and increased feed intake tend to have

increased rates of passage. Diets with a high fiber content and high specific gravity tend to decrease rate of passage. Conflicting results on the effect of feeding frequency have been reported, but the direct effect of feeding frequency on feed efficiency is well established. Other factors which affect rate of passage are temperature, gut microflora and water absorption. Given the TME assay as described by Sibbald (1976a), rate of passage of digesta may have a profound effect on TME values. The TME assay bird is unique because of the low feed input (20 to 40g) and the extended starvation period prior to, and after force feeding. The factors which affect rate of passage have been illustrated in the full fed bird, but these factors may not necessarily represent the conditions of the TME assay bird.

Sibbald (1979a) determined the rate of passage of several feedstuffs under standard TME assay procedures. The duration of starvation prior to force feeding affected rate of passage of the maintenance diet, but 24 hours were found to be satisfactory to clear the alimentary tract. When cereal grains were force fed 10, 20 or 30g, feed input affected the rate of passage, but all feed residue clearance was complete by 24 hours. Meat meal was shown to require 30 hours for complete clearance. These findings suggested that for some test materials, the excreta collection period needed to be extended beyond 24 hours. This was confirmed by Sibbald (1979c), and Muztar and Slinger (1979b) and Sibbald (1980c). Like Sibbald (1979a), Muztar and Slinger (1979b) showed that rate of passage is affected by feed input. They concluded that for input levels of 30g or greater, a collection period of 36 hours was required to clear the gut.

If the excreta collection period does not allow for complete passage of test material feed residues, the observed TME value is over estimated. An extension of the collection period results in a reduction of

the excreta collection period has a more significant effect on AME values (Muztar and Slinger 1980d). The reason for this is that during the initial portion of the collection period, FE + IE consist of both FiE + UiE and FmE + UeE. After complete clearance of the test material, only FmE + UeE is voided. For extended collection periods, FE + UE becomes larger and is charged against the feed, therefore decreasing the AME value. In the TME assay, after complete clearance of the gut, FiE + UiE remains constant. Total FmE + UeE will increase but is not charged against the feed and therefore does not affect the TME value.

Even though TME values are unaffected by extended collection periods, Salmon (1983) suggests that adoption of greatly extended collection periods is unnecessary. Such practise only extends the rest period required between assays. A rest period between assays is required to allow the birds to regain initial body weight (Sibbald 1978a). The duration of that period is dependant upon the length of the collection period (Salmon 1983). Sibbald (1978a) and Schang et al. (1982a) have suggested that a rest period of 12 days is satisfactory. However, Muztar and Slinger (1980b) found that after a 32 day rest period, only 63% of the birds had regained their initial body weight. Slinger and Muztar (1980) consider 4 weeks necessary to allow for complete body weight recovery.

Sibbald (1976a) initially proposed a 24 hour excreta collection period, but later this was extended, depending upon the test material fed (Sibbald 1979d). Kessler and Thomas (1981) proposed the adoption of a standard 48 hour collection period, which is presently considered appropriate for most test materials (Sibbald 1982a; 1983).

x) Drying of Excreta

Variations in moisture content of collected excreta can affect TME

values. To avoid any errors, excreta is dried, equilibrated with atmospheric moisture, weighed, ground and mixed prior to analysis. Dried poultry excreta is very hydroscopic. If samples are weighed immediately after drying, excreta will gain moisture between the time of weighing and the time of analysis. Therefore, dried excreta must be equilibrated with atmospheric moisture before weighing. Grinding and mixing is necessary to produce a homogenous sample (Sibbald 1982a).

The preferred method of drying excreta is freeze drying (Sibbald 1983) but drying in a forced-air oven may also be used (Dale and Fuller 1980). Sibbald (1979e) determined the TME value of wheat and a laying hen ration using excreta that was freeze dried or oven dried at 65, 80 or 95 °C. The drying procedure was shown to have no effect on the TME values.

xi) Pooling of Excreta Samples

Measurement of the gross energy of feed and excreta is the most expensive aspect of the TME assay. Any reduction in the cost of the assay increases the potential use of TME in practical feed formulation. Dale and Fuller (1981) found that pooling excreta samples within each treatment decreased the total number of individual samples and therefore decreased the cost of analysis.

Pooling samples may be undesirable because the variation among individual birds is ignored. Dale and Fuller (1981) suggest that variation in TME values is affected to a greater extent by individual excreta weights, than by the gross energy of individual samples. They suggest that a measure of TME variation can be maintained by recording individual excreta weights prior to pooling. Sibbald and Morse (1982) recommended dividing birds within a treatment into subgroups and then pooling the excreta of each subgroup before calorimetry. This gives a better estimate of among

bird variation, but does increase the number of samples for analysis.

Dale and Fuller (1981) recommend the use of pooling excreta and reported that pooling reduced the number of energy determinations by 80%. The TME values obtained by pooling are precise and in agreement with individual TME values. Sibbald and Morse (1982) also support the use of pooling excreta, but question the use in a research laboratory.

xii) Practical Use

In practical feed formulation, feed manufacturers would like to predict the BE content of any feed mixture by assuming additivity of the gross energy content of individual feed ingredients. Several indirect assays for AME have been developed, but fall short of practical use since AME values have proven to be nonadditive (Sibbald 1980a).

Early work by Sibbald and Price (1976b, 1977) attempted to predict TME values from physical and chemical data. Predicted TME values obtained for wheat and oats did not correspond to experimental values. However, the TME values of barley could be accurately predicted from bulk density, crude fiber, starch, starch and sugar and ash. Sibbald et al. (1980b) predicted TME values of 419 commercial poultry feeds and 41 mixtures of feedstuffs using multiple linear regression equations. The variables used to predict TME where fat, crude fiber and ash or fat, crude fiber and nitrogen free extract. Equations with four or more variables were not significantly different from experimental values. Any difference between observed and predicted TME values were said to result from errors in mixing, changes in moisture content or analytical variation (Sibbald et al. 1980a).

Sibbald (1977a) and Dale and Fuller (1980) established the additivity of TME values for simple mixtures of feedstuffs. This, combined with successful prediction of TME values from nutrient composition suggests the

posibility that feed manufacturers may predict the TME values of practical poultry diets without the expense of a direct TME assay.

Once the TME value of a feed is determined, it must be proven to be an applicable measure of BE under commercial poultry production standards. Dale and Fuller (1982a) assayed five practical poultry diets for a 3 week period using chicks. Comparing feed efficiency and TME and AMEn content of the diets, it was concluded that TME values reflected chick performance better than AMEn. In a second study, diets were formulated based on the TME and crude protein content of individual feed ingredients. The 3 week performance of chicks was compared to chicks on an isocaloric, isonitrogenous practical diet. At the end of the test period all chicks had identical body weight gains and feed conversion ratios. Calculated TME values of diets were within 1.6% of assayed TME values. These results indicate that TME values are a valid measure of the energetic content of feeds, and the TME values can be used accurately to formulate broiler diets.

Success of the TME assay, has prompted the adoption of the assay procedure to other species and nutrients. Sibbald et al. (1983) compared the ADE values measured with pigs and TME values measured with adult cockerels. The TME values were closely related to ileal ADE values and fecal ADE values for six feedstuffs. The results suggest that TME values as determined with adult cockerels can be used to predict the ADE values in the formulation of practical pig diets.

Likuski and Dorrell (1978) proposed the use of a standard TME assay to determine true available amino acids (TAAA). Sibbald (1979b) later showed that both TME and TAAA could be measured in a single TME assay. The standard TME assay procedure has proved equally useful to determine true available minerals (TAM)(Sibbald 1983) and true available lipids(TAL) (Sibbald and Kramer 1980). The use of TAAA, TAM and TAL in practical diet

formulations has not yet been investigated. However, it is thought that the practical use of these latter applications will parrallel that of the TME assay.

xiii) Criticism

Despite the extensive data which supports the use of the TME assay as a measure of BE for poultry, several authors are highly critical of the assay. It is therefore necessary to examine such criticsm.

The additivity of TME values has been shown to be the basis for acceptance of the TME values in practical poultry ration formulation. However, one major problem in diet formulation is that the TME value of fats are not additive (Sibbald and Kramer 1977, Sibbald et al. 1961). Also TME assays show the extra-caloric effect of fat (Mateos and Sell 1980), such that the addition of fat to a diet increases the BE value above expected calculated values (Sell et al. 1976). In addition fat TME values have been shown to vary according to the reference diet with which they are fed (Sibbald and Kramer 1980).

Another objection to changing from AME to TME is that existing energy requirements of poultry are expressed in AME. To prevent the expense of time required to obtain TME requirement data, Sibbald (1980a) proposed that data can be converted from AME to TME by a factor of 1.097. The conversion factor is derived from the fact that TME values are 1.097 times (or 9.7%) higher than AME values. Pesti and Edwards (1982) argue that the reason for a higher TME value is due to differences in age, strain and physiological state of assay birds. This is supported by Farrell (1981).

Pesti and Edwards (1983) state that there is no conceptual difference between AME and TME values. In the AME assay, metabolic and endogenous energy losses are assumed to be the same for the test and reference diets,

just as in the TME assay. Quantification of these losses is not necessary since the correction would need to be subtracted from both test and reference diets, thereby cancelling each other. In addition, Pesti and Edwards (1982) state that metabolic and endogenous energy losses are measured in an AME assay, and in an ad libitum feeding condition, are a better estimate of FmE + UeE than is the fasted TME bird. Sibbald (1982a) maintains that the AME assay makes no correction for FmE + UeE and therefore these losses are charged against the feed.

The most important critcism has come from Farrell (1981) who argues that a fasted bird cannot be used to measure FmE + UeE losses of full fed birds. This criticism remains very controversial and inadequately tested, and as such, deserves detailed examination.

III. Metabolic and Endogenous Energy

Harris (1966) first proposed the true metabolizable energy system. The fundamental difference between TME and AME is that FmE + UeE losses are considered body maintenance costs, and therefore should not be charged against the feed. Sibbald (1975) proposed that the FmE + UeE losses in fasted birds are always positive, relatively constant and equal to the FmE + UeE losses of fed birds. Sibbald (1976a) developed this concept into a working model to determine TME in which starved birds are used to measure the FmE + UeE losses of fed birds. The precision of the TME assay depends upon how accurately the FE + UE losses of unfed birds measures the FmE + UeE losses of fed birds. This has been the target of extensive criticism.

Fasted birds overestimate metabolic and endogenous losses due to abnormal physiological conditions resulting from starvation. In a standard

TME assay, birds are first starved 24 hours to clear the gut of any feed residues. During this period, the birds enter a post absorptive state where glycogen stores are exhausted. Birds are then selected and either force fed a small quantity of test material (20 to 40g) or remain unfed. The unfed birds must rely entirely on tissue catabolism to meet basal energy requirements. The by-products of catabolism are high energy nitrogen compounds which are excreted in the urine (UeE), thereby increasing total FmE + UeE (Dale and Fuller 1982b). If body fat is degraded for energy, the end product is carbon dioxide which does not contribute to FmE + UeE. Therefore the nature of tissue degradation will affect FmE + UeE losses (Sibbald 1981c). In the fed bird, tissue catabolism may be spared depending on the extent to which the test material can provide dietary energy. It has been established that FmE + UeE losses are inversely proportional to caloric intake when birds are in a negative energy balance (Dale and Fuller 1982b). The result is that assay birds are in different stages of energy balance, so the assumption that metabolic and endogenous energy losses of fed and unfed birds are equal is questioned. Furthermore, one may question the validity of using a 20 to 40g fed bird as a measure of BE for an ad libitum fed bird.

Guillaume and summers (1970) first determined metabolic and endogenous energy losses of adult cockerels to be 21.76kJ per body weight per day. Sibbald (1975) predicted FmE + UeE by plotting the regression line of gross energy of excreta voided and weight of wheat consumed. The intercept of the regression line was positive and gave an estimate of FmE + UeE of 35.57 kJ per day. Further studies have shown the value to be highly variable. Patchell and Edmundson (1977a) observed the FmE + UeE values of 6 birds to range from 33.9 to 101.2 kJ per bird per day with a mean of 57.0 kJ. In a subsequent report Patchell and Edmundson (1977b) observed a range in

values of 25.8 to 62.9 kJ per day with a mean of 41.5 kJ for 48 birds. Sibbald (1982a) argues that these values are too large and may be due to an inadequate fasting period prior to the assay. Farrell (1978) also showed large variation in FmE + UeE of 32.5 to 82.0 kJ per bird per day. Sibbald and Price (1978) recorded FmE + UeE losses for 300 birds over 38 TME assays and observed a range of values of 25.0 to 69.3 kJ per bird per day with a mean of 43.7 kJ. The variation observed was reported to be due to the variation between assays. More recently Campbell et al. (1983) reported variation of FmE + UeE ranging from 40.85 to 66.44 kJ per bird per day. On the other hand, Sibbald and Price (1980) and Sibbald (1981a) have reported low variability in FmE + UeE losses. Sibbald (1981c) examined 808 FmE + UeE values and found a wide range, but suggested the variation was due to the differences among birds and experiments. Sibbald (1982a) states that if the FmE + UeE value used to calculate TME is the mean of several observations, the error in the TME value due to the among bird variation is negligable.

Sibbald (1976a) found FmE + UeE losses to vary among birds within a population and therefore recommended that birds within a treatment should be selected on the basis of similar body weight. An effect of body weight on FmE + UeE was observed by Shires et al. (1979) and Campbell et al. (1983). Miski and Quazi (1981) found FmE + UeE tovary with age, which Sibbald 1982a) explained was really a body weight:age effect. Sibbald (1981c) explained the effect of body weight on FmE + UeE losses by assuming that lighter birds have a smaller fat reserve, and therefore when fasted, must catabolize more tissue protein per unit body weight. The result is increased nitrogen and energy excretion in the urine (UeE), and therefore increased FmE + UeE losses. However, several authors have reported no effect of weight on FmE + UeE (Patchell and Edmundson 1977a; Farrell 1978; Muztar

and Slinger 1980b; Sibbald and Price 1980; Bilgili and Arscott 1982). There is also no correlation of body weight on FmE + UeE losses in geese (Storey and Allen 1982). Edmundson (1983, personal communication) notes that in theory, one expects to find a direct correlation between body weight and FmE + UeE losses, but such an effect is masked by the day- to-day variation in FmE + UeE losses of any single bird. To prevent the among bird variation associated with body beight when performing a TME assay, Edmundson et al. (1978) recommended using a "self-pairing design", where each bird acts as its own measure of FmE + UeE. In this manner body weight will not effect FmE + UeE values.

Sibbald (1979d) observed that FmE + UeE loss of fasted birds are affected by duration of starvation. After an initial 24 hour fast, birds were starved for an additional 24, 48, 72 and 96 hours. The FmE + UeE loss was 45.02 $\pm$ 2.05, 37.03 $\pm$ 1.63, 34.94 $\pm$ 1.84 and 34.48 $\pm$ 2.93 kJ per bird respectively. This decrease in FmE + UeE with the duration of starvation is supported by Shires et al. (1979) and Muztar and Slinger (1980b).

Dale and Fuller (1981) showed a direct effect of environmental temperature on FmE + UeE. Losses tend to be higher in winter (5°C) lowest in summer (30°C). Similar findings were reported by Dale and Fuller (1982b). This reflects the response in changes of tissue catabolism to meet changes in energy maintenance requirements. As a result, TME values are directly affected by temperature (Yamazaki and Zi-Yi 1982). Sibbald (1982a) suggested that environmental temperature and duration of starvation do affect FmE + UeE losses, but will have no effect on TME values provided all assay birds are maintained under the same conditions. This has yet to be confirmed.

There are indications that FmE + UeE losses are influenced by the amount and type of feedstuff consumed. This stems from the findings of

Hallsworth and Coates (1962) that increased intake of dietary fiber increased the erosion of the intestinal lining, thereby increasing FmE and in turn FmE + UeE. However, Rolls et al. (1978) found that addition of high fiber to diets did not affect the rate of removal of intestinal epithelium. This is probably due to the decreased rate of passage of high fiber diets. To illustrate this, Sibbald (1981a) fed a cellulose:carboxymethyl cellulose mixture or sawdust as high fiber test materials but could show no effect on FmE + UeE loss.

Farrell (1981) reported an effect of the neutral detergent fiber (NDF) of test materials on FmE + UeE. FmE + UeE losses increased in a linear fashion as NDF increased, but only to a level of about 12% NDF. At levels greater than 12%, FmE + UeE losses remained constant and did not exceed a value of about 87.86 kJ per bird per 32 hours. Sibbald (1982a) argued that because of the slow rate of passage of high fiber (or NDF) test materials, a collection period of 32 hours is not sufficient for complete clearance of the gut. Therefore the data presented by Farrell (1981) is "obscure". Feeding cellulose or sand in combination with various test materials has also been investigated (Sibbald 1980b), but no significant results were obtained. Farrell (1981) explained this, concluding that sand was being retained by the gizzard. Tenesaca and Sell (1978) fed silica gel either alone or in combination with corn and found increased FmE + UeE, but gave no explanation for such results.

The effect of feed input on FmE + UeE loss was investigated by Sibbald and Morse (1983a). Adult cockerels were force fed single test materials at various input levels and FE + UE was measured cumulatively. A linear relationship existed at all input levels provided the collection period was sufficient to allow complete clearance of all feed residues. FmE + UeE were determined by regression to zero feed intake. The inter-

cepts were not different from the FmE + UeE values measured by unfed birds. It was therefore concluded that feed input does not affect FmE + UeE losses.

When a completely digestible energy source such as glucose, destrose or corn oil is force fed to fasted roosters, energy excretion tends to vary with the level fed (Dale and Fuller 1982b; Campbell et al. (1983). From this, Dale and Fuller (1982b) concluded that FmE + UeE is inversley proportional to the energy intake of roosters in a negative energy balance. The reason for this is that dietary energy is used to meet the birds maintenance energy requirements, which spares tissue catabolism. The result is FmE + UeE losses are significantly different between birds depending upon the extent to which tissue catabolism is spared. Sibbald and Morse (1983c) force fed a variety of purified diets as supplemental energy sources to reduce the severity of the fast and reduce the variation in nitrogen and energy loss among birds. Energy sources were also offered free choice, but feed spillage, excreta contamination and highly variable intake proved force feeding to be the better feeding method. The supplemented energy reduced nitrogen losses due to degradation of body protein and therefore reduced FmE + UeE.

Sibbald and Morse (1983c) state that "there is an inevitable metabolic fecal plus endogenous urinary N loss that cannot be reduced by the provision of supplementary E". They also comment that supplemental purified energy sources are not completely digestible. The result is that FmE + UeE and subsequently TME values are biased depending upon the level of feed input. Therefore the method is not recommended. Sibbald and Morse (1983a, 1983c) suggest the best method to determine FmE + UeE is by force feeding various levels of the test material and then measuring the regression of excreta energy on feed input.

It is important to note that the initial reason for using TME as compared to AME was that TME values are not affected by feed input (Sibbald 1975). However, as seen from the data presented above, provision of supplemental energy directly affected FmE + UeE and TME values. There is essentially no difference between force feeding a test material in a TME assay and force feeding supplemental energy to the "unfed bird". In both conditions, dietary energy intake reduces the extent of tissue catabolism, which reduces FmE + UeE and directly affects the TME value. As a result, validity of the TME assay may be questioned.

IV. Nitrogen Correction

Classical AME assays included a nitrogen correction factor to account for protein storage in the carcass during the assay period. In the AME assay, growing chicks are fed ad libitum. The retained nitrogen (RN) is positive and AME values are larger than nitrogen corrected values (AMEn) (Wolynetz and Sibbald 1984). In the TME assay, adult cockerels are in severe negative nitrogen balance (RN is negative) so the rationale of a nitrogen correction was not originally applied (Sibbald 1976a).

The FmE + UeE loss of a fasted TME assay bird depends upon the extent of tissue catabolism (Sibbald and Morse 1983b), metabolic body size (Sibbald and Wolynetz 1984b) and is inversely proportional to caloric intake (Dale and Fuller 1982b). Therefore, the nitrogen and energy excretion among fasted and fed birds is highly variable (Sibbald and Morse 1983c). The problem of the TME assay, is the difference in the large nitrogen loss of fasted birds compared to the small loss or gain of the force fed birds. The result is fasted birds tend to over-estimate the FmE + UeE of fed birds (Parsons et al. 1982). However, it should be possible to re-

duce the variation in FmE + UeE by correction to zero nitrogen balance (Sibbald and Morse 1983b).

TME values are corrected to zero nitrogen balance as follows:

$$TMEn = \frac{IE - ((FE + UE) + (Ni - Ne)k) - ((FmE + UeE) + (Ni - Ne)k)}{g \text{ intake}}$$

where (Ni - Ne) = nitrogen balance (Dale and Fuller 1984). The value of conversion factor (k) is controversial. Hill and Anderson (1958) proposed a value of 34.39 kJ per gram of RN, which is the energetic value of uric acid nitrogen. The assumption is that uric acid is the sole nitrogen excretary compound. Titus (1956) proposed a factor 36.53 kJ per gram of RN. This value is based on the fact that catabolism of body tissue produces urea, creatinine, ammonia and other nitrogenous compounds in addition to uric acid. The value was obtained by determining the gross energy of a sample of nondescript chicken urine. More recently Sibbald and Wolynetz (1984b) estimated the coefficient of fecal and urinary nitrogen (FN + UN) by regression equation to determine an appropriate correction factor. The estimated values of 38.13 $\pm$ 0.17 kJ for meat type birds and 34.68 $\pm$ 1.57 kJ for SCWL cockerels are similar to the value proposed by Titus (1956). Sibbald and Wolynetz (1984b) observed that the coefficient of FN + UN varies among birds and over time, which suggests that the energetic value of nitrogen in chicken urine is not constant. More importantly, this implies that the proportions of nitrogenous end products of tissue catabolism also vary.

Shires et al. (1980) determined TMEn using chicks and cockerels and found the correction to reduce TME values by 8 and 6% respectively. Muztar and Slinger (1981) determined the TMEn values to be consistently higher than TME values. Sibbald and Morse (1983b) point out that Muztar and Slinger (1981) failed to correct the unfed bird to zero nitrogen balance and therefore TMEn values are falsely inflated. Correction of the FmE + UeE

value is essential because of the severe negative nitrogen balance of the unfed bird. Dale and Fuller (1984) corrected the unfed bird using the factor 36.53 kJ/g RN and reduced FmE + UeE by more than 50%. Sibbald and Morse (1983b) also showed a reduction in FmE + UeE from 127.3 to 56.0 kJ per bird per 72 hours. By definition of the TMEn formula, when the reduced value is subtracted from FE + UE, TMEn will always be less than TME. The effect is the reverse for fed birds. FE + UE are reduced by the correction factor which will increase the TMEn value. Therefore, the larger the negative nitrogen balance of the fed bird, the higher the TMEn value (Dale and Fuller 1984). As such, the magnitude of the correction will decrease as the fatness of the unfed bird increases, and will vary according to the ability of the test material to prevent tissue catabolism (Sibbald 1982a).

Dale and Fuller (1984) observed a positive relationship between the protein content of test materials and the difference between TME and TMEn values. As protein intake increased, the per cent difference between TME and TMEn values increased. The reason for this is that as nitrogen intake increased, the fed bird enters a greater positive nitrogen balance which decreased TMEn. This is supported by Muztar and Slinger (1981) who showed that the magnitude of nitrogen correction is affected by the type and level of protein fed. However, they reported that only 46 to 48% of the variation in TMEn data is due to the change in nitrogen input levels. Sibbald and Morse (1983b) suggested that any difference in FmE + UeE between fed and unfed birds may be due to differences in the type and amount of feed, but because nitrogen represents the greatest proportion of FmE + UeE, correction to zero nitrogen balance will reduce any bias and TMEn will not be influence.

To minimize the negative nitrogen balance of the TME assay bird, a

completely digestible energy source may be fed. Dale and Fuller (1981), du Preez et al. (1981) and Campbell et al. (1983) found the excretion of energy and nitrogen to be significantly affected. Sibbald (1975, 1976a) was not able to show any effect on nitrogen correction. The reason for these differences may be due to the degree of fatness of the bird (Sibbald 1982a). If the energy sources are not completely digested, TME and TMEn are biased depending upon the level of input. Sibbald and Morse (1983c) suggest the procedure of supplemental energy is not necessary because the nitrogen correction reduces any variance due to differences in nitrogen balance.

V. Errors in Balance Studies

The true metabolizable energy assay developed by Sibbald (1976a) is a balance study by definition. A balance study is an experiment that measures input minus output. As a balance situation, both positive and negative values are possible. If input is equal to output, then balance is zero. The TME assay measures only fecal and urinary output so it is termed gross balance. A net balance study includes dermal and respiratory losses. True balance studies account for metabolic and endogenous losses. Therefore, by definition, the TME assay is a gross true balance study (Asplund 1979).

The recent addition of a nitrogen correction to the TME assay has created the need for a precise measure of nitrogen balance. In theory, if all nitrogen inputs and outputs are accurately measured, then the difference between input and output will equal the total nitrogen retention (positive or negative). Nitrogen balance is equal to nitrogen retention. (Asplund 1979).

Davidson and Williams (1963) determined nitrogen retention in chicks by comparative slaughter and balance study. A difference of 11 to 14% was observed between the nitrogen retention values determined by the two methods. Retention values determined by balance studies are typically higher than direct studies. The reason for the difference is because of the large degree of error associated with balance trials.

Several errors exist in balance studies which are common to the TME assay. The subject has been reviewed by Asplund (1979). Errors in moisture loss, sedimentation of nitrogen compounds in the excreta and quantification of excreta loss effect retention values. If birds are restricted water after force feeding, large quantities of dry matter will accumulate in the digestive tract and decrease rate of passage. This gives an erroneously high nitrogen retention value. The 24 hour fast prior to force feeding causes nitrogen depletion of the assay bird, resulting in a higher nitrogen retention than observed in continuous fed birds. It has been suggested that most balance trials are invalid because they do not incorporate a diet adaption period. It is generally accepted that a 7 to 10 day adaption period is necessary so that rate of passage and nutrient utilization are at a "steady state" during the assay period. Any imbalance during the assay period will effect nitrogen retention and utilization. A steady state is also essential to ensure that the urine and feces collected during the collection period is representative of the feed. The steady state concept is important to the TME assay, especially with regard to the criticism of feeding a small quantity of feed (20 to 40g) to starved birds. In classical balance trials, in order to determine any difference in availability of the feed, the nutrient considered (nitrogen) must be fed at less than the requirement level. If fed above the requirement level, the retention value is a reflection of the state of the bird, rather than a re-

flection of the feed. Also nitrogen must be the first limiting nutrient in the diet. If another nutrient is first limiting, nitrogen availability and retention may be affected by the other nutrient.

The most important error of nitrogen balance studies is the loss of nitrogen which occurs during the drying of excreta. Davidson and Williams (1968) determined that about half of the 11 to 14% difference between nitrogen retention values determined by direct and indirect methods was due to the volatile losses of nitrogen upon drying of excreta. Denitrification and volatilization are considered the major errors in nitrogen balance studies resulting in under estimation of fecal and urinary nitrogen losses (Asplund 1979). This error is critical to the validity of a nitrogen corrected TME assay.

Although freeze drying is preferred, no standard method of drying excreta for BE assays has been established. This prompted concern over the effect of drying procedures on excreta energy and nitrogen loss. Flatt (1957) compared fresh cow feces dried in an oven at 80°C and reported an energy loss of 3.27 $\pm$ 3.08%. Colovos et al. (1957) compared fresh cow feces with feces dried in a convection oven at about 65°C and showed a significant loss of nitrogen - 2.5 to 34.2% in addition to a significant loss of energy 4.1 to 20.9%. Bratzler and Swift (1959) dried cow feces in a forced-air oven at 65°C for 22 hours and compared this to fresh feces. Only a small loss of nitrogen was reported 5.15 $\pm$ 1.89% and no loss of gross energy. This contradiction to previous work was due to the large error in sampling of fresh samples. Fenner and Archibald (1959) dried cow feces in an oven at 80°C and showed only a 0.61% loss of energy due to drying. It was therefore concluded that drying procedure did not affect the energy content of cow feces. It was also concluded that if any nitrogen is lost, it is in the form of ammonia (NH_3). These findings are supported

by Martin (1966) who found no loss of nitrogen from the feces of sheep, but did report a significant loss of nitrogen from the urine, in the form of NH_3. Such losses are considerable and are directly affected by environmental temperature, frequency of urination, bacterial population, proportion of preformed NH_3 and pH of the urine.

Prior to 1964 the loss of energy and nitrogen from chicken excreta had not been investigated. Manoukas et al. (1964) compared fresh hen excreta with excreta dried in a convection oven at 65°C for 24 hours. The hen excreta showed a significant loss of energy 1.2 to 20.2% with a mean of 12% and a significant loss of nitrogen -7.1 to 15.2% with a mean of 5.45%. Losses of this magnitude caused concern about problems associated with ME determinations. Shannon and Brown (1969) compared fresh hen excreta with excreta freeze dried at 40°C and excreta dried in a forced-air oven at 60, 100 and 120°C. All of the drying methods showed significant losses of energy and nitrogen. The smallest energy loss (1.3%) occured when excreta was freeze dried. When excreta was dried in the forced-air oven, energy loss increased from 2.8 to 5.5% as oven temperature decreased from 120°C to 60°C. The smallest nitrogen loss occured when excreta when excreta was freeze dried (4.8%) or oven dried at 60°C (4.6%). Excreta loss of nitrogen increased from 4.6 to 10.6% as drying temperature increased from 60°C to 120°C. All of the energy lost on freeze drying could be accounted for by nitrogen loss in the form of ammonia.

Sibbald (1979e) investigated the effect of excreta drying procedure on the TME value of various feedstuffs. Excreta of force fed and unfed SCWL cockerels was freeze dried or oven dried at 65, 80 and 95°C. The excreta of laying hens and feces from cows, pigs, horses and sheep were also dried by the same procedures. For all of the excreta samples under each different drying procedure, no effect of drying could be found. Sim-

ilar findings were reported by Dale and Fuller (1983b) who examined the effect of freeze drying compared to forced-air oven drying at 60°C on the excreta of SCWL cockerels.

Wallis and Balnave (1983) compared the loss of energy and nitrogen when broiler excreta was freeze dried or dried in a forced-air oven at 60°C and 80°C. Freeze drying and oven drying at 60°C both caused significant losses of energy (3 to 4%) and nitrogen (4 to 8%). Wallis and Balnave (1983) also examined the effect of excreta collection period on excreta energy and nitrogen by comparing 12 to 24 hour collection periods. Significant increases of energy (1 to 4%) and nitrogen (2 to 6%) resulted from extension of the collection period to 24 hours.

It should be noted that Sibbald (1979e), Dale and Fuller (1983b) and Wallis and Balnave (1983) all failed to determine the energy and nitrogen content of fresh excreta. Therefore the real effect of drying procedure on excreta energy and nitrogen content was not determined. This is essential in order to properly evaluate the effect of drying method and collection period on BE assays and specifically the TME and TMEn assays.

The loss of energy and nitrogen from the feces and urine of ruminants is well established. As a result there have been several attempts to try to prevent such losses. Flatt (1957) and Jacobson et al. (1959) found canning to be an appropriate method of preserving fresh excreta until samples were required for analysis. Martin (1966) trapped urinary volatile NH_3 of sheep directly in a boric acid solution (2% w/v). However the problem of boric acid is that acid hydrolysis of urea may occur and therefore effect total nitrogen. Rocks (1977) suggested that to prevent loss of nitrogen and hydrolysis of urea, a preservative must consist of a weak acid to buffer the urine at low pH and an inorganic bacteriostat. Evaluation of six different possible preservatives showed mercuric chloride (0.3 per

cent w/v) in glacial acetic acid and boric acid powder plus (0.3 per cent w/w) mercuric chloride to be equally effective in maintaining total nitrogen, urea nitrogen and ammonia at initial levels. Refrigeration at 5°C or less did prove adequate as a method of preserving unpreserved urine, but but only for a period of up to 14 days.

Little or no work on the preservation of poultry excreta has been published to date. Muramatsu and Okamura (1979) cited the use of 2.5N sulfuric acid to prevent microbial fermentation and loss of ammonia, but no comparative analysis of the effectiveness of the preservative was mentioned. Smith et al. (1978) and Narasimhalu et al. (1981) evaluated the use of chemical preservatives on layer and broiler litter to reduce microbial populations for the sole purpose of refeeding the litter to ruminants. Smith et al. (1978) found formaldehyde, an acetic-propionic acid mixture and an acetic-propionic acid-formaldehyde mixture to each be equally effective in reducing litter deterioriation and nitrogen loss up to 14 days. Narasimhalu et al. (1981) found sodium tannic acid to be effective in reducing microbial populations. However, neither Smith et al. (1978) or Narasimhalu et al. (1981) investigated the loss of energy or nitrogen from the treated litter.

MATERIALS AND METHODS

I. General

A. Experimental Objectives

Seven experiments were designed to quantitatively examine variations of nitrogen compounds in excreta of adult SCWL cockerels and determine the contribution of such compounds to a nitrogen correction factor for the true metabolizable energy assay. The effects of feed input (experiment 2 and 7), length of excreta collection period (experiment 3) and excreta drying procedure (experiment 4) on the proportions of the various nitrogen compounds in excreta were determined. Experiments 5, 6 and 7 were conducted to study the use of preservatives to prevent the loss of excreta nitrogen over time or as a result of drying procedure.

B. Experimental Birds and Management

All trials employed adult SCWL cockerels (Shaver 288) which ranged in age from 273 to 360 days. All birds used within any one trial were, however, of the same age. The birds were obtained from a commercial hatchery at one day of age and fed typical chick starter and grower diets until mature. With the exception of experiment 1, all birds were dubbed within the first week of life. After maturity all birds were placed in pens located in an environmentally controlled barn that provided 14 hours of light per day and a temperature of 20°C. The birds were fed ad libitum a standard maintenance diet (Table 1) in mash form. The vitamin and mineral premixes are presented in Tables 2a and 2b, respectively. Feed was

Table 1. Standard maintenance diet

Ingredients	Kg
Barley	689.6
Soybean meal (44%)	183.1
Alfalfa	10.0
Calcium Carbonate	43.0
Biophos	17.0
Oyster Shell	19.9
Tallow	17.4
Vitamin premix (standard)[1]	10.0
Mineral premix (standard)[2]	5.0
Grit	5.0
	1,000.0

Chemical analysis:

	Air Dry Basis	Dry Matter Basis
Dry Matter (%)	89.24	100.00
Energy (MJ/Kg)	14.71	16.49
Nitrogen (%)	2.80	3.14
Crude Protein (%)	17.50	19.61

[1]Standard Vitamin Premix (Table 2a)

[2]Standard Mineral Premix (Table 2b)

Table 2a. Standard vitamin premix

Ingredients	3/Kg Premix
Vitamin A (500,000 I.U./g)	1.65
Vitamin D3 (200,000 I.C.U./g)	0.45
Vitamin E (20,000 I.U. /lb)	12.40
Vitamin B12 (60 mg/lb)	8.50
Vitamin B58 (2-4-6-100)[1]	50.00
DL-Methionine (98-99%)	50.00
Santoquin (50%)	25.00
Animal tallow	10.00
Wheat middlings	842.00

[1]Vitamin B58 contains per Kg:

Riboflavin	4,400 mg
Ca Pantothenate	8,800 mg
Niacin	13,200 mg
Choline Chloride	220,000 mg

Table 2b. Standard mineral premix

Ingredients	g/Kg Premix
Manganese Oxide	33.00
Zinc Oxide	11.00
Iodized Salt	956.00

available ad libitum from tube type feeders (40cm diameter) and water was available ad libitum from 15 cm diameter dish waterers. In all experiments, birds were randomly selected from the maintenance (spare) group.

With the exception of experiment 1, experimental birds were housed in individual wire cages (60 x 45 x 40 cm) raised off the floor. The wire cages were located in an environmentally controlled barn that provided 24 hour continuous lighting and a temperature of 20°C. The purpose of such a lighting scheme was to prevent any diurnal effects on excretion. When not on test the birds were fed a standard maintenance diet (Table 1) ad libitum from feeders (8 x 10 x 15 cm). Water was available ad libitum from an automatic watering cup system.

In experiment 1, birds were housed in individual floor type wire cages (45 x 45 x 45 cm) located in an environmentally controlled barn that provided 12 hours of light per day and a temperature of 20°C. When not on test the birds were fed a standard maintenance diet (Table 1) ad libitum from feeders (8 x 10 x 15 cm). Water was available ad libitum from similar containers.

Daily management procedure of the spare birds included cleaning wet litter, shaking down feeders, cleaning water dishes and cull birds. Daily management of the caged birds included cleaning water containers and cups, feeding, manure handling and culling birds. Mortalities which occured in the caged birds were replaced by birds selected at random from the spare group of birds.

C. Experimental Procedure

Assay birds were not used in any feeding trials until acclimatized to the cage environment for a period of not less than 3 weeks. In experiments 1, 2 and 7, birds were force fed following the general procedure as

described by Sibbald (1983). Any birds which were found to have impacted crops, or which regurgitated as a result of the force feeding procedure were not used. The assay birds in experiments 3, 4, 5 and 6 were fed standard maintenance diets (Table 1) throughout the experiment.

The excretory nitrogen compounds determined were expreseed in two ways: 1) grams of nitrogen of the compound per 100 grams of total excreta, and 2) grams of nitrogen of the compound per 100 grams of total nitrogen excreted. Expressing the excreta nitrogen compounds on the basis of total excreta allowed for an evaluation of variations in the proportion of the nitrogen compounds between treatments without bias excreta weight. Expressing the excreta nitrogen compounds on the basis of total nitrogen excreted allowed for the calculation of the ocntribution of each nitrogen compound to a nitrogen correction factor for the TME assay.

In experiment 1, the total excreta uric acid energy was calculated and was expressed as a per cent of total excreta energy. In this manner, the energetic contribution of uric acid to the total excreta energy was determined.

D. Chemical Analysis

Gross energy of the feed and excreta samples were determined using a Parr Oxygen Bomb Calorimeter. Total nitrogen (macro Kjeldahl) and moisture were measured according to A.O.A.C. (1980) methods.

In experiment 1, excreta uric acid was determined by a high-performance liquid chromatographic (HPLC) method as developed by Marquardt et al. (1983) and by a spectrophotometric method as developed by Marquardt (1983). Very close agreement in the uric acid values was obtained by the two analytical procedures (< 1% difference between means). As a result, in subsequent experiments, excreta uric acid was determined only by the spectro-

photometric method.

Excreta urea nitrogen and ammonia nitrogen were determined according to A.O.A.C. (1980) methods. Because of the small sample size of the freeze dried excreta, only 1g of finely ground excreta was used per replicate analyzed.

Excreta creatinine was determined by modification of the procedure described in the Sigma Technical Bulletin No. 555 (1982). The modification involved weighing 0.5g of finely ground freeze dried excreta, or 2.0g of fresh excreta into a 125ml flask. Exactly 50ml of 0.02 N HCL was added and the flask was tightly stoppered. The solution was mixed continuously for 1 hour at room temperature at 250 RPM and then centrifuged for 10 minutes at 10,000 RPM. The precipitate was discarded and the remaining solution was analyzed spectrophotometrically by the method of the Sigma Technical Bulletin No. 555 (1982).

Excreta amino acids were analyzed using a Beckman 119C Amino Acid Analyzer following the Beckman standard method. All samples were hydrolyzed using 6 N HCI at 121°C for 16 hours. Samples analyzed for cysteine and methionine were oxidized with performic acid (Hirs 1967) prior to being hydrolyzed.

E. Statistical Analysis

For all experiments, analysis of variance and test of treatment differences were computed using S.A.S. - General Linear Model Procedure (Computer Science Department - University of Manitoba). Comparison of treatment means were carried out using Tukey's Test. For all experiments, except experiment 4, treatment means were analyzed by one way analysis of variance. Treatment means for experiment 4 were analyzed by two way analysis of variance.

II. Experiment 1. True metabolizable energy values of different cultivars of corn and barley.

Experiment 1 was designed to compare the TME values of several different cultivars of corn and barley using adult SCWL cockerels. Excreta uric acid levels were measured to determine the energetic contribution of uric acid to the total excreta energy. The contribution of uric acid nitrogen to a nitrogen correction factor for the TME assay was also determined.

Different cultivars of corn and barley were assayed using a modification of the standard TME assay procedure (Sibbald 1983). The test materials assayed were samples of Alberta corn, Manitoba corn, U.S. corn and 4 cultivars of barley; IFD, Betzes, Bonanza and Klages (Table 3). Eighteen birds were randomly selected and divided into 2 groups of 9 birds and housed in wire cages. Prior to force feeding, birds were fasted for 24 hours. Eight birds were then weighed and force fed 25g of a pre weighed sample. Four replicates per feed sample were employed. One bird, chosen at random, per group of 9 was weighed and left unfed to obtain a measure of metabolic and endogenous losses. Immediately after force feeding, a clean stainless steel excreta collection tray was placed under the bird and the time was recorded. Exactly 30 hours later, excreta was collected quantitatively. Excreta of the unfed bird was also collected for exactly 30 hours. The excreta was then frozen, freeze dried, equilibrated with atmospheric moisture, weighed, ground, mixed and stored in a freezer until analyzed. Test materials were analyzed for gross energy, total nitrogen and moisture. Excreta samples were analyzed for gross energy, total nitrogen, moisture and uric acid.

Table 3. Test Materials - Experiment 1

Sample	Dry Matter (%)	Total Nitrogen (%)	Crude Protein (%)	Gross Energy (MJ/Kg)
		----------Air Dry Basis----------		
Alberta Corn 1	88.66	1.51	9.44	16.78
Alberta Corn 2	88.49	1.56	9.73	16.89
Alberta Corn 3	88.63	1.60	10.00	16.59
Alberta Corn 4	88.56	1.40	8.72	16.80
Alberta Corn 5	88.33	1.39	8.71	16.61
Alberta Corn 6	88.55	1.51	9.45	16.71
Manitoba Corn 1	87.38	1.46	9.14	16.65
Manitoba Corn 2	86.92	1.46	9.13	16.56
Manitoba Corn 3	87.18	1.42	8.87	16.50
Manitoba Corn 4	87.37	1.56	9.73	16.53
Manitoba Corn 5	86.86	1.40	8.75	16.45
U.S. Corn 1	86.88	1.64	10.23	16.32
U.S. Corn 2	87.44	1.90	11.89	16.19
U.S. Corn 3	87.68	1.48	9.32	16.54
Barley c.v. IFd 1	87.61	1.89	11.81	16.43
Barley c.v. IFd 2	87.95	1.88	11.75	16.44
Barley c.v. IFd 3	88.07	1.88	11.76	16.53
Barley c.v. IFd 4	87.56	1.96	12.23	16.69
Barley c.v. Bonanza 1	89.40	1.90	11.88	16.59
Barley c.v. Bonanza 2	89.32	1.92	12.01	16.54
Barley c.v. Bonanza 3	89.27	1.90	11.90	16.62
Barley c.v. Bonanza 4	88.31	1.84	11.49	16.57
Barley c.v. Betzes 1	88.61	1.93	12.06	16.46
Barley c.v. Betzes 2	88.63	1.85	11.59	16.48
Barley c.v. Betzes 3	89.10	1.92	11.99	16.67
Barley c.v. Betzes 4	88.62	2.12	13.26	16.65
Barley c.v. Klages 1	87.74	1.56	9.74	16.24
Barley c.v. Klages 2	88.24	1.63	10.21	16.33
Barley c.v. Klages 3	87.73	1.59	9.91	16.27

III. Experiment 2. Quantification of nitrogen compounds in poultry excreta and the contribution to a nitrogen correction factor for the TME assay.

This experiment was designed to determine the effect of feed input on the proportion of excretory nitrogen compounds. The contribution of these compounds to a nitrogen correction factor for the TME assay was also determined.

Twenty four birds were randomly distributed among 24 wire cages. Birds were divided into 3 groups of 8 birds per treatment. The 3 treatment groups were as follows: 1) full fed (ad libitum) standard maintenance diet (Table 1); 2) force fed 30g of cracked wheat (Table 4a); 3) unfed.

The force fed and unfed birds were starved for 24 hours. Full fed birds continued on ad libitum feed intake throughout the assay period. After the 24 hour fast, body weights of all birds were recorded. The force fed birds were then fed 30g of a pre weighed sample of wheat. Immediately after force feeding, a human colostomy bag was attached around the cloaca to collect excreta (Sibbald 1983). Bags were attached using a strong adhesive (3M - EC847). Collection bags were also attached to the full fed and unfed birds and the time of bag attachment was recorded. Exactly 48 hours later, bags were removed and samples were frozen. Excreta samples were then freeze dried, equilibrated with atmospheric moisture, weighed, group and mixed. Prior to chemical analysis, all excreta samples were pooled in pairs on the basis of bird body weight. This resulted in 4 replicate excreta samples per treatment for analysis. Excreta was analyzed for gross energy, total nitrogen, moisture, uric acid, urea, ammonia, creatinine and amino acids. Feed samples were analyzed for gross energy, total nitrogen and moisture.

Table 4a. Cracked Wheat - Experiment 2

Chemical Analysis	Air Dry Basis	Dry Matter Basis
Dry Matter (%)	89.66	100.00
Total Nitrogen (%)	2.46	2.74
Crude Protein (%)	15.35	17.12
Gross Energy (MJ/Kg)	16.38	18.27

Table 4b. Cracked Wheat - Experiment 7

Chemical Analysis	Air Dry Basis	Dry Matter Basis
Dry Matter (%)	88.48	100.00
Total Nitrogen (%)	2.35	2.66
Crude Protein (%)	14.69	16.60
Gross Energy (MJ/Kg)	16.49	18.64

IV. Experiment 3. Effect of the duration of excreta collection period on the proportion of nitrogen compounds in poultry excreta.

Experiment 3 was designed to determine the effect of duration of excreta collection period on the proportions of nitrogen compounds in poultry excreta. The effect of changes in the proportions of nitrogen compounds on a nitrogen correction factor for the TME assay was examined.

Experiment 3 was performed in two trials, A and B. In trial A, 24 birds housed in wire cages were fed ad libitum standard maintenance diet (Table 1) throughout the assay period. Clean collection paper was placed under each bird and the time recorded. Exactly 1 hour later, excreta from all 24 birds was collected, pooled into a single sample and homogenized. The sample was then divided into 3 subsamples of equal weight and allocated to 1 of 3 treatments: 1) 1 hour sample; 2) 24 hour sample; 3) 48 hour sample. The 1 hour sample was immediately frozen. The 24 hour and 48 hour samples were left untouched at room temperature (20°C) for the designated time period, after which, the sample was homogenized and frozen. This procedure was repeated 4 times.

Trial B employed the same 24 birds as in trial A. All birds received standard maintenance diet (Table 1) throughout the trial. Birds were randomly allocated to 1 of 3 treatments: 1) 4 hour collection period; 2) 24 hour collection period; 3) 48 hour colleciton period. Birds were weighed, a collection bag was securely placed over the cloaca and the time was recorded. At the end of the treatment collection period, excreta collection bags were removed and weighed. Excreta samples for each treatment were pooled in pairs on the basis of bird body weight. This resulted in 4 pooled excreta samples per treatment. In the 48 hour collection period 2 of the 8 collection bags fell off, resulting in only 3 samples after

pooling. Pooled samples were homogenized and frozen until analyzed.

Excreta samples for both trial A and trial B were analyzed for total nitrogen, moisture, uric acid, urea, ammonia and creatinine. Excreta samples were not freeze dried, rather they were analyzed as is, or "fresh".

V. Experiment 4. Effect of freeze drying on the proportion of nitrogen compounds in poultry excreta.

Experiment 4 was designed to investigate the loss of excreta nitrogen compounds as a result of drying procedure. This experiment was set up to quantify such losses and identify the nitrogen compounds most affected by drying. The effect of nitrogen losses on a nitrogen correction factor for the TME assay was examined.

This experiment was conducted in two trials, A and B. In trial A, 24 birds were fed standard maintenance diet (Table 1) ad libitum throughout the assay period. Clean collection paper was placed under each bird and the time recorded. Exactly 1 hour later, excreta from all 24 birds was collected, pooled into a single sample and homogenized. The sample was divided into 4 sub samples of equal weight, and allocated to 1 of 4 treatments: 1) 1 hour fresh; 2) 1 hour freeze dry; 3) 48 hour fresh; 4) 48 hour freeze dry. The two 1 hour samples were immediately frozen. The two 48 hour samples were left untouched at room temperature (20°C) for the designated time period. After 47 hours, plus 1 hour collection period, the samples were homogenized and frozen. The two treatments, 1 hour freeze dry and 48 hour freeze dry were freeze dried, equilibrated with atmospheric moisture, weighed, ground, mixed and frozen. This procedure was repeated 4 times.

In trial B, the same 24 birds as used in trial A were again used.

Birds were randomly allocated to 4 treatments; 6 birds per treatment: 1) 4 hour fresh; 2) 4 hour freeze dry; 3) 48 hour fresh; 4) 48 hour freeze dry. All birds were fed a standard maintenance diet (Table 1) ad libitum throughout the assay period. Birds were weighed, a collection bag was securely attached over the cloaca and the time was recorded. Collection bags were removed at the end of the appropriate collection period. Excreta samples in each treatment were pooled in pairs on the basis of bird body weight, resulting in 3 replicates per treatment. In the 48 hour fresh treatment, 2 of the bags had holes due to pecking and were eliminated from the trial, leaving 2 replicates for the 48 hour fresh treatment. Pooled samples were homogenized, weighed and frozen. The two treatments, 4 hour freeze dry and 48 hour freeze dry were freeze dried, equilibrated with atmospheric moisture, weighed, ground, mixed and frozen. All samples of trial A and trial B were analyzed for total nitrogen, moisture, uric acid, urea, ammonia and creatinine.

VI. Experiment 5. Use of a preservative - mercuric chloride (0.3 per cent w/v) in glacial ace acid - to prevent the loss of nitrogen from poultry excreta over time and as a result of freeze drying.

This experiment was designed to investigate the potential use of a preservative to prevent losses of ammonia and hydrolysis of urea in poultry excreta as a result of an extended collection period and/or drying procedure. The preservative investigated was mercuric chloride (0.3 per cent w/v) in glacial acetic acid.

Thirty birds were fed ad libitum a standard maintenance diet (Table 1) throughout the assay period. Clean excreta collection paper was placed under each bird and the time was recorded. Exactly 1 hour later,

excreta from all 30 birds was collected, pooled into a single sample and homogenized. A sub sample was taken to represent 1 hour fresh excreta, weighed and frozen. Preservative was added to the remaining pooled sample until the excreta pH was less than 3. The amount of preservative required to reduce the pH was equal to approximately 1ml per 1g of excreta. This excreta was then divided into 2 sub samples of equal weight and allocated to 2 treatments: 1) 48 hour fresh plus preservative; 2) 48 hour freeze dry plus preservative. These samples were left untouched at room temperature (20°C) for 47 hours, plus the 1 hour initial collection period. Samples were then homogenized, weighed and frozen. The 48 hour freeze dry treatment was freeze dried, equilibrated with atmospheric moisture, weighed, ground, mixed and frozen. This procedure was repeated 6 times. All samples were analyzed for pH, moisture, total nitrogen, uric acid, urea, ammonia and creatinine.

VII. Experiment 6. Use of a preservative - boric acid powder plus (0.3 per cent w/w) mercuric chloride - to prevent the loss of nitrogen from poultry excreta over time and as a result of freeze drying.

Like experiment 5, this experiment was designed to investigate the potential use of a preservative to prevent losses of ammonia and hydrolysis of urea as a result of extended collection periods and/or drying procedure. The preservative investigated was boric acid powder plus 0.3 per cent w/w mercuric chloride.

The preservative used was made by dissolving 1g of boric acid powder plus 0.3 per cent w/w mercuric chloride in 20ml of water. The boric acid powder was brought into solution by mixing on a hot plate for approximately a half hour.

Experiment 6 was conducted in two trials, A and B. Experimental procedure for trial A was the same as that for experiment 5. The only difference was the type of preservative used. In trial A, 50ml of boric acid solution was added per day to the 2 treatments: 1) 48 hour fresh plus preservative; 2) 48 hour freeze dry plus preservative. Excreta samples were stirred upon the addition of preservative. Use of the boric acid preservative did not require determination of excreta pH. The procedure for trial A was repeated 6 times.

In trial B, 24 of the 30 birds of trial A were housed in wire cages. The birds were randomly allocated to 1 of 3 treatments, 8 birds per treatment: 1) 4 hour fresh; 2) 48 hour fresh plus preservative; 3) 48 hour freeze dry plus preservative. All birds received standard maintenance diet (Table 1) ad libitum thoughout the assay period. Birds were weighed, an excreta collection bag was attached over the cloaca and the time was recorded. The bags were attached by a generous application of adhesive around the opening of the bag, except for a small portion near the top of the bag. A long stem funnel was placed inside the bag via the gap in adhesive and 60ml of the boric acid solution was added at 0 and 24 hours. The 4 hour fresh and 48 hour fresh plus preservative treatment samples were frozen immediately after collection. The 48 hour freeze dry plus preservative samples were frozen, freeze dried, equilibrated with atmospheric moisture, weighed, ground, mixed and refrozen. In both 48 hour collection periods, 2 bags per treatment fell off resulting in 6 replicates per treatment. All samples in trials A and B were analyzed for total nitrogen, moisture, uric acid, urea, ammonia and creatinine.

VIII. Experiment 7. Quantification of nitrogen compounds in poultry excreta with added preservative and the contribution to a nitrogen correction factor for the TME assay.

Experiment 7 was designed to determine the effect of feed input on the proportion of excretory nitrogen compounds in poultry excreta collected in a preservative - boric acid powder plus 0.3 per cent w/w mercuric chloride. The contribution of the excretory nitrogen compounds to a nitrogen correction factor for the TME assay was also determined.

This experiment was designed identical to experiment 2 so that a direct effect of a preservtitive on excretory nitrogen compounds could be established. The cracked wheat that was force fed (Table 4b) was the same sample as was used in experiment 2 (Table 4a). Experiment 7 differed from experiment 2 in the following ways. Collection bags were attached to 30 birds as described in experiment 6, trial B. The preservative was the same as that used in experiment 6 and was added to the excreta collection bags (60ml) at 0 and 24 hours for a total of 120ml per collection bag. In all treatments, full fed, force fed and unfed, several bags fell off, resulting in 6,5 and 9 samples per treatment respectively. All excreta samples were analyzed for total nitrogen, gross energy, moisture, uric acid, ammonia and creatinine. Addition of the preservative reduced sample organic matter thereby preventing burning of the sample in the oxygen bomb calorimeter. This problem was overcome by adding benzoic acid to the sample in a 1:1 ratio.

RESULTS

I. Experiment 1. True metabolizable energy values of different cultivars of corn and barley.

The mean TME and TMEn values of different cultivars of corn and barley assayed by standard procedures are presented in Table 5a. Experimental values are compared to typical literature values presented by Sibbald (1983) in Table 5b. The TME values of the corn samples assayed fit within the expected range of values, except for the TME value of U.S. corn (16.61 MJ/Kg) which was less than the lowest literature value (16.64 MJ/Kg). The TME values determined for all of the barley cultivars were less than the literature TME data. The TMEn values determined for all of the corn samples were less than the respective values listed in Table 5b. All corn TMEn values were less than 15.92 MJ/Kg. The TMEn values for barley cultivars were less than the literature TMEn values, except for the value of Klages barley (12.52 MJ/Kg) which was within the literature TMEn range of values (12.41 to 14.28 MJ/Kg).

Excreta weight ranged from 7.86g to 8.49g for corn fed birds and from 10.33g to 11.08g for the barley fed birds (Table 6). There were no significant ($P > 0.05$) differences among values within each range. The mean excreta weight of the unfed birds was 5.99g. Excreta weight was significantly ($P < 0.05$) different among dietary treatments. There were, however no significant ($P > 0.05$) differences in excreta weights among birds fed Alberta corn, U.S. corn and Betzes barley. There was also no signifi-

Table 5a. The TME and TMEn values of different cultivars of corn and barley assayed by standard procedure[1] - Experiment 1.

Feedstuff	TME	TMEn
	MJ/Kg dry matter	
Alberta Corn	16.89	14.81
Manitoba Corn	17.18	14.85
U.S. Corn	16.61	14.83
Barley c.v. Ifd	15.20	12.12
Barley c.v. Betzes	15.28	12.39
Barley c.v. Bonanza	15.45	12.15
Barley c.v. Klages	15.72	12.52

[1] Sibbald (1983).

Table 5b. Literature TME and TMEn values of different cultivars of corn and barley.[1]

	TME		TMEn	
Feedstuff	Range	Mean	Range	Mean
	MJ/Kg dry matter			
Corn. ground, yellow	16.64-17.56	17.10	15.92-16.62	16.27
Barley. whole, ground	12.75-14.76	14.03	12.41-14.28	13.38

[1] Sibbald (1983)

Table 6. The contribution of uric acid nitrogen to excreta weight, excreta total nitrogen and total excreta energy in excreta collected under standard TME assay procedure - Experiment 1.[1]

Feedstuff	Excreta Weight	Total Nitrogen	Uric Acid[2] Nitrogen	Uric Acid[3] Nitrogen	Excreta Energy	Uric Acid[4] Energy
	(g)	(g)	(g)	(g)	(kJ)	(%)
Alberta Corn	8.25^{ab}[5]	1.01^{a}	12.01^{ab}	99.05^{a}	97.66^{a}	34.99^{a}
Manitoba Corn	7.86^{ad}	1.01^{a}	11.93^{ab}	92.74^{ab}	92.89^{a}	35.04^{a}
U.S. Corn	8.49^{ab}	1.07^{a}	10.50^{b}	83.37^{b}	93.72^{a}	32.81^{ab}
Barley c.v. IFd	11.08^{c}	1.18^{a}	8.62^{b}	83.30^{b}	150.12^{b}	22.00^{b}
Barley c.v. Betzes	10.33^{bc}	1.18^{a}	9.45^{b}	82.63^{b}	150.58^{b}	24.41^{ab}
Barley c.v. Bonanza	10.84^{c}	1.14^{a}	8.81^{b}	84.25^{b}	144.77^{b}	22.74^{ab}
Barley c.v. Klages	10.88^{c}	1.24^{a}	9.53^{b}	83.30^{b}	143.68^{b}	24.96^{ab}
Unfed	5.99^{d}	1.19^{a}	18.23^{a}	87.81^{ab}	61.97^{c}	57.95^{c}
Root MSE	1.01	0.23	3.02	6.09	2.51	5.63

[1] all values expressed on a dry matter basis

[2] expressed as grams of nitrogen per 100 grams of total excreta

[3] expressed as grams of nitrogen per 100 grams of nitrogen excreted

[4] expressed as % of total excreta energy

[5] means within a column followed by the same superscript are not significantly different at $P< 0.05$

cant (P> 0.05) differences between excreta weights of birds fed Manitoba corn (7.86g) and that of unfed birds (5.99g).

Excreta total nitrogen of the corn fed birds ranged from 1.01g to 1.07g and from 1.14g to 1.24g for barley fed birds (Table 6). The mean excreta total nitrogen for the unfed birds was 1.19g. There was no significant (P> 0.05) differences among any of the values.

To prevent any bias which may occur due to differences in excreta weight, uric acid nitrogen was expressed as grams of nitrogen per 100 grams of total excreta (Table 6). No significant (P> 0.05) differences in uric acid nitrogen values between corn fed and barley fed birds was observed. The unfed birds excreted uric acid nitrogen (18.23g) at a level which was significantly (P< 0.05) different from all other treatments, except Alberta corn (12.01g) and Manitoba corn (11.93g).

To prevent any bias which may occur due to differences in excreta total nitrogen, uric acid nitrogen was expressed as grams of nitrogen per 100 grams of total nitrogen (Table 6). Excreta uric acid nitrogen levels were similar for all treatments. The only exception was for Alberta corn fed birds (99.05g) which were significantly (P< 0.05) different from all other treatments except the Manitoba corn fed birds (92.74g) and the unfed treatment (87.81g). In all treatments, uric acid nitrogen comprised the majority (82.63g to 99.05g) of total nitrogen excreted.

Total excreta energy was determined by oxygen bomb calorimetry and the mean values are presented in Table 6. Excreta energy for the corn fed birds ranged from 92.89 kJ to 97.66 kJ, for the barley fed birds the range was 143.68kJ to 150.78kJ and for the unfed birds mean excreta energy was 61/79kJ. There was no significant (P> 0.05) differences among values within a range. There were, however, significant (P< 0.05) differences among dietary treatments.

No significant (P> 0.05) differences within diet means were observed for uric acid energy expressed as a per cent of total excreta energy (Table 6). In general, there were significant (P< 0.05) difference among diets. The exception to this was excreta of U.S. corn fed birds which was not significantly (P> 0.05) different from any barley treatment values, and Klages and Betzes barley which were not significantly (P> 0.05) different from any corn treatment values. Excreta of the unfed birds was significantly (P< 0.05) different from all other values.

II. Experiment 2. Quantification of nitrogen compounds in poultry excreta and the contribution to a nitrogen correction factor for the TMEn assay.

Excreta weight and excreta total nitrogen are listed in Tables 7 and 8, respectively. Excreta weight of birds fed ad libitum maintenance diet (83.94g) was significantly (P< 0.05) larger than that of force fed (21.81g) and unfed birds (17.18g), which did not differ (P> 0.05) significantly (Table 7). Regardless of the large differences in excreta weight, there were no significant (P> 0.05) differences in total nitrogen excretion among full fed (4.79g), force fed (3.98g) and unfed (3.89g) birds (Table 8).

The excreta nitrogen containing compounds, uric acid, urea, ammonia, creatinine and amino acids presented in Table 7 are expressed as grams of nitrogen per 100 grams of total excreta. Uric acid nitrogen was highest in the unfed bird excreta (21.06g) and lowest in excreta of birds fed ad libitum maintenance diets (4.36g). Excreta of the force fed birds contained an intermediate level (15.62g) of uric acid nitrogen. All treatment means were significantly (P< 0.05) different from each other.

Urea nitrogen was highest in excreta of the unfed birds (0.33g), but this was not significantly (P> 0.05) different from the urea nitrogen level

Table 7. Effect of feed input on the major excretory nitrogen compounds in poultry excreta, expressed on the basis of total excreta weight - Experiment 2.

Treatment	Excreta Weight	Grams of nitrogen per 100 grams of total excreta: Uric Acid	Urea	Ammonia	Creatinine	Amino Acids
	(g)					
Full Fed	83.94[a1]	4.36[a]	0.01[a]	0.77[a]	0.02[a]	0.88[a]
Force Fed	21.81[b]	15.62[b]	0.07[ab]	2.31[b]	0.04[b]	0.43[b]
Unfed	17.18[b]	21.06[c]	0.33[b]	2.09[b]	0.05[b]	0.31[c]
Root MSE	14.29	0.98	0.14	0.15	0.01	0.06

[1] means within a column follwed by the same superscript are not significantly different at $P< 0.05$

Table 8. Effect of feed input on the major excretory nitrogen compounds in poultry excreta, expressed on the basis of total excreta nitrogen - Experiment 2.

Treatment	Total Nitrogen	Grams of nitrogen per 100 grams of total nitrogen excreted				
		Uric Acid	Urea	Ammonia	Creatinine	Amino Acids
	(g)					
Full Fed	4.79[a1]	74.96[a]	0.16[a]	13.11[a]	0.29[a]	15.16[a]
Force Fed	3.98[a]	86.28[b]	0.27[ab]	12.85[a]	0.22[ab]	2.40[b]
Unfed	3.89[a]	93.37[c]	1.45[b]	9.25[b]	0.21[b]	1.38[b]
Root MSE	0.78	2.22	0.64	1.17	0.04	0.71

[1] means within a column followed by the same superscript are not significantly different at $P < 0.05$

in excreta of the force fed birds (0.07g). Excreta urea nitrogen of the full fed birds (0.01g) was low, but not significantly ($P > 0.05$) different from that of the force fed birds. There was a significant ($P < 0.05$) difference, however, between the excreta urea nitrogen levels of unfed and full fed birds.

Ammonia nitrogen levels were high, but not significantly ($P > 0.05$) different between excreta of the force fed (2.31g) and unfed birds (2.09g). Both of these values were significantly ($P < 0.05$) larger than the excreta ammonia nitrogen value of the full fed birds (0.77g).

Excreta creatinine nitrogen levels were relatively low in all three treatments. Creatinine nitrogen in the excreta of full fed birds (0.02g) was significantly ($P < 0.05$) less than that in the excreta of force fed (0.04g) and unfed (0.05g) birds which did not differ significantly ($P > 0.05$).

The amounts of amino acid nitrogen excreted by birds in all three treatments was low and differed significantly ($P < 0.05$) among treatments. The levels which occured were 0.88g in the full fed birds excreta, 0.43g in the force fed birds excreta and 0.31g in the excreta of the unfed birds.

The excreta nitrogen containing compounds were also expressed as grams of nitrogen per 100 grams of total nitrogen and are presented in Table 8. Excreta uric acid nitrogen was 93.37g for the unfed birds, 86.28g for the force fed birds and 74.96g for the full fed birds. All values were significantly ($P < 0.05$) different.

Urea nitrogen was highest in the excreta of the unfed birds (1.45g) which did not differ significantly ($P > 0.05$) from the excreta urea nitrogen value of the force fed birds (0.27g). Excreta of the full fed birds contained 0.16g of urea nitrogen which was not significantly ($P > 0.05$) different from the excreta of the force fed birds (0.27g), but significantly ($P < 0.05$) different from that of the unfed birds (1.45g).

Ammonia nitrogen was high in the excreta of full fed (13.11g) and force fed (12.85g) birds and these values did not differ significantly ($P > 0.05$). The lowest value of ammonia nitrogen was in the unfed bird excreta (9.25g) which was significantly ($P < 0.05$) different from the other two treatments.

Creatinine nitrogen excretion was low and relatively constant, ranging from a low of 0.21g and 0.22g per 100 grams of excreta total nitrogen for the unfed and force fed birds, respectively, to a high of 0.29g for the full fed birds. Only the difference between the full fed and unfed bird values was significant ($P < 0.05$).

The amino acid nitrogen excretion level was high in the excreta of full fed birds (15.16g). This value was significantly ($P < 0.05$) different from that for the force fed (2.40g) and unfed (1.38g) birds, which did not differ significantly ($P > 0.05$)

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined and is presented in Table 9a. In all three treatments, uric acid nitrogen represents the largest, but not the sole portion of the nitrogen correction factor. Ammonia nitrogen was also shown to make an important contribution to the correction factor. Both urea nitrogen and creatinine nitrogen provided only a small contribution. This trend was expected considering the grams of nitrogen of each compound excreted (Table 8) and the energetic value of each compound (Table 9b). The correction factors which were determined were 36.92 kJ/g RN for full fed birds, 40.57 kJ/g RN for force fed birds and 40.28 kJ/g RN for birds which were unfed.

Table 9a. Contribution of individual nitrogen containing compounds to a nitrogen correction factor for the TME assay - Experiment 2.

Treatment	Uric Acid	Urea	Ammonia	Creatinine	Correction Factor
	----------kJ/g N----------				
Full Fed	25.78	0.04	10.94	0.16	36.92
Force Fed	29.67	0.06	10.72	0.12	40.57
Unfed	32.11	0.33	7.72	0.12	40.28

Table 9b. Energetic Value of Excreta nitrogen compounds[1]

Compound	kJ/g	kJ/g N
Uric Acid	11/46	34.39
Urea	10.50	22.55
Ammonia[2]	68.62	83.43
Creatinine	20.84	56.11

[1] from C.R.C. Handbook of Chemistry and Physics, 63rd edition (1982-1983)
[2] from Comprehensive Inorganic Chemistry, Vol. 2. (1976.)

III. Experiment 3. Effect of the duration of excreta collection period on the proportion of nitrogen compounds in poultry excreta.

A. Trial A.

Excreta weight and excreta total nitrogen are presented in Table 10. Treatment samples in trial A were sub samples of equal weight taken from a single pooled excreta sample from 24 birds collected for a period of 1 hour. As a result, mean excreta weight for each treatment was not significantly ($P > 0.05$) different. Values ranged from 16.10g to 16.82g. Similarly, there were no significant ($P > 0.05$) differences in excreta total nitrogen among treatments. Values ranged from 1.04g to 1.08g.

Excreta uric acid, urea, ammonia and creatinine were determined and expressed as grams of nitrogen per 100 grams of total excreta. The mean values for each treatment are presented in Table 10. Uric acid nitrogen did not differ significantly ($P > 0.05$) among treatments, however, the control sample tended to have a higher uric acid nitrogen level (6.41g) than excreta left exposed to the atmophere for the 24 hour (5.46g) or 48 hour (5.66g) time periods.

Excreta urea nitrogen in the control sample was 0.16g which was not significantly ($P < 0.05$) different from the 24 hour sample (0.13g) but significantly ($P < 0.05$) different from the 48 hour sample (0.02g). There was no significant ($P > 0.05$) difference between the 24 hour and 48 hour samples, but the 48 hour sample tended to be lower. This data showed a significant ($P < 0.05$) decrease in excreta urea nitrogen level as a result of increased time of exposure of the excreta to atmospheric conditions.

Time had an opposite effect on ammonia nitrogen level in comparison to the effect on urea. There was a significant ($P < 0.05$) increase of excreta ammonia nitrogen as the length of time of exposure of the excreta to

Table 10. Excreta total nitrogen and nitrogen compounds in poultry excreta collected on trays for 1 hour and subsequently left exposed to atmospheric conditions for 24 and 48 hours, expressed on the basis of total excreta weight - Experiment 3 (trial A).

Treatment	Excreta Weight	Total Nitrogen	Grams of nitrogen per 100 grams of total excreta: Uric Acid	Urea	Ammonia	Creatinine
	(g)	(g)				
1 Hour Excreta [1]	16.10[a2]	1.05[a]	6.41[a]	0.16[a]	0.44[a]	0.03[a]
24 Hour Excreta	16.82[a]	1.08[a]	5.46[a]	0.13[ab]	0.53[ab]	0.04[a]
48 Hour Excreta	16.67[a]	1.04[a]	5.66[a]	0.02[b]	0.87[b]	0.04[a]
Root MSE	6.16	0.41	0.93	0.06	0.18	0.004

[1] control treatment

[2] means within a column follwed by the same superscript are not significantly different at $P < 0.05$

the atmosphere increased. There was no significant ($P > 0.05$) difference between control (0.44g) and 24 hours (0.53g) samples, but there was a significant ($P < 0.05$) difference between control (0.44g) and 48 hour (0.87g) samples. The 24 hour and 48 hour excreta samples were not significantly ($P > 0.05$) different.

The length of time which excreta samples were exposed to the atmosphere was shown to have no effect of excreta creatinine nitrogen levels.

B. Trial B.

Excreta weight and excreta total nitrogen are presented in Table 11. Mean excreta weight and total nitrogen values were significantly ($P < 0.05$) different among treatments. As expected, the total excreta weight voided increased as the length of time the samples were collected increased from 4 hours (control)(5.15g) to 24 hours (34.09g) and48 hours (76.93g). Similarly, as the length of excreta collection period increased, the excreta total nitrogen content increased. Excreta total nitrogen of the 4 hour (control) bird (0.39g) increased to 2.68g after 24 hours, and to 6.29g after 48 hour collection periods.

The excreta nitrogen compounds, uric acid, urea, ammonia, and creatinine expressed as grams of nitrogen per 100 grams of total excreta are presented in Table 11. Length of the excreta collection period did not affect uric acid nitrogen levels. Values ranged from 6.33g to 7.09g, with no significant ($P > 0.05$) differences among treatments.

Urea nitrogen values, however, were significantly ($P < 0.05$) affected by the length of the excreta collection period. There was a significant ($P < 0.05$) decrease in urea nitrogen from 4 hours (0.34g) to 24 hours (0.15g). The 48 hour value (0.19g) was less than the 4 hour (control) value, but this difference was not significant ($P > 0.05$). There was no significant

Table 11. Effect of duration of collection period on the excreta nitrogen compounds in poultry excreta collected in colostomy bags, expressed on the basis of total excreta weight - Experiment 3 (trial B)

Treatment	Excreta Weight	Total Nitrogen	Grams of nitrogen per 100 grams of total excreta			
			Uric Acid	Urea	Ammonia	Creatinine
	(g)	(g)				
4 Hour Excreta[1]	5.15[a2]	0.39[a]	6.33[a]	0.34[a]	0.36[a]	0.04[a]
24 Hour Excreta	34.09[b]	2.68[b]	7.09[a]	0.15[b]	0.74[b]	0.04[a]
48 Hour Excreta	76.93[c]	6.29[c]	6.61[a]	0.19[ab]	1.06[c]	0.04[a]
Root MSE	7.63	0.79	1.35	0.08	0.12	0.01

[1] control treatment

[2] means within a column followed by the same superscript are not significantly different at $P < 0.05$

($P > 0.05$) difference between the 24 hour and 48 hour excreta values.

Length of the excreta collection period significantly ($P < 0.05$) increased from 0.36g to 1.06g by extending the collection period from 4 hours to 48 hours. All collection period values were significantly ($P < 0.05$) different from each other.

Creatinine nitrogen was not affected by duration of the collection period. At all time intervals, the excreta creatinine nitrogen level was 0.04g.

IV. Experiment 4. Effect of freeze drying on the proportion of nitrogen compounds in poultry excreta.

A. Trial A.

Excreta weight and excreta total nitrogen are presented in Table 12. As expected, due to the method of sample collection, there were no significant ($P > 0.05$) differences among the excreta weights of the various treatments. Mean excreta values ranged from 10.34g to 11.78g. Similarly, excreta total nitrogen did not differ significantly ($P > 0.05$) among treatments. Values ranged from 0.62g to 0.67g.

The excreta nitrogen containing compounds, uric acid, urea ammonia and creatinine are expressed as grams of nitrogen per 100 grams of total excreta and are presented in Table 12. Freeze drying of excreta had no effect on the level of uric acid nitrogen. The freeze dried samples tended to have less uric acid nitrogen than fresh excreta samples of a corresponding time period, but this difference was not significant ($P > 0.05$). There was no effect of the length of time that excreta samples were exposed to the atmosphere on excreta uric acid nitrogen values.

Conflicting results of the effect of freeze drying on excreta urea

Table 12. Effect of freeze drying on the excreta nitrogen compounds in poultry excreta collected on trays for 1 hour and left exposed to atmospheric conditions for 48 hours, expressed on the basis of total excreta weight - Experiment 4 (trial A)

Treatment	Excreta Weight	Total Nitrogen	Grams of nitrogen per 100 grams of total excreta: Uric Acid	Urea	Ammonia	Creatinine
	(g)	(g)				
1 Hour Fresh[1]	11.03[a2]	0.62[a]	4.79[a]	0.06[a]	0.35[a]	0.03[a]
48 Hour Fresh	10.34[a]	0.67[a]	4.83[a]	0.04[a]	0.48[b]	0.04[b]
1 Hour Freeze Dry	11.78[a]	0.62[a]	4.46[a]	0.12[b]	0.12[c]	0.04[b]
48 Hour Freeze Dry	11.28[a]	0.61[a]	4.43[a]	0.05[a]	0.26[a]	0.04[b]
Root MSE	1.65	0.09	0.45	0.02	0.05	0.003

[1] control treatment

[2] means within a column followed by the same superscript are not significantly different at $P < 0.05$

Analysis of Varience Table

Source	DF	Excreta Weight	Total Nitrogen	Uric Acid	Urea	Ammonia	Creatinine
		PR>F					
Treatment	1	0.3301	0.5555	0.2584	0.0074	0.0001	0.0022
Time	1	0.4862	0.6748	0.6367	0.0010	0.0003	0.0032
TRT x Time	1	0.9115	0.5485	0.7729	0.0139	0.7410	0.4124
Error	12				*		

*Significant interaction at $P < 0.05$

nitrogen level wereobserved. Excreta urea nitrogen increased significantly ($P< 0.05$) in the control samples from 0.06g to 0.12g as a result of freeze drying. In the 48 hour samples, no significant ($P> 0.05$) effect of freeze drying was observed. Urea nitrogen in both the fresh and freeze dried samples decreased as the length of time the excreta samples were exposed to atmospheric conditions increased. For the fresh samples, urea nitrogen decreased from 0.06g to 0.04g, but this difference was not significant ($P< 0.05$). For the freeze dried samples, urea nitrogen decreased significantly ($P< 0.05$) from 0.12g to 0.05g.

The results of trial A (Table 12) indicated a significant ($P< 0.05$) effect of freeze drying on excreta ammonia nitrogen. Freeze drying significantly ($P< 0.05$) decreased excreta ammonia nitrogen level from 0.35g to 0.12g for the control samples and from 0.48 to 0.26g for the 48 hour samples. A marked effect of the length of time that excreta samples were exposed to the atmosphere on excreta ammonia nitrogen levels was also demonstrated. Excreta ammonia nitrogen significantly ($P<0.05$) increased after 48 hours from 0.35g to 0.48g for the fresh excreta and from 0.12g to 0.26g for the freeze dried excreta.

No consistant significant ($P> 0.05$) effect of freeze drying, or of time period could be shown for excreta creatinine. For all treatments the creatinine nitrogen level was constant at 0.04g, except for the control sample (0.03g) which was significantly ($P<0.05$) different from all the other treatments.

In experiment 4, trial A (Table 12), the interaction between time period and drying procedure was studied. The interaction was not significant ($P> 0.05$) for any experimental parameters except urea nitrogen. The significant ($P< 0.05$) interaction for urea nitrogen suggests that freeze drying had a greater effect on excreta which was collected for 1 hour then

on excreta which was collected for 48 hours.

B. Trial B.

Excreta weight and excreta total nitrogen are presented in Table 13. As expected, the longer the excreta was collected, the larger sample excreta weights became. For both the fresh and freeze dried excreta samples, extension of the collection period to 48 hours signigicantly ($P < 0.05$) increased excreta weight. Excreta weight increased from 5.25g to 67.84g from the fresh excreta and from 10.67g to 96.09g for the freeze dried excreta. The data presented in Table 13 indicated that freeze drying increased excreta weight from 5.25g to 10.67g for the 4 hour samples and from 67.84g to 96.09g for the 48 hour samples. This effect was only significant ($P < 0.05$) for the 48 hour samples and may not be a true treatment effect. Like excreta weight, excreta total nitrogen (Table 13) increased significantly ($P < 0.05$) by extension of the collection period to 48 hours for both fresh and freeze dried samples. Excreta total nitrogen increased from 0.51g to 5.31g for the fresh excreta and from 0.88g to 7.12g for the freeze dry excreta. No significant ($P > 0.05$) effect of freeze drying on excreta total nitrogen was observed, however, freeze dried samples tended to have more total nitrogen than non-freeze dried samples. This may not be a true treatment effect of freeze drying, which parallels the freeze drying effect noted with excreta weight.

The excreta nitrogen containing compounds uric acid, urea, ammonia and creatinine were expressed as grams of nitrogen per 100 grams of total excreta and are presented in Table 13. Excreta uric acid nitrogen was relatively constant, ranging from 6.21g to 7.36g, except for the 4 hour fresh excreta sample (9.33g) which was significantly ($P < 0.05$) different from all other treatments. Uric acid nitrogen in the 4 hour excreta samples de-

Table 13. Effect of freeze drying on the excreta nitrogen compounds in poultry excreta collected in colostomy bags for 4 hours or 48 hours, expressed on the basis of total excreta weight - Experiment 4 (trial B).

			Grams of nitrogen per 100 grams of total excreta			
Treatment	Excreta Weight	Total Nitrogen	Uric Acid	Urea	Ammonia	Creatinine
	(g)	(g)				
4 Hour Fresh[1]	5.25[a2]	0.51[a]	9.33[a]	0.41[a]	0.35[a]	0.04[a]
48 Hour Fresh	67.84[b]	5.31[b]	6.41[b]	0.10[b]	0.78[b]	0.04[a]
4 Hour Freeze Dry	10.67[a]	0.88[a]	7.36[b]	0.28[ab]	0.29[a]	0.04[a]
48 Hour Freeze Dry	96.09[c]	7.12[b]	6.21[b]	0.06[b]	0.59[b]	0.04[a]
Root MSE	8.96	0.69	0.57	0.10	0.07	0.005

[1] control treatment

[2] means within a column followed by the same superscript are not significantly different at $p < 0.05$

Analysis of Varience Table

Source	DF	Excreta Weight	Total Nitrogen	Uric Acid	Urea	Ammonia	Creatinine
Treatment	1	0.0181	0.0358	0.0171	0.2075	0.0285	0.7396
Time	1	0.0001	0.0001	0.0006	0.0042	0.0001	0.1605
TRT x Time	1	0.0759	0.1298	0.0381	0.5047	0.2038	0.4367
Error	12			*			

*Significant interaction at $P<0.05$

creased significantly ($P< 0.05$) from 9.33g to 7.36g after freeze drying. Excreta uric acid nitrogen in the 48 hour samples was not affected by freeze drying. Extension of the excreta collection period from 4 hours to 48 hours caused a decrease in the excreta uric acid nitrogen content for both the fresh (9.33g to 6.41g) and freeze dried samples (7.31g to 6.21g), however, only the decrease in the fresh excreta samples was significant ($P< 0.05$).

Freeze drying of excreta tended to decrease urea nitrogen in both the 4 hour (0.41g to 0.28g) and 48 hour samples (0.10g to 0.06g), but neither decrease was significant ($P> 0.05$). Extension of the excreta collection period from 4 hours to 48 hours resulted in a decrease in excreta urea nitrogen for the fresh (0.41g to 0.10g) and freeze dry samples (0.28g to 0.06g). Only the loss of urea nitrogen in the fresh samples was significant ($P< 0.05$).

Freeze drying of excreta tended to decrease the ammonia nitrogen level in the 4 hour excreta from 0.35g to 0.29g and in the 48 hour excreta from 0.78g to 0.59g, but neither loss was significant ($P> 0.05$). Excreta ammonia nitrogen was affected by time, such that extension of the collection period from 4 hours to 48 hours significantly ($P< 0.05$) increased ammonia nitrogen in both the fresh (0.35g to 0.78g) and freeze dry (0.29g to 0.59g) samples.

Excreta creatinine nitrogen was not affected by drying procedure, or by extension of the excreta collection period. For all excreta samples, the excreta creatinine nitrogen value was consistant at 0.04g, regardless of experimental treatment.

In experiment 4, trial B (Table 13), the interaction between excreta collection period and drying procedure was studied. The interaction was not significant ($P> 0.05$) for any experiment of parameters except uric acid.

The significant ($P< 0.05$) interaction for uric acid suggests that freeze drying had a greater effect on excreta which was collected for 4 hours than on excreta which was collected for 48 hours.

V. Experiment 5. Use of a preservative - mercuric chloride (0.3 per cent w/v) in glacial acetic acid - to prevent the loss of nitrogen from poultry excreta over time and as a result of freeze drying.

Excreta weight and excreta total nitrogen are presented in Table 14. Treatment samples were sub samples of equal weight taken from a single pooled excreta sample from 24 birds collected for a period of 1 hour. As a result there was no significant ($P> 0.05$) difference between treatment means. Excreta weight values ranged from 19.29g to 22.12g. Similarly, excreta total nitrogen was not significantly ($P> 0.05$) different among treatments. Values ranged from 0.95g to 1.08g.

The excreta nitrogen containing compounds uric acid, urea, ammonia and creatinine presented in Table 14 were expressed as grams of nitrogen per 100 grams of total excreta. The 48 hour fresh (3.25g) and 48 hour freeze dry (3.47g) excreta samples tended to be lower in uric acid nitrogen content than the control sample (3.98g), but these differences were not significant ($P> 0.05$).

The excreta urea nitrogen level decreased from 0.08g for the control sample to 0.05g and 0.04g for the 48 hour fresh and 48 hour freeze dry samples, respectively. This decrease, although not significant ($P> 0.05$) indicated a lack of effectiveness of the preservative in preventing hydrolysis of urea. There was no significant ($P> 0.05$) difference between urea nitrogen levels of the 48 hour fresh and 48 hour freeze dry samples. As such, no effect of freeze drying on excreta urea nitrogen was observed.

Table 14. Effect of time period and freeze drying procedure on the excreta nitrogen compounds in poultry excreta collected on trays for 1 hour and left exposed to atmospheric conditions for 48 hours with added preservative (mercuric chloride 0.3 per cent w/v in glacial acetic acid), expressed on the basis of total excreta weight - Experiment 5.

Treatment	Excreta Weight	Total Nitrogen	Uric Acid	Urea	Ammonia	Creatinine	pH
		Grams of nitrogen per 100 grams of total excreta					
	(g)	(g)					
1 Hour Excreta[1]	19.29[a2]	1.05[a]	3.98[a]	0.08[a]	0.31[a]	0.03[a]	7.0
48 Hour Fresh plus preservative	22.01[a]	1.08[a]	3.25[a]	0.05[a]	0.20[b]	0.02[b]	3.0-3.5
48 Hour ~~Fresh~~ Frozen plus preservative	22.12[a]	0.95[a]	3.47[a]	0.04[a]	0.09[c]	0.03[a]	5.5-6.0
Root MSE	4.66	0.19	0.37	0.04	0.04	0.003	

[1] control treatment

[2] means with a column followed by the same superscript are not significantly different at $P < 0.05$

Excreta ammonia nitrogen was highest in the control excreta (0.31g) and lowest in the 48 hour freeze dried excreta (0.09g). The 48 hour fresh excreta sample had an intermediate level of ammonia nitrogen (0.20g). All treatment values were significantly ($P< 0.05$) different. This data indicated a lack of effectiveness of the added preservative.

Excreta creatinine nitrogen was the same for the control (0.03g) and 48 hour freeze dried samples (0.03g). The creatinine nitrogen level of the 48 hour fresh sample, however, was significantly ($P< 0.05$) lower (0.02g) than the other treatment means.

Preservative was added to the 48 hour fresh and 48 hour freeze dried samples at a volume sufficient to decrease the excreta pH to 3.0 or less. At the end of the 48 hour time period, excreta pH was determined. Treatment values are presented in Table 14. The excreta pH of the control excreta was 7.0, which represents excreta which did not have preservative added. The pH of the 48 hour fresh excreta was 3.0 - 3.5. Freeze drying of the 48 hour preserved excreta increased the pH to 5.5 - 6.0.

VI. Experiment 6. Use of a preservative - boric acid powder plus (0.3 per cent w/w) mercuric chloride - to prevent the loss of nitrogen from poultry excreta over time and as a result of freeze drying.

A. Trial A.

Excreta weight and excreta total nitrogen are presented in Table 15. Due to the method of sample collection, there were no significant ($P> 0.05$) differences among excreta weights for the various treatments. Mean excreta weights ranged from 15.85g to 17.42g. Similarly, excreta total nitrogen was not significantly ($P> 0.05$) different among treatments. Treatment mean values ranged from 0.91g to 1.02g.

Table 15. Effect of time period and freeze drying procedure on the excreta nitrogen compounds in poultry excreta collected on trays for 1 hour and left exposed to atmospheric conditions for 48 hours with added preservative (boric acid powder plus 0.3 per cent w/w mercuric chloride), expressed on the basis of total excreta weight - Experiment 6 (trial A).

			Grams of nitrogen per 100 grams of total excreta			
Treatment	Excreta Weight	Total Nitrogen	Uric Acid	Urea	Ammonia	Creatinine
	(g)	(g)				
1 Hour Excreta[1]	15.85[a2]	0.91[a]	4.30[a]	0.23[a]	0.36[a]	0.04[a]
48 Hour Fresh plus preservative	17.32[a]	0.92[a]	4.29[a]	0.21[a]	0.40[a]	0.04[a]
48 Hour Freeze Dry plus preservative	17.42[a]	1.02[a]	4.30[a]	0.18[a]	0.34[a]	0.04[a]
Root MSE	1.88	0.13	0.31	0.07	0.06	0.005

[1] control treatment

[2] means within a column followed by the same superscript are not significantly different at $P < 0.05$

The excreta nitrogen containing compounds uric acid, urea, ammonia and creatinine presented in Table 15 are expressed as grams of nitrogen per 100 grams of total excreta. Excreta uric acid nitrogen values were not significantly (P> 0.05) different among treatments. Addition of a preservative to the 48 hour fresh (4.29g) and 48 hour freeze dry (4.30g) excreta samples maintained uric acid nitrogen at a level similar to that of the 1 hour fresh excreta (4.30g)

The excreta urea nitrogen value for the 1 hour fresh excreta was 0.23g. Extension of the collection period to 48 hours (0.21g), or freeze drying of the 48 hour excreta (0.81g) did not significantly (P> 0.05) affect the urea nitrogen value of excreta samples with added preservative.

There were no significant (P>0.05) differences among treatments for excreta ammonia nitrogen values. Ammonia nitrogen of the 1 hour fresh excreta (0.36) was not significantly (P>0.05) different from the 48 hourfresh excreta value (0.40g). After freeze drying of the 48 hour excreta, there was a slight decrease in excreta ammonia nitrogen (0.34g) but this decrease was not significant (P>0.05).

Excreta creatinine nitrogen was constant at 0.04g. Creatinine nitrogen was not affected (P>0.05) by time period, freeze drying or the addition of boric acid powder plus (0.3 per cent w/w) mercuric chloride as a preservative.

B. Trial B.

Excreta weight and excreta total nitrogen are presented in table 16. Excreta that was collected for 4 hours weighed 5.34g which was significantly (P>0.05) less than the weight of excreta collected for 48 hours. There was no significant (P>0.05) difference between the 48 hour fresh (81.03) and 48 hour freeze dry (68.52g) excreta weights. Excreta total nitrogen followed the same pattern as excreta weight. The total nitrogen

Table 16. Effect of excreta collection period and freeze drying procedure on the excreta nitrogen compounds inpoultry excreta collected in colostomy bags with added preservative (boric acid powder plus 0.3 per cent w/w mercuric chloride), expressed on the basis of total excreta weight - Experiment 6 (trial B).

Treatment	Excreta Weight	Total Nitrogen	Grams of nitrogen per 100 grams of total excreta: Uric Acid	Urea	Ammonia	Creatinine
	(g)	(g)				
4 Hour Fresh[1]	5.34[a2]	0.40[a]	6.89[a]	0.28[a]	0.25[a]	0.04[a]
48 Hour Fresh plus preservative	81.30[b]	5.18[b]	5.76[a]	0.20[a]	0.36[a]	0.04[a]
48 Hour Freeze Dry plus preservative	68.52[b]	4.79[b]	5.57[a]	0.09[b]	0.34[a]	0.04[a]
Root MSE	10.64	0.66	1.18	0.06	0.11	0.006

[1] control treatment

[2] means within a column followed by the same superscript are not significantly different at $P < 0.05$

excreted after 4 hours was 0.40g, which was significantly ($P < 0.05$) less than the total nitrogen excreted in 48 hours for both the fresh (5.81g) and freeze dry excreta (4.79g). There was no significant ($P > 0.05$) difference between the 48 hour fresh and 48 hour freeze dry excreta total nitrogen values.

Treatment means for excreta uric acid, urea, ammonia and creatinine expressed as grams of nitrogen per 100 grams of total excreta are presented in Table 16. Excreta uric acid nitrogen for the 4 hour fresh sample was 6.89g. Extension of the collection period to 48 hours (5.76g) and freeze drying of the excreta (5.57g) decreased excreta uric acid nitrogen values slightly, however, this decrease was not significant ($P > 0.05$). There was no significant ($P > 0.05$) difference between the 48 hour fresh and 48 hour freeze dry samples.

There was no significant ($P > 0.05$) difference between the urea nitrogen levels of the 4 hour fresh (0.28g) and 48 hour fresh (0.20g) excreta samples with added preservative. Both of the 4 hour fresh and 48 hour fresh values were significantly ($P < 0.05$) different from the 48 hour freeze dry excreta sample (0.09g), which suggests a failure of the preservative to prevent an effect of freeze drying on excreta urea nitrogen.

Excreta ammonia nitrogen values, ranged from 0.25g to 0.36g, and were not significantly ($P > 0.05$) different among treatments. Ammonia nitrogen values were not affected by duration of the excreta collection period or freeze drying procedure for excreta samples with added preservative.

Creatinine nitrogen was not affected by duration of the collection period on freeze drying procedure following addition of the preservative to the excreta samples. There was no significant ($P > 0.05$) difference among the treatment means which were constant at 0.04g.

VII. Experiment 7. Quantification of nitrogen compounds in poultry excreta with added preservative and the contribution to a nitrogen correction factor for the TME assay.

Excreta weight and excreta total nitrogen are presented in Tables 17 and 18, respectively. Excreta weight of birds fed ad libitum maintenance diet (82.82g) was significantly ($P< 0.05$) different from that of force fed (14.37g) and unfed birds (12.26g), which did not differ significantly ($P> 0.05$)(Table 17). Total nitrogen excretion followed the same pattern as that of excreta weight. The full fed birds excreted 4.82g of total nitrogen which was significantly ($P< 0.05$) different from that of the force fed (2.03g) and unfed birds (1.89g). There was no significant ($P> 0.05$) difference between the force fed and unfed birds (Table 18).

Treatment means for excreta uric acid, urea, ammonia and creatinine expressed as grams of nitrogen per 100 grams of total excreta are presented in Table 17. Excreta uric acid nitrogen was lowest for the full fed birds (5.19g). This value was significantly ($P< 0.05$) less than the uric acid nitrogen level for the force fed (13.37g) and unfed birds (14.43g) which did not differ signigicantly ($P> 0.05$).

The excretion of urea nitrogen was relatively constant for all 3 treatments, with values ranging between 0.04g to 0.08g. The urea nitrogen level of the full fed birds (0.08g) was significantly ($P< 0.05$) different from that of the unfed birds (0.06g). There was no significant ($P> 0.05$) difference between the excreta urea nitrogen value of the force fed and unfed birds.

The excreta ammonia nitrogen content differed significantly ($P< 0.05$) among treatments and was inversely proportional to the level of feed intake, such that, as feed intake decreased the level of excreta ammonia ni-

Table 17. Effect of feed input on the excretory nitrogen compounds in poultry excreta with added preservative (boric acid powder plus 0.3 per cent w/w mercuric chloride), expressed on the basis of total excreta weight - Experiment 7.

Treatment	Excreta Weight	Uric Acid	Urea	Ammonia	Creatinine
	(g)	Grams of nitrogen per 100 grams of total excreta			
Full Fed	82.82[a1]	5.19[a]	0.08[a]	0.19[a]	0.04[a]
Force Fed	14.37[b]	13.37[b]	0.04[b]	0.47[b]	0.05[a]
Unfed	12.26[b]	14.43[b]	0.06[ab]	0.86[c]	0.05[a]
Root MSE	5.51	2.87	0.02	0.19	0.01

[1] means within a column followed by the same supercript are not significantly different at $P < 0.05$

Table 18. Effect of feed input on the excretory nitrogen compounds in poultry excreta with added preservative (boric acid powder plus 0.3 per cent w/w mercuric chloride), expressed on the basis of total excreta nitrogen - Experiment 7.

Treatment	Total Nitrogen	Grams of nitrogen per 100 grams of total nitrogen excreted: Uric Acid	Urea	Ammonia	Creatinine
	(g)				
Full Fed	4.82[a1]	89.10[a]	1.31[a]	3.25[a]	0.73[a]
Force Fed	2.03[b]	92.44[a]	0.27[b]	3.66[ab]	0.34[b]
Unfed	1.89[b]	95.20[a]	0.36[b]	5.65[b]	0.32[b]
Root MSE	0.56	4.43	0.16	1.42	0.10

[1] means within a column followed by the same superscript are not significantly different at $P < 0.05$

trogen increased. Excreta ammonia nitrogen was 0.19g for the full fed birds, 0.47g for the force fed birds and 0.86g for the unfed birds.

The excretion of creatinine nitrogen was unaffected by feed input and relatively constant for all 3 treatments. The full fed birds excreted 0.04g of creatinine nitrogen which was not significantly ($P > 0.05$) different from the creatinine nitrogen excretion of the force fed (0.05g) and unfed birds (0.05g). Excreta creatinine nitrogen was not significantly ($P > 0.05$) different between force fed and unfed birds.

Treatment means for excreta uric acid, urea, ammonia and creatinine expressed as grams of nitrogen per 100 grams of total nitrogen are presented in Table 18. Uric acid nitrogen made up the majority of the total nitrogen excreted by all birds in all 3 treatments, with values ranging from 89.10g to 95.20g. Although there were no significant ($P > 0.05$) differences among treatments, the proportion of total nitrogen as uric acid nitrogen was inversely proportional to feed input. Excreta uric acid nitrogen was 89.10g for the full fed birds, 92.44g for the force fed birds and 95.10g for the unfed birds.

In excreta of the full fed birds, the proporiton of total nitrogen as urea nitrogen was 1.31g, which was significantly ($P < 0.05$) greater than that of the force fed (0.27g) and unfed birds (0.36g). There was no significant ($P > 0.05$) difference between the excreta values for the force fed and unfed birds.

The excretion of ammonia nitrogen was greatly affected by the feed input level (Table 17), yet the proportion of total nitrogen as ammonia nitrogen remained relatively constant (Table 18). The ammonia nitrogen value for the full fed birds (3.25g) was not significantly ($P > 0.05$) different from that of the force fed birds (3.66g), but significantly ($P > 0.05$) different from that of the unfed birds (5.65g). The proportion of excreta

nitrogen as ammonia was not significantly ($P< 0.05$) different between force fed and unfed birds.

The excretion of creatinine nitrogen remained constant for the most part (Table 17), however the proporiton of total nitrogen as creatinine nitrogen (Table 18) was affected by feed input. Excreta creatinine nitrogen was highest in the full fed birds (0.73g) which was significantly ($P< 0.05$) different from force fed (0.34g) and unfed (0.32g) bird excreta values. The proportion of excreta total nitrogen as creatinine nitrogen was not significantly ($P> 0.05$) different between force fed and unfed birds.

The contribution of each excreta nitrogen compound to a nitrogen correction factor for the TME assay was determined and treatment values are presented in Table 19. In all 3 treatments, uric acid nitrogen represented the largest contribution to the nitrogen correction factor. Ammonia nitrogen was also shown to make an important contribution to the correction factor. Both urea nitrogen and creatinine nitrogen provided only a small contribution. The correction factors determined were 34.06kJ/g RN for the full fed birds, 35.10 kJ/g RN for the force fed birds and 37.70 kJ/g RN for the unfed birds.

Table 19. Contribution of individual nitrogen containing compounds to a nitrogen correction factor for the TME assay - Experiment 7.

Treatment	Uric Acid	Urea	Ammonia	Creatinine	Correction Factor
	kJ/g N				
Full Fed	30.64	0.30	2.71	0.41	34.06
Force Fed	31.79	0.06	3.06	0.19	35.10
Unfed	32.74	0.08	4.71	0.17	37.70

DISCUSSION

I. Experiment 1. True metabolizable energy values of different cultivars of corn and barley.

The TME values of the different cultivars of corn assayed in experiment 1 (Table 5a) were shown to fit within the expected range of literature values (Table 5b). The only exception to this was the TME value of U.S. corn (16.61MJ/Kg) which was slightly less than the lowest literature value (16.64MJ/Kg), however, not significantly different. The TME values of the different cultivars of barley assayed in experiment 1 (Table 5a) were greater than the literature range of values (Table 5b). This difference may be due to serveral reasons. Larger than normal TME values are observed whenever the collected excreta is less than the actual excreta output level. This will occur in situations of incomplete passage of the test material (Sibbald 1983). These problems may be prevented by using an excreta collection time period of appropriate duration specific to the test material (Sibbald 1979d) and by the use of colostomy bags to ensure total excreta collection. Other reasons for the observed differences may be the errors associated with nitrogen balance trials.

The TMEn values of the different cultivars of corn and barley assayed (Table 5a) were less than the literature data (Table 5b). Several reasons for this may exist. Dale and Fuller (1984) have shown TMEn value to be affected by the magnitude of the negative nitrogen balance of the fed bird. Sibbald (1982a) suggested that TMEn values are affected by the fatness of the unfed bird and vary according to the ability of the test material to

prevent tissue catabolism in the fed bird. Therefore, differences in determined TMEn values and the literature data may be due to differences in the nitrogen balance of the fed and unfed birds among trials. Other factors which may affect the TME nitrogen corrected assay are incomplete passage of the test material (Sibbald 1979), contamination of the excreta with energy and nitrogen containing compounds such as scales and feathers (Farrell 1981; Sibbald 1982a) and the errors associated with nitrogen balance trials.

The total excreta weight (Table 6) voided by birds was directly affected by the type of feed and feed input level. The corn, barley and unfed diets produced significantly different excreta weights with no significant differences among values within each range. The barley fed birds voided the largest amount of excreta, due to the high fiber content of barley. The unfed birds excreted the smallest excreta weight due to the zero feed input level. The excreta weights of Alberta corn, U.S. corn and Betzes barley fed birds were not significantly different. Conflicting results on the effect of feed input on rate of passage have been reported. Sibbald (1977b) observed no difference in the rate of passage in birds fed 10g to 100g of feed input. However, Sibbald (1980c) suggested feed input directly affects rate of passage. Factors which have been reported to affect rate of passage, at any given feed input level, are physical form of the feed (Sibbald 1979a), duration of starvation prior to feed input (Sibbald 1980c). The relationship between feed intake and rate of passage will then in turn affect excreta output. The result is a direct relationship between feed input level and total excreta weight.

There was a tendency to group total excreta nitrogen values (Table 6) according to the type of diet fed, however, there were no significant differences in the total nitrogen excretion among birds in any trial. It is

important to note that total nitrogen excretion is not an absolute measure of nitrogen balance or degree of tissue catabolism. However, both of these directly affect excreta total nitrogen (Shires et al. 1979).

Although excreta uric acid nitrogen, expressed on the basis of total excreta weight (Table 6), did not differ significantly among the force fed birds, there was a tendency to group uric acid nitrogen excretion on the basis of the type of diet fed. These results support the conclusion of Dale and Fuller (1984) that nitrogen excretion is related to the degree to which the test material can spare tissue catabolism. The large proportion of uric acid nitrogen excreted by the unfed birds was expected since the unfed bird must catabolize a greater proportion of tissue protein to meet maintanance requirements compared to the fed bird. The result is an increased uric acid excretion in the unfed birds as documented by Okumura and Tasaki (1969).

Comparing the protein content of the force fed diets (Table 3) with the uric acid excretion levels in Table 6, the data suggests that as dietary protein decreased, uric acid nitrogen excretion decreased. Such results are in conflict with literature data. Tasaki and Okumura (1964) showed the excretion of uric acid nitrogen to be directly correlated to protein intake, such that, as dietary protein intake increased uric acid nitrogen excretion increased. Similarly, as dietary protein intake decreased, uric acid nitrogen excretion decreased (Teekell et al. 1968). Any discrepancy between the results of experiment 1 and the literature data can be explained by the negative nitrogen balance of the assay birds which are force fed only 25g of feed. In the corn fed treatments, the protein content of the corn is low and may not be sufficient to offset tissue catabolism. Consequently uric acid nitrogen excretion is high among the corn fed birds, and in the case of Alberta corn and Manitoba corn fed birds not significantly differ-

ent from that for the unfed birds. For the birds force fed the barley diets, tissue catabolism may be spared to a certain degree by the higher protein content of the barley. As a result, uric acid nitrogen excretion was less in the barley fed birds when compared to the corn fed birds. Therefore, the degree to which the force fed material can spare tissue catabolism will directly affect uric acid excretion, nitrogen balance and ultimately TMEn.

Expressing uric acid nitrogen on the basis of total nitrogen (Table 6) was necessary to determine the contribution of uric acid to a nitrogen correction factor for the TMEn assay. The correction factor 34.39kJ/g of RN proposed by Hill and Anderson (1958) is based on the assumption that uric acid is the sole nitrogen excretory product of the chicken. From the data presented in Table 6, for all treatments, uric acid nitrogen represents the greatest proportion (over 80%) but not the sole excretory nitrogen product. These results are supported by several other authors (Table 20). Urea, ammonia and creatinine also make a significant contribution to excreta total nitrogen. Therefore, Titus (1956) calculated a nitrogen correction factor which included all excretory nitrogen containing compounds in addition to uric acid. By determining the gross energy of a sample of nondescript chicken urine from a full fed bird, Titus (1956) determined the nitrogen correction factor to be 36.53 kJ/g of RN.

It is important to note that in the TMEn assay both feces and urine are collected together quantitatively. The data presented in Table 20 and the correction factors proposed by both Hill and Anderson (1958) and Titus (1956) are based only on the analysis of urine. To accurately determine bioavailable energy, it is essential to calculate a nitrogen correction factor for the TMEn assay based on the contribution of the various nitrogen compounds in a mixed excreta (feces and urine) collected from birds

Table 20. Major urinary nitrogen compounds of the chicken

Reference	Percent nitrogen of total urinary nitrogen						
	Uric Acid	Urea	Ammonia	Creatinine	Amino Acids	Purines	Other
Minkowski 1886[1]	60-70	3-4	9-18				
Milroy 1903[1]	60-65		20-25				
Paton 1910[2]				1.5			
Sharpe 1912[2]	30.0		5.6				
Katayama 1924[2]	82.0		5.6				
Mayers 1924[2]	65.7		6.0				
Davis 1927[2]	62.9	10.4	17.3	8.0			1.4
Coulson and Hugh 1930	65.8	6.5	7.6	4.6		13.4	2.8
Edwards and Wilson 1954[2]	60.0				10.0	20.0	
Spector 1956	63-87	0.9-10	1.5-17	8.0	6.0	8.0	28.0
O'Dell et al. 1960	80.7	4.5	10.5	0.9	2.2	1.2	
Teekell et al. 1968[3]	60.0	6.0	23.0	4.0	2.0		
Sykes 1971	84.1	5.2	6.8	0.5	1.7		1.7 Fed
	57.8	2.9	23.0	4.3	2.8		9.2 Unfed
FASEB 1974	60-80	5-10	8-15	0.2-8.0	1.7-2.2		1.2-2.8
McNab and McNab 1975	55-72	2-11	11-21				
Krogdahl and Dalgard 1981	88.0	3.0	7.0				2.0

[1] cited in Coulson and Hughes (1930)

[2] cited in O'Dell et al. (1960)

[3] cited in Sturkie (1976)

force fed a low amount of feed (25g) or left unfed. In Table 6, the data illustrates that in a mixed excreta, uric acid is the major excreta nitrogen containing compound (82.63% to 99.05%), but not the sole nitrogen containing excretory product. Therefore, for the TMEn assay, the correction factor proposed by Hill and Anderson (1958) is invalid. Yet, this does not validate the correction factor proposed by Titus (1956).

Total excreta energy (Table 6) was shown in experiment 1 to be directly related to feed input level and to the type of test material fed. These results were as expected considering the direct effect of feed input and test material on total excreta weight. It would therefore seem only logical that as total excreta weight increases, the total energetic value of that excreta would also increase. The energetic contribution of uric acid to the total excreta energy was calculated (Table 6). Birds which were force fed a test material (corn or barley) excreted uric acid at varying proportions, dependant upon the ability of the test material to spare tissue catabolism. The contribution of uric acid to the total excreta energy for the fed birds was 22% to 35%. The contribution of uric acid to total excreta energy for the unfed birds was 57.95%. These results are similar to those of Parsons et al. (1982) who determined the energy associated with total nitrogen excretion for fasted male and female SCWL birds and found values of 37% and 58%, respectively. Considering the rationale of a nitrogen corrected TME assay, these results are important for two reasons. First, the data further illustrates that uric acid is not the sole energy compound in the excreta of birds subject to standard TME assay procedures. Second, the data illustrates that a uric acid energy based correction factor for the TME assay will not bring the fed and unfed birds to a state of zero energy balance or zero nitrogen balance.

II. Experiment 2. Quantification of nitrogen compounds in poultry excreta and the contribution to a nitrogen correction factor for the TMEn assay.

Total excreta weight voided by birds, listed in Table 7, was direcly related to feed input. As expected, birds which received ad libitum feed intake excreted significantly more excreta than birds force fed or unfed. Force fed birds tended to produce more excreta than unfed birds, but this difference was not sufficient.

Uric acid excretion (Table 7) was directly related to feed input level. Specifically, uric acid excretion is directly correlated to total dietary protein intake (Tasaki and Okumura 1964; Teekell et al. 1968; Hevia and Clifford 1977). The metabolic end product of protein utilization is uric acid (Sibbald 1982a). In situations of low to zero feed intake, stored body fat and body tissue protein are catabolized to meet the birds maintenance requirements. There is no preferential utilization of body fat vs body tissue protein (Siregar and Farrell 1980). For the unfed birds, catabolism of tissue protein results in a marked increase in uric acid excretion (Okumura and Tasaki 1969) as seen in Table 7. For the force fed birds, an intermediate level of uric acid was excreted, dependant on the degree to which dietary protein was able to spare tissue catabolism.

Data presented in Table 7 shows that as feed intake decreased, urea excretion significantly increased. However, at any given feed intake level the contribution of urea to the total excreta output is small. Under ad libitum conditions, thesource of urea in chicken urine in dietary arginine which is hydrolyzed in the kidney and liver by arginase to produce urea and ornithine. Orthinine is involved in detoxification reactions and ornithine excretion accounts for approximately 40% of dietary arginine (Sykes 1971).

Urea is excreted, but more than 99% of the excreted urea is reabsorbed in the renal tubules. Yet, urea plays only a minor role in renal osmotic regulation (Skadhauge 1983). In general, urea serves no useful purpose (Sykes 1971) and is excreted only in small quantities (Table 20), which supports the data presented in Table 7. Sykes (1971) reported that an increased urea excretion will occur only when the urea to inulin clearance ratio increases above unity, such as in the case of excess plasma arginine, or when uric acid synthesis is blocked.

Ammonia nitrogen excretion presented in Table 7 was shown to be significantly increased in the fasted bird. This is supported by Tasaki and Okumura (1964) and Teekell et al. (1968) who showed ammonia excretion to parallel that of uric acid excretion. In actual fact, uric acid and ammonia nitrogen excretion are both related to dietary protein intake. Urinary ammonia of the chicken is formed by deamination of protein in the tubule cells (Shoemaker 1972). Ammonia diffuses into the acidic luminal fluid to form NH_4^+ which is then excreted with anions (Sykes 1971). As such, urinary ammonia is involved in acid-base balance and the conservation of cations (Shoemaker 1972). Okumura and Tasaki (1968; 1969) showed ammonia excretion to increase with the acidemia associated with prolonged fasting.

The data presented in Table 7 indicated that creatinine excretion significantly increased as feed input decreased. In general creatinine excretion is low and fairly constant. Several authors (Table 20) have found creatinine to account for only a minor contribution to total urinary nitrogen. Sturkie (1976) suggested that creatinine nitrogen formation and excretion in the urine of the chicken is negligable. The significant increase in creatinine excretion of the force fed and unfed birds may be the result of utilization of phosphocreatine as an energy form. In the kidney, glycine and arginine are hydrolyzed to form ornithine and guanido-

acetic acid. Ornithine is involved in detoxification reactions. Guanidoacetic acid is transmethylated in the liver by S-adenosylmethionine to form creatine. In the presence of ATP, creatine forms phosphocreatine which is a high energy phosphate storage compound in muscle tissue. Phosphocreatine may then be used for energy following a loss of phosphoric acid which produces creatinine which is then excreted into the urine. Creatinine excretion is directly correlated to metabolic body weight (Lehninger 1975). Teekell et al. (1968) reported that creatinine excretion may be highly variable on a daily basis, while maintaining a constant level over long periods of time. These facts may explain the significant differences in excreta creatinine nitrogen level between full fed and unfed birds, simply because the excreta collection period may have been too short to show true excreta creatinine levels.

The amino acid content of excreta samples in experiment 2 decreased as feed input decreased (Table 7). This decrease was expected since the force fed birds received a low level of feed input (30g). In general, amino acid excretion in the urine, and subsequently excreta, of the chicken is small (O'Dell et al. 1960; Sykes 1971) (Table 20) and usually represents nutritional waste (Sykes 1971). Parsons et al. (1982) reported that 25% of the amino acid content of poultry excreta is made up by excreted intestinal microflora. By comparison, 50% of fecal amino acids in swine may be of microbial origin (Mason et al. 1976). The data presented in Table 7 shows that the unfed birds excreted significantly less amino acids than the full fed or force fed birds. These results are supported by Sibbald (1980b) who found amino acid excretion of unfed SCWL cockerels to decrease with the duration of the fast.

Total nitrogen excretion by birds in experiment 2 is presented in Table 8. There was a slight trend for total nitrogen excretion to decrease

as feed input decreased, but this trend was not significant. As observed in Table 7, total excreta weight was directly related to feed input level. A similar relationship between excreta total nitrogen and feed input was expected. The lack of such an effect may be explained by the negative nitrogen balance of the force fed and unfed birds. Full fed birds are assumed to be at zero energy balance and zero nitrogen balance (Asplund 1979). The force fed and unfed birds are in a state of negative energy balance and negative nitrogen balance (Sibbald 1982a), thus the reason for a nitrogen correction factor. The excessive uric acid, urea and ammonia nitrogen values shown in Table 7 illustrate the negative nitrogen balance of birds receiving low feed input levels (30g to 0g). The magnitude of the negative nitrogen balance was such that the total nitrogen excretion of birds with low to zero feed intake was not significantly different from that of a bird on full feed. These results illustrate that the total nitrogen content of excreta is not an indication of nitrogen balance. The nitrogen correction factor however is applied to the excreta total nitrogen value, and on this basis alone, the full fed and unfed birds are similar. As such the nitrogen correction factor proposed by Titus (1956) may be appropriate for all feed input levels. However, Titus (1956) developed the correction factor 36.53 kJ/g RN by analysis of urine from full fed birds. The data in Tables 7 and 8 indicate that although total nitrogen excretion does not significantly change with feed input level, the excretion of individual nitrogen containing compounds are significantly affected. Therefore, the effect of feed input on the TMEn assay will not be on the basis of total nitrogen excretion. Rather, feed input will directly affect the TMEn assay by altering the excreta nitrogen containing compounds, and therefore the nitrogen correction factor.

The proportion of total nitrogen as uric acid nitrogen (Table 8) sig-

nificantly increased as feed input decreased. These results reflect the effect of feed input on the actual weight of uric acid excreted (Table 7). The results in Table 8 confirm the results of experiment 1, that uric acid is not the sole nitrogen excretory product in chicken excreta. The results also indicate that the contribution of uric acid nitrogen to a nitrogen correction factor is different depending upon the feeding condition of the bird. Therefore, in a standard TMEn assay the nitrogen correction factors determined using full fed birds as proposed by Hill and Anderson (1958) or Titus (1956) may not be appropriate. Rather these results indicate that the force fed and unfed birds of the TMEn assay require specific individual nitrogen correction factors.

Urea nitrogen excretion by the chicken was small (Table 7) and as such only accounted for a small proportion of the total nitrogen excreted (Table 8). Urea nitrogen excretion was shown to increase as feed input decreased (Table 7) and the proportion of total nitrogen as urea nitrogen was similarly affected (Table 8). However, urea nitrogen provides only a small contribution to excreta total nitrogen. As a result, the significant changes in the proportion of excreta nitrogen as urea do little to affect the nitrogen correction factor (Table 9a).

Ammonia nitrogen excretionper 100g of total nitrogen was shown to increase as feed input decreased (Table 7). However, the data presented in Table 8 indicates that the proportion of total nitrogen as ammonia nitrogen decreased as feed input decreased. This can be explained by the fact that uric acid nitrogen makes up such a large proporiton of the total excreta nitrogen (> 75%). On a proportional basis, as uric acid nitrogen increases, the proportion of ammonia nitrogen and other nitrogen compounds must decrease. This has very important implications to a nitrogen correction factor for the TMEn assay since ammonia nitrogen has the highest ener-

getic value of any of the excreta nitrogen containing compounds (Table 9b). In general, ammonia makes the second largest contribution of nitrogen to total urinary nitrogen excretion (Ward et al. 1975) and generally accounts for 1% to 25% (Table 20) of total urinary nitrogen. As a result, a small change in the proportion of total nitrogen as ammonia will significantly affect the nitrogen correction factor (Table 9a).

The proportion of total nitrogen as creatinine (Table 8) significantly decreased as feed intake decreased. In general, like urea, creatinine nitrogen excretion was small. Similar findings have been reported by several authors (Table 20). Sturkie (1976) reported the excretion of creatinine by poultry to be small and accounted for only a minor contribution to total nitrogen excretion. Although creatinine nitrogen provides a substantial energetic contribution to the nitrogen excreted (Table 9b), the small excretion of total creatinine does not significantly affect the nitrogen correction factor (Table 9a).

Amino acid nitrogen excretion decreased as feed input decreased (Table 7), and as a result, the proportion of total nitrogen as amino acid nitrogen decreased as feed input decreased (Table 8). In the full fed birds, amino acid nitrogen accounted for a major portion of the total nitrogen excreted, but in the force fed and unfed birds, amino acid nitrogen excretion was low. This data is supported by Sykes (1971) who reported amino acid excretion to represent primarily nutritional waste. The high nitrogen content of excreta (27.22% for the unfed bird) significantly interfered with amino acid analysis. The large error involved in amino acid analysis prevented detailed analysis of the contribution of amino acid nitrogen to a correction factor for the TMEn assay.

The contribution of individual nitrogen containing compounds to a nitrogen correction factor for the TMEn assay were calculated and are pre-

sented in Table 9a. Only the factor determined for full fed birds (36.92 kJ/g RN) is similar to that of Titus (1956) (36.53 kJ/g RN). These results were not unexpected considering that the value proposed by Titus (1956) was determined using full fed birds. However, the TMEn assay is unique because of low feed inputs (30g or 0g) of the assay birds. At low dietary input levels, birds must catabolize body tissue to meet maintenance requirements. As a result, assay birds are in a negative nitrogen balance (Sibbald 1982). Therefore, the correction factor for the force fed and unfed birds should be larger than that for the full fed birds (Table 9a). It is interesting to note that there was little difference between the factors determined for the force fed (40.57 kJ/g RN) and unfed birds (40.28 kJ/g RN).

In order to fully realize the implication of an incorrect nitrogen correction factor, using the data of experiment 2, the TME and TMEn values of the cracked wheat were calculated (Table 21). The TME value of the cracked wheat sample was 15.64 MJ/Kg. Using the correction factor 36.53 kJ / g RN as proposed by Titus (1956) the TMEn value was 14.76 MJ/Kg. However, using individual correction factors for force fed (40.57 kJ/g RN) and unfed birds (40.28 kJ/g RN), the TMEn value was determined to be 14.70 MJ/Kg. Using these individual correction factors as determined in experiment 2 the TME value was decreased 6.01% after applying the nitrogen correction. This can be compared to a 5.63% decrease after correction with the value of Titus (1956) and a 5.31% decrease after correction with the value of Hill and Anderson (1958). According to the data of experiment 2, the negative nitrogen balance of the assay bird is greater than that assumed by Hill and Anderson (1958) or Titus (1956). This would suggest that correction of the TME value with a factor determined using full fed birds is not only inappropriate, but incorrect.

Table 21. TME and TMEn values of cracked wheat determined by standard procedure with and without the addition of a preservative.[2]

Treatment	Parameter	Correction Factor[3] k_1	k_2	k_3	k_4	k_5
		MJ/Kg				
Experiment 2[1]	TME	15.64				
	TMEn		14.81	14.76	14.70	
Experiment 7[2]	TME	16.94				
	TMEn		16.21	16.16		16.01

[1] no preservative added to excreta samples
[2] preservative (boric acid powder plus 0.3 per cent w/w mercuric chloride) added to excreta samples
[3] Correction factors (kJ/g RN): $K_1 = 0$
$k_2 = 34.39$ - Hill and Anderson (1958)
$k_3 = 36.53$ - Titus (1956)
$k_4 = 40.57$ (force fed bird) + 40.28 (unfed bird) - Experiment 2
$k_5 = 35.10$ (force fed bird) + 37.10 (unfed bird) - Experiment 7

III. Experiment 3. Effect of duration of excreta collection period on the proportion of nitrogen compounds in poultry excreta.

Several authors (Martin 1966; Davidson and Williams 1968; Asplund 1979) have detected important errors in nitrogen balance trials using sheep and cattle. These errors are the denitrification of excretory nitrogen (urea) in the presence of bacteria of intestinal origin, and the subsequent production and potential loss of ammonia nitrogen. These errors are important because they may result in incorrect nitrogen balance data.

The TMEn assay, by definition, is a nitrogen balance trial, and therefore the same balance trial errors found using sheep and cattle may occur using poultry. However, conflicting data regarding similar losses from poultry excreta exist. It is therefore essential to clarify such data as any errors in nitrogen balance trials is critical to the validity of a nitrogen corrected TME assay.

A. Trial.

Total excreta weight of each treatment did not differ significantly (Table 10) as samples for each treatment were subsamples of equal weight taken from a larger pooled sample of excreta collected from 24 birds. Any differences which were observed in excreta weight may have been due to incomplete mixing of the initial pooled sample. Asplund (1979) suggested that sedimentation of compounds (nitrogen) in moist excreta is a major potential error of balance trials.

The uric acid nitrogen content of excreta samples did not change significantly with the length of time the samples were exposed to atmospheric conditions (Table 10). There was a slight decrease in the excreta uric acid nitrogen content which may have been due to uric acid degrading bacteria of cecal origin present in the excreta samples. Parson et al. (1982)

reported that intestinal microflora account for 11% of excreta dry matter. The degradative ability microbes in the gut and ceca is well documented (Jayne-Williams and Fuller 1971; McNab 1973; March 1979). However, the extent of bacterial degradation of uric acid over time in excreta samples is not well established. Okumura et al. (1976) noted that microorganisms will degrade endogenous nitrogen preferentially to dietary nitrogen.

Excreta urea nitrogen significantly decreased as the length of time the samples were exposed to the atmosphere increased (Table 10). After 48 hours, urea nitrogen in the excreta decreased from 0.16g to 0.02g, which represents an 87.5% loss of urea nitrogen. The decrease of urea which occured with time was probably the result of urea degrading bacteria of cecal origin in the excreta. In the intestine and ceca bacterial urease acts freely on urea. The end product of bacteria ureolysis is ammonia. In the intestine, this ammonia would be absorbed and excreted as uric acid (Okumura et al. 1976). In the excreta, however, bacterial liberation of ammonia would result in an increased excreta content of ammonia nitrogen. The data presented in Table 10 shows that after 48 hours, excreta ammonia nitrogen increased from 0.44g to 0.87g, which represents a 97.7% increase. These results are consistent with the findings of Okumura et al. (1976) that bacterial degradation of urea results in the substantial production of ammonia. An increase of such magnitude would have a significant effect on the nitrogen correction factor for the TMEn assay, particularly since ammonia nitrogen has the highest energetic value of all the excreta nitrogen containing compounds (Table 9b). As such, these results suggest an important effect of duration of the excreta collection period on the TMEn assay.

Excreta creatinine nitrogen remained constant regardless of the length of time samples were left exposed to the atmosphere (Table 10). These results were not as expected since creatinine is a relatively high energy compound (Table 9b). As a result, one would expect microbial degradation of the

compound as a readily available energy source, but such was not the case. To date, bacterial degradation of creatinine in the intestine or ceca has not been documented in the literature.

Excreta total nitrogen was not significantly different among treatments (Table 10). Excreta total nitrogen remained constant regardless of the bacterial activity on uric acid and urea, and the subsequent increase in ammonia nitrogen. Wallis and Balnave (1983) found a significant increase of excreta energy (1% to 4%) and nitrogen (2% to 6%) by extension of the collection period from 12 to 24 hours. Although the data of trial A and of Wallis and Balanve (1983) do not correspond, these results serve to illustrate a major potential problem with the standard TMEn assay corrected using the factor of Hill and Anderson (1958) or Titus (1956). The nitrogen correction factor is applied only to the quantity of total nitrogen. As seen in Table 10, after extreme shifts in the excreta content of urea and ammonia, excreta total nitrogen content remained constant. However, the data of Wallis and Balnave (1983) illustrates that excreta total nitrogen content may be directly affected by these shifts in the excreta nitrogen compounds. The error of a standard TMEn assay exists then, not in the amount of total nitorgen to be corrected, rather in the correction factor which is applied. If after a typical 48 hour excreta collection period, the proportion of the high energy compounds, urea and particularily ammonia (Table 9b), are changed, then the nitrogen correction factor may also change. It is the magnitude of the nitrogen correction factor which is directly affected by shifts in the individual excreta nitrogen compounds. It is this factor which is then applied to the excreta total nitrogen content.

B. Trial B.

Experiment 3, trial B, was designed to duplicate the excreta col-

lection method of a standard TMEn assay (Sibbald 1983). Total excreta weight of each treatment for trial B (Table 11) was significantly different. As the duration of the collection period increased the total excreta weight collected increased. These results were as expected due to the method of excreta collection.

Excreta uric acid content (Table 11) was not significantly affected by duration of the excreta collection period. These results were not as expected, since in trial A, uric acid nitrogen tended to decrease with time. If excreta uric acid is degraded by bacteria, as indicated by Barnes and Impey (1974), it stands to reason that the environment of an enclosed colostomy bag would promote bacterial activity. The data, however, does not support this.

As illustrated by the data presented in Table 11, excreta urea nitrogen significantly decreased as duration of the excreta collection period increased. This data is consistant with the findings of trial A. Extension of the collection period decreased excreted urea from 0.34g to 0.19g, which represents a 44.1% loss of urea nitrogen. This loss of urea nitrogen was less than the 87.5% loss observed in trial A. The difference can be explained by the method of sample collection. In trial B, as the duration of excreta collection period increased, the total amount of urea excreted would also increase. However, at the same time, urea is being degraded by bacteria. The rate of urea excretion offsets the rate of urea loss caused by bacterial degradation, and the net loss of urea in trial B is less than that observed in trial A.

Excreta ammonia significantly increased as the duration of excreta collection period increased (Table 11). After only 24 hours, excreta ammonia nitrogen increased from 0.36 to 0.74g, or 105.6%. Extension of the collection period to 48 hours increased ammonia nitrogen to 1.06g, which

represents a total increase of 194.4%. The increase in excreta ammonia nitrogen which occured in trial B greatly exceeds that of trial A. These results were not as expected considering that in trial B uric acid and urea, the source of ammonia, are degraded to a lesser extent than in trial A. These results were not as expected considering that in trial B uric acid and urea, the source of ammonia, are degraded to a lesser extent than in trial A. It is possible that the source of ammonia may be bacterial degradation of amino acids. Parsons et al. (1982b; 1982c) observed substantial deamination of amino acids by microflora and the subsequent production of ammonia in excreta samples has not been documented.

Excreta creatinine level was not affected by duration of the excreta collection period (Table 11). Regardless of the length of time excreta was collected, the creatinine nitrogen level was 0.04g of nitrogen per 100 grams of total excreta. This data supports the conclusions of Sturkie (1976) and Teekell et al. (1968) that the excretion of creatinine by the chicken is small and relatively constant.

As the length of the excreta collection period increased, the total grams of nitrogen collected increased (Table 11). As a result, the excreta total nitrogen level was significantly different among treatments. This data is somewhat misleading as it indicates a direct treatment effect of time upon excreta nitrogen content. In fact, the significant increase in excreta total nitrogen is due to the method of excreta collection. It is also important to note that total excreta nitorgen content is not an accurate reflection of the effect of duration of collection period on the individual excreta nitrogen compounds. Yet, any changes in the amount of the excreta nitorgen compounds directly affects the excreta total nitrogen content. It is for this reason that the excreta content of individual nitrogen compounds must be expressed per 100 grams of total excreta nitrogen.

From the data presented in Tables 10 and 11, duration of the excreta collection period does not significantly affect excreta uric acid nitrogen or creatinine nitrogen. Increasing the excreta collection period significantly decreased excreta urea nitrogenand significantly increased ammonia nitrogen. Since the energetic value of ammonia nitrogen is greater than that of urea nitrogen (Table 9b), and because the net change in total grams is greater for ammonia nitrogen than urea nitrogen, extension of the excreta collection period will increase the nitrogen correction factor. In addition, because the urea nitrogen and ammonia nitrogen content of excreta is changing over time, nitrogen balance will also be affected.

To date several authors (Sibbald 1976a; 1978a; 1979d; 1982a; 1983; Kessler and Thomas 1981; Schange et al. 1982b; Salmon 1983) have determined the effect of duration of the excreta collection period on TME and TMEn values. Work by these researchers has been concentrated entirely on the effect of the excreta collection period on the rate of passage, and gut clearance of the test material in question. These experiments have generated important data in terms of establishing optimal collection times for specific test materials. However, the question of nitrogen balance and the contribution of excreta nitrogen compounds to a nitrogen correction factor for the TME assay have been overlooked. The data presented in experiment 3 shows significant changes in the high energy excreta nitrogen compounds (urea and ammonia) over time. These changes directly affect total excreta energy and nitrogen. More importantly, these changes alter the contribution of the nitrogen compounds to a nitrogen correction factor and therefore directly affect the TMEn value.

VI. Experiment 4. Effect of freeze drying on the proportion of nitrogen compounds in poultry excreta.

Data presented in experiment 3 indicated that as duration of the excreta collection period increased, the bacterial degradation of urea increased, which produced a substantial increase in the excreta ammonia nitrogen level. Asplund (1979) reported that volatilization of ammonia produces a major error in nitrogen balance studies. Manoukas et al. (1964) and Shannon and Brown (1969) observed significant losses of nitrogen and energy from poultry excreta as a result of the drying of excreta. However, Sibbald (1979e) and Dale and Fuller (1983b) did not find similar results. Because of the possible error associated with nitrogen balance trials and the impact of these errors on the TMEn assay, it is important to investigate the problem further.

A. Trial A.

The total excreta weight for each treatment in experiment 4 (Table 12) was not significantly different. As per the experimental procedure, excreta samples for each treatment were subsamples of equal weight drawn drom a single pooled excreta sample. Differences which occured were likely due to sampling error and incomplete mixing of the initial excreta sample. Because of the slight differences which occured in treatment excreta weights, the excreta nitrogen compounds were expressed on the equal basis of 100g of total excreta.

Excreta uric acid nitrogen (Table 12) was not affected by the length of time that the excreta was exposed to the atmosphere. The results correspond to those of experiment 3, trial B. Barnes and Impey (1974) suggested the possibility of uric acid utilizing microbes of cecal origin

being present in the excreta. The results of experiment 3, trial A support this finding. However, a lack of consistency of data may raise doubts as to the ability of microbes of intestinal origin to degrade excreta uric acid. Excreta samples that were freeze dried tended to have a lower level of uric acid nitrogen than non-freeze dried samples. Two possible explanations exist. First, the freeze drying process requires approximately a two to three day period until completion. This extra time period may have been advantageous to the bacterial population and helped to facilitate uric acid degradation. Second, the nonsignificant difference may have been due simply to excreta sampling error and analytical error.

Excreta urea nitrogen was affected by time in the same manner as observed in experiment 3. Extending the length of time that samples were exposed to the atmosphere increased microbial degradation of excreta urea (Table 12). This effect has been well documented in the literature. However, the observed decrease of excreta urea nitrogen was greater in the excreta which was freeze dried. This may be explained by the additional time required for the freeze drying process, and therefore increased opportunity for bacterial degradation. Freeze drying produced conflicting results regarding excreta urea nitrogen content. The 1 hour samples showed a significant increase in excreta urea nitrogen as a result of freeze drying. However, no effect of freeze drying was observed in the 48 hour excreta samples. The results produced a significant interaction for urea nitrogen, but the reason for such an interaction was not evident.

The data of experiment 4 (trial A) support the data of experiment 3 which illustrate an effect of time on excreta ammonia nitrogen (Table 12). As the length of time which the excreta samples were exposed to the atmosphere increased the excreta ammonia nitrogen level also increased. The increase in excreta ammonia nitrogen is associated with a decrease of ex-

creta urea nitrogen. The increase of ammonia nitrogen as a result of bacterial degradation of urea is supported by several other authors (Jayne-Williams and Fuller 1971; Okumura et al. 1976; Asplund 1979). The data presented in Table 12 also shows that freeze drying significantly decreased excreta ammonia nitrogen. Freeze drying decreased excreta ammonia nitrogen of the 1 hour samples 65.7% and 45.8% in the 48 hour samples. This data supports the observations of Martin (1966) that drying of excreta significantly increases the loss of excreta nitrogen, and that the loss of nitrogen is in the form of ammonia. Manoukas et al. (1964), Shannon and Brown (1969) and Wallis and Balnave (1983) also reported significant losses of nitrogen from the excreta of poultry. The magnitude of the nitrogen loss reported by these authors was not as great as that observed in experiment 4. The reason for this difference was probably due to the method of drying, oven drying vs. freeze drying. The vacumn environment of the freeze dryer results in a substantial loss of the volatile ammonia, greater than that which would occur in oven drying techniques. Sibbald (1979e) and Dale and Fuller (1983b) investigate the loss of nitrogen from poultry excreta as a result of drying procedure, but found no effect. It should be noted that neither Sibbald (1979e) or Dale and Fuller (1983b) evaluated fresh excreta. As a result, an initial base level for the excreta nitrogen containing compounds was not established.

Excreta creatinine nitrogen was not affected by either duration of the excreta collection period or by freeze drying procedure (Table 12). These results were as expected and support the data of experiment 3.

Excreta total nitrogen level (Table 12) was not significantly affected by duration of the collection period or by freeze drying procedure. These results were not as expected considering the significant shifts in urea and ammonia nitrogen over time, and the significant loss of nitrogen

(ammonia) as a result of drying. A significant loss of nitrogen resulting from the drying of poultry excreta was observed by Manoukas et al. (1964), Shannon and Brown (1969) and Wallis and Balnave (1983). Reasons for the lack of freeze drying effect observed in Table 12 for total nitrogen was not evident. One possible explanation may be that data in Table 12 documents the total grams of excreta nitrogen, and therefore the values are subject to the errors associated with sampling.

B. Trial B.

Like experiment 3, trial B, experiment 4, trial B, was designed to duplicate the excreta collection method of a standard TMEn assay (Sibbald 1983). Due to the method of excreta collection, total excreta weight increased according to the length of the excreta collection period. The data presented in Table 13 indicated that freeze drying increased total excreta weight, however, this effect was probably due to among bird variation.

In trial B (Table 13) uric acid nitrogen tended to be constant, regardless of the duration of the collection period or freeze drying procedure. The exception to this was the 4 hour fresh value (9.33g) which was significantly greater than other treatment values. Statistically this value indicates significant treatment effects of collection period and freeze drying procedure which produced a significant interaction value. The results of trial A and of experiment 3 suggest that uric acid nitrogen is not significantly affected by these treatments. Asplund (1979) reported that the main error associated with the analysis of fresh excreta are moisture loss and the sedimentation of nitrogen compounds. Although the experimental procedure of trial B was designed to try to reduce such errors, they may have occured and contributed to the variation

observed.

As expected, the excreta urea nitrogen level decreased as the duration of the collection period increased due to bacterial degradation. Freeze drying of the excreta tended to decrease urea nitrogen content (Table 13). These results are contrary to the data presented in experiment 3. It is possible that freeze drying per se does not directly affect excreta urea content. Rather, because the freeze drying process involves heating the excreta for a prolonged period of time (usually 2 to 3 days), this may in itself facilitate bacterial degradation of urea (Table 13).

In trial B, excreta ammonia nitrogen increased as the duration of the excreta collection period increased. These results were as expected and support the data of the previous experiments. Support for these findings are well established in the literature as previously cited. The freeze drying procedure in trial B decreased excreta ammonia nitrogen, but not to the same extent as in trial A. The loss of ammonia nitrogen in the 4 hour samples was 17.1% and the loss in the 48 hour samples was 24.4%. Martin (1966) observed that the rate of loss of ammonia from excreta was dependant upon temperature, pH and frequency of excretion. In trials A and B, temperature should not have been a factor contributing to the observed differences in ammonia nitrogen loss. In both trials temperature was maintained at a constant level in the barn, during sample storage and during the freeze drying process. Excreta pH also should not have been a factor contributing to the observed trial differences. In both trials, all birds were fed ad libitum a maintenance diet (Table 1). Therefore, the acidemia associated with prolonged fasting (Okumura and Tasaki 1968; 1969) which occurs in a standard TMEn assay, should not have affected excreta pH or ammonia nitrogen. The differences in excreta ammonia loss which occured between trials A and B were most likely due to the differences in fre-

quency of excretion. In trial B (cumulative collection of excreta in colostomy bags) the continued addition of new excreta to the sample would result in a lower loss of ammonia on a unit weight basis when compared to trial A (a single collection of excreta). It must be assumed that the rate of loss of ammonia in the environment of a freeze dryer is the same for both trials. This data is important to the TMEn assay which employs the use of colostomy bags for the cumulative collection of excreta.

Excreta creatinine nitrogen (Table 13) was constant at 0.04g and was unaffected by duration of the excreta collection period or freeze drying procedure. This data is supported by experiment 3 and confirms the observation of Sturkie (1976) that creatinine excretion in poultry is small and constant. Lehninger (1975) reported that creatinine excretion is directly correlated to metabolize body weight. Birds in trial B fed ad libitum maintenance diet neither gained nor lost body weight so therefore, creatinine excretion would be constant. Birds that are force fed or unfed must catabolize body tissue protein to meet maintenance energy requirements, and in the process probably lose body weight. The small differences in creatinine nitrogen excretion observed in experiment 2 may then be a relection of changes in metabolic body weight of birds involved in a TMEn assay.

Excreta total nitrogen (Table 13) significantly increased as the duration of the excreta collection period increased. These results were as expected due to the method of excreta collection. The data in Table 13 indicates that freeze drying tended to increase total excreta nitrogen content. This data is not supported by that of trial A. The significant loss of ammonia nitrogen as a result of freeze drying of excreta would be expected to decrease excreta total nitrogen content. As observed in experiment 3 (trial B), the significant treatment effect which occured for

excreta total nitrogen may have been due to the error associated with the sampling of fresh excreta.

V. Experiment 5. Use of a preservative - mercuric chloride (0.3 per cent w/v) in glacial acetic acid - to prevent the loss of nitrogen from poultry excreta over time and as a result of freeze drying.

The loss of energy and nitrogen from the feces and urine of ruminants is well established (Flutt 1957; Colovos et al. 1957; Fenner and Archibald 1959; Martin 1966). As a result, there have been several attempts to prevent such losses. Rocks (1977) developed two preservatives which consisted of a weak acid and an organic bacteriostat. The preservatives were: 1) mercuric chloride (0.3 per cent w/v) in glacial acetic acid; and 2) boric acid powder plus (0.3 per cent w/w) mercuric chloride. Both preservatives were equally effective in maintaining total nitrogen, urea nitrogen and ammonia nitrogen at initial levels following a storage period of 14 days at 20-25°C.

Little or no work on the preservation of poultry excreta has been published to date. The data of experiments 3 and 4 indidcated that significant losses of nitrogen containing compounds from poultry excreta occur in standard balance trials (TMEn). It is therefore essential to employ the use of proven preservatives in an experimental trial to determine the potential of such preservatives for the standard TMEn assay.

The excreta of each treatment in experiment 5 (Table 14) was not significantly different by weight. Excreta samples of equal weight for each treatment were expected due to the procedure of sampling excreta from a single larger pooled sample. Any variation which did occur was probably due to the errors associated with sampling and the problems of dry matter

sedimentation in high moisture excreta.

Excreta uric acid nitrogen was not significantly affected by collection period or freeze drying. There was a slight trend for uric acid values to be depressed due to increased exposure of the sample to the atmosphere. It is possible that this difference may be the result of bacterial degradation of uric acid as documented by Barnes and Impey (1974). However, inconsistency of data from the previous experiments suggest bacterial degradationof uric acid nitrogen may not be a factor. The depressed uric acid values are probably due to the addition of the acidic preservative. The data in Table 14 shows that low uric acid nitrogen values are associated with low excreta pH values. This would then suggest that the added preservative is interfering with the pH dependant uric acid analysis.

Excreta urea nitrogen (Table 14) tended to decrease as a result of prolonged exposure of the excreta to the atmosphere. This difference was not statistically significant but the observed decrease of 50% after 48 hours seems relevant. The degradation of urea over time is well established. The data presented in Table 14 illustrates a failure of the preservative to prevent such bacterial degradation. The low excreta pH which occured upon addition of the preservative may serve to actually increase the loss of urea, by means of acid hydrolysis (Martin 1966; Rocks 1977). Freeze drying did not significantly affect excreta urea nitrogen. These findings are supported by the data of experiment 4.

Excreta ammonia nitrogen (Table 14) decreased 35.5% after extending the length of time that samples were exposed to the atmosphere from 1 hour to 48 hours. A decrease in excreta ammonia nitrogen over time had not previously been observed (experiments 3 and 4). The reason for the decrease in excreta ammonia may be the result of the addition of preservative to the excreta. Following addition of the preservative, excreta pH

was decreased to 3.0 which may then interfere with the pH dependant ammonia analysis. Freeze drying significantly decreased excreta ammonia nitrogen from 0.31g to 0.09g, which represents a 71.0% loss. This may be explained by the volatile nature of glacial acetic acid (C.R.C. Handbook of Chemistry and Physics, 63rd ed. 1982-1983). The added preservative decreased excreta pH (to 3.0 - 3.5), and in doing so, trapped excreta ammonia (Rocks 1977). The vacumn environment of the freeze drier draws off the glacial acetic acid, as evident by the increase in excreta pH (5.5 - 6.0) after freeze drying. As a result, the trapped ammonia would also be lost due to volatilization. Therefore, this data suggests that use of the preservative mercutic chloride (0.3 per cent w/v) in glacial acetic acid functions to increase the loss of ammonia nitrogen in excreta subjected to freeze drying.

Excreta creatinine nitrogen (Table 14) was not affected by time, freeze drying procedure or addition of a preservative to the excreta. The significant loss of creatinine nitrogen in the 48 hour fresh sample was probably due to the low excreta pH interfering with the pH dependant creatinine nitrogen analysis.

Data presented in Table 14 show excreta total nitrogen content to not be significantly affected by excreta collection period, freeze drying or addition of a preservative to excreta samples. There was a slight trend for total nitrogen values to be low in the 48 hour freeze dried treatment. These results may be a reflection of the loss of ammonia nitrogen which was observed as a result of freeze drying excreta with added volatile preservative. These results may also have been due simply to the sampling error associated with high moisture excreta.

From the above data, it can be concluded that mercuric chloride (0.3 per cent w.w) in glacial acetic acid is unacceptable as a preservative in

poultry excreta subject to prolonged atmospheric exposure and/or freeze drying. Thus the evaluation of the preservative in excreta collected in colostomy bags (trial B) was unnecessary.

VI. Experiment 6. Use of a preservative - boric acid powder plus (0.3 per cent w/w) mercuric chloride - to prevent the loss of nitrogen from poultry excreta over time and as a result of freeze drying.

Data presented for experiment 5 proved mercuric chloride (0.3 per cent w/v) in glacial acetic acid to be ineffective as a preservative in poultry excreta. Therefore, experiment 6 was designed to evaluate boric acid powder plus (0.3 per cent w/w) mercuric chloride under similar experimental conditions.

A. Trial A.

Mean total excreta weight (Table 15) of samples for each treatments were not significantly different. This data was expected due to the method of sample collection. The small variation which did occur between treatments was probably due to the error associated with sampling.

As indicated previously, the sampling error of high moisture excreta may be unavoidable. In this regard excreta nitrogen containing compounds are expressed on the basis of 100 grams total dry matter to minimize the sampling error. Asplund (1976) suggested that the sedimentation of nitrogen compounds in fresh excreta samples can potentially cause large analytical errors. The data presented in Table 15 and in previous experiments show a nonsignificant difference in the excreta total nitrogen values among treatments. This would suggest good sample uniformity and indicates that little or no sedimentation of nitrogen compounds occured.

Addition of the preservative boric acid powder plus (0/3 per cent w/w) mercuric chloride to excreta samples prevented any change in excreta nitrogen containing compounds, due to time or freeze drying procedure. These results correspond to the data presented by Rocks (1977). The preservative was successful in maintaining excreta uric acid nitrogen, urea nitrogen, ammonia nitrogen and creatinine nitrogen at a level equal to initial values. Only urea nitrogen deviated from linearity, showing a slight decrease after freeze drying. This loss of urea was probably due to a small degree of bacterial degradation during the extended time period required for the freeze drying process. Such an effect may be prevented be increasing the volume of preservative added to the excreta.

The volume of preservative required in experiment 5 and 6 was based on the results of Rocks (1977). In experiment 5, the volume of mercuric chloride (0.3 per cent w/v) in glacial acetic acid added to the excreta was determined by the ability of the preservative to decrease excreta pH to 3.0. In experiment 6, the volume of boric acid powder plus (0.3 per cent w/w) mercuric chloride (in solution) added to the excreta was based on the recommendation by Rock (1977) of 20 g/day used in the urine of sheep. To extrapolate this to a mixed excreta of adult SCWL cockerels the following calculations were made. Sheep, on average, excrete 25 ml of urine/Kg body weight (FASEB 1974). Assuming a body weight of 40 Kg, sheep used in the study by Rocks (1977) excreted 0.32g of total ammonia (based on an average excretion of 170 mg/Kg body weight (FASEB 1974). Following the results of experiment 4, adult cockerels require 2.5g of preservative per 24 hours. Since the preservative is in solution, at 1g of boric acid powder per 20ml of water, a volume of 50 ml/day of preservative was used in experiment 6. As evident by the data of trial A, 2.5g of boric acid powder per 24 hours may not be adequate to completely prevent bacterial degradation of urea nitrogen in poultry excreta. As a

result the level of added preservative should be increased. This becomes increasingly important when excreta is collected in a colostomy bag where uniform mixing of preservative and excreta may be more difficult to achieve.

B. Trial B.

In trial B, excreta was collected using colostomy bags. As expected, the longer the collection bag was left attached to the bird, the larger the quantity of excreta collected (Table 16). There was a trend for excreta weight to decrease as a result of freeze drying, but this effect was not significant. The observed trend may be explained by among bird variation. In trial B, experimental birds were of similar body weights. Under ad libitum feeding conditions birds of similar metabolic body size have similar body maintenance requirements (Guillaume and Sommers 1970). Therefore it was assumed that birds in experiment 7 consumed similar quantities of feed and excreted similar quantities of waste. The observed differences which occured are due to among bird variation in metabolic body weight, feed consumption and waste excretion.

Uric acid nitrogen in excreta with added preservative was not significantly affected by duration of the collection period or freeze drying (Table 16). There was a trend for uric acid nitrogen to decrease with extension of the collection period to 48 hours, possibly due to bacterial degradation as suggested by Barnes and Impey (1974). These results were not as expected since thepreservative consists of an inorganic bacteriostat, mercuric chloride (Rocks 1977). From the calculations described in trial A, an adequate amount of preservative was added to each colostomy bag in trial B (60ml per day). Therefore, the slight decrease in uric acid nitrogen may be explained by failure to achieve complete mixing of the excreta and preservative, possible resulting in some bacterial activity.

Excreta urea nitrogen was not affected by duration of the collection period. Data in Table 16 indicate that the excreta urea nitrogen was significantly decreased by freeze drying. Similar results were observed in experiment 4 (trial B) and experiment 6 (trial A). As discussed above, the loss of urea probably results from the extended time period associated with freeze drying rather than the freeze drying process per se. The results of trial B indicate a failure of the preservative to completely prevent bacterial degradation. However, the results of trial A indicated that the preservative was effective in maintaining the excretory nitrogen compounds at a level equal to their initial values. In addition, if bacterial degradation of urea nitrogen and uric acid nitrogen had occured in trial B, excreta ammonia nitrogen content would have increased as a product of the bacterial degradation.

Ammonia nitrogen was not significantly affected by collection period or freeze drying following addition of the preservative to excreta samples (Table 16). Excreta ammonia nitrogen did increase slightly by extension of the collection period to 48 hours, but the observed increase did not correspond to the magnitude of urea nitrogen loss. In the presence of bacterial urease, one molecule of urea produces two molecules of ammonia (Morrison and Boyd 1975). The molecular weight of urea is 60.06 and the molecular weight of ammonia is 17.03 (C.R.C. Handbook of Chemistry and Physics, 63rd ed. 1982-1983). On a gram for gram basis, the loss of 1g of urea would produce 1g of ammonia. Therefore the reason for the highly significant loss of urea nitrogen which was observed as a result of freeze drying is unexplained.

As observed in all of the previous experiments, excreta creatinine nitrogen (Table 16) was unaffected by duration of the excreta collection period or by the freeze drying procedure. Addition of the preservative

did not interfere with creatinine nitrogen analysis. Interference by the preservative on the analysis of excreta nitrogen containing compounds was the reason for the failure of the preservative mercuric chloride (0.3 per cent w/w)in glacial acetic acid (experiment 5).

Due to the method of excreta collection, excreta total nitrogen (Table 16) significantly increased as the duration of the excreta collection period increased. Excreta total nitrogen was not significantly affected by freeze drying prodecure, but the freeze dried samples did tend to have less total nitrogen than nonfreeze dried samples. Birds in all treatments were fed ad libitum, and therefore are assumed to be at zero nitrogen balance (Sibbald 1982a). Total nitrogen excretion followed the same pattern as total excreta weight. As a result, the slight difference in excreta total nitrogen observed between 48 hour fresh and 48 hour freeze dried samples was probably due to among bird variation as described for excreta weight.

From the data of experiment 6 it can be concluded that the preservative, boric acid powder plus (0.3 per cent w/w) mercuric chloride, prevents the bacterial degradation of uric acid and urea, and prevents the loss of ammonia over time and as a result of freeze drying. The data also illustrates an important precaution for the use of preservatives in excreta collection bags. In a collection bag, it is essential that any added chemical preservative (in solution) must be added in a sufficient volume to ensure thorough and complete mixing of the preservative and excreta. On the other hand, the volume of the preservative added must not exceed the physical limitations of the collection bag or adhesive. In trial B, 4 colostomy bags (16%) fell off due to failure of the adhesive and/or feathers of the bird to support the weight of excreta plus preservative.

Success of the boric acid preservative for poultry excreta suggests a potential use in nitrogen balance trials, specifically the TMEn assay. Use of the preservative would prevent the errors associated with nitrogen balance trials. As a result, a precise nitrogen correction factor for the TMEn assay could be established.

VII. Experiment 7. Quantification of nitrogen compounds in poultry excreta with added preservative and the contribution to a nitrogen correction factor for the TMEn assay.

Data presented in experiment 6 proved the preservative boric acid powder plus (0.3 per cent w/w) mercuric chloride to be effective for the preservation of nitrogen compounds in poultry excreta. Therefore, experiment 7 was designed identical to experiment 2 to accurately quantify the nitrogen compounds in poultry excreta and determine their effect on a nitrogen correction factor for the TMEn assay.

Total excreta weight voided by birds was directly related to feed intake (Table 17). Birds which received feed ad libitum excreted significantly more excreta than birds force fed only 30g or those left unfed. The unfed birds excreted less total excreta than the birds receiving 30g of feed, but this difference was not significant. These results were as expected and correspond to those of experiment 2.

Uric acid excretion (Table 17) was directly related to dietary protein intake. This data is supported by literature data (Tasaki and Okumura 1964; Teekall et al. 1968; Hevia and Clifford 1977) and by data of experiment 2. The full fed birds excreted uric acid at a level corresponding to the dietary protein content of the standard maintenance diet (Table 1). The unfed birds, which received no dietary protein, must catabolize body tissue protein to meet maintenance requirements. This produces an in-

crease in uric acid excretion (Okumura and Tasaki 1968) as observed in Table 17. The force fed birds excreted an intermediate level of uric acid dependent on the degree to which dietary protein was able to spare tissue catabolism. It is interesting to note that there was no significant difference in the uric acid excretion between the force fed and unfed birds. This would suggest that the magnitude of the negative nitrogen balance for force fed and unfed birds is similar. This data would also suggest that the nitrogen balance of both force fed and unfed birds is significantly different from that of full fed birds. This data is important because the nitrogen correction factors proposed by Titus (1956) and Hill and Anderson (1958) were determined using full fed birds.

Excreta urea nitrogen (Table 17) was highest in the full fed birds. Urea excretion decreased as feed intake decreased, which corresponds to the data of experiment 2. In general, for both experiments 2 and 7, urea nitrogen was excreted in only a very small quantity. This is consistant with the data of Sykes (1971).

The excreta of ammonia nitrogen (Table 17) significantly increased as dietary feed intake decreased. Ammonia nitrogen excretion is involved in acid-base balance (Shoemaker 1972). This explains the increase in excretory ammonia nitrogen as feed intake decreases, due to the acidemia associated with prolonged fasting (Okumura and Tasaki: 1968, 1969). Similar results were reported in experiment 2.

In experiment 7, creatinine nitrogen excretion (Table 17) was low and constant and unaffected by feed intake. This data is consistant with results obtained for experiments 2 through 6 and are supported by Teekel et al. (1968).

The proportion of total nitrogen as uric acid nitrogen (Table 18) tended to increase as feed intake decreased, however, this trend was significant. In experiment 2, as feed intake decreased the proportion of

total nitrogen as uric acid nitrogen significantly increased. For both experiments 2 and 7, these results were expected since the birds receiving low feed input must catabolize tissue protein, which results in an increased excretion of uric acid (Okumura and Tasaki 1969). The primary difference between experiment 2 and experiment 7 is the magnitude of theincrease in uric acid nitrogen excretion. These results suggest that by adding a preservative to excreta samples, the contribution of uric acid nitrogen to a nitrogen correction factor remains relatively constant (89 to 95%) for all feed input levels. The constant level of excreta uric acid nitrogen in experiment 7 was not necessarily due to the ability of the preservative to prevent bacterial degradation of uric acid. Rather, the preservative probably prevented the degradation of urea and subsequent production of ammonia. On a proportional basis, this results in a larger proportion of the total nitrogen being made up by uric acid nitrogen.

The proportion of total nitrogen as urea nitrogen, presented in Table 18, followed the trends observed in Table 17. The proportion of urea nitrogen was highest for full fed birds and significantly less for force fed and unfed birds. In all treatments, the proportion of total nitrogen as urea nitrogen was low. This data is supported by several other authors (Table 20). Urea nitrogen provides the smallest energetic contribution of all the excreta nitrogen containing compounds (Table 9a) and as a result, the changes which occured have little effect on the TMEn nitrogen correction factor.

The proportion of total nitrogen as ammonia nitrogen (Table 18) parralleled the trends observed for the total nitrogen proportion of uric acid. As expected, as the acidemia of the birds increased, due to low or zero feed intake, the proportion of total nitrogen as ammonia nitrogen increased. It is important to note that the proportion of total nitrogen as

ammonia nitrogen in experiment 7 is considerably less than that observed in experiment 2. Addition of a preservative to the excreta samples in experiment 7 prevented bacterial degradation of urea which results in the production of ammonia. These results are important because ammonia has the highest energetic value of all of the excreta nitrogen compounds (Table 9b). This would suggest that addition of the preservative significantly changed the proportion of total nitrogen as ammonia nitrogen, by as much as 10% in the full fed treatment. This would have a significant effect on the nitrogen correction factor.

The total excretion of creatinine nitrogen, presented in Table 17, was not significantly affected by feed input level. However, the proportion of total nitrogen as creatinine nitrogen significantly decreased as feed input decreased (Table 18). This data is misleading. The results of experiments 2 to 6, supported by several authors (Table 20), confirm the observation of Teekell et al. (1968) and Sturkie (1976) that creatinine nitrogen excretion is small and constant. The significant changes in the proportion of total nitrogen as creatinine nitrogen which occured are probably due to the shifts in the total nitrogen proportion of the other excreta nitrogen compounds. Therefore, although creatinine excretion was not affected by feed input (Table 17), the contribution of creatinine nitrogen to a nitrogen correction factor was significantly affected.

Excreta total nitrogen for experiment 7 is presented in Table 18. As feed input decreased, the total nitrogen content of excreta also decreased. These results are contrary to the data of experiment 2 (Table 8). Following experiment 2, it was concluded that the negative nitrogen balance of the force fed and unfed birds significantly affected the individual nitrogen containing compounds, which in turn directly affected total nitrogen excretion. The result was excreta total nitrogen of force fed

and unfed birds was not significantly different from that of full fed birds. In experiment 7, addition of a preservative reduced the effect of feed intake on the individual nitrogen containing compounds of excreta. The result was total nitrogen excretion of force fed and unfed birds was significantly different from that of full fed birds. Therefore, following the addition of a preservative to excreta samples, excreta content of the nitrogen containing compounds which contribute to the nitrogen correction factor were significantly different among treatments. In addition, the amount of nitrogen to be corrected to zero was significantly different among treatments. This data suggests that nitrogen balance and the nitrogen correction factor which is to be applied to zero the nitrogen balance is different for unfed, force fed and full fed birds.

The contribution of individual nitrogen containing compounds to a nitrogen correction factor for the TMEn assay was determined and are presented in Table 19. Uric acid nitrogen provided the largest energetic contribution to the correction factor, followed by ammonia nitrogen. Urea nitrogen and creatinine nitrogen both provided very low contributions to the factor. These results were as expected considering the data presented in Table 18. The correction factor proposed by Titus (1956) (36.53kJ/g RN) falls between the factors determined for the force fed birds (35.10kJ/g RN) and unfed birds (37.70 kJ/g RN). The correction factor proposed by Hill and Anderson (1958) (34.39 kJ/g RN) falls between the factors determined for the full fed birds (34.06 kJ/g RN) and force fed birds (35.10 kJ/g RN). This would suggest that use of the nitrogen correction factor proposed by either Titus (1956) or Hill and Anderson (1958) is inappropriate and that individual nitrogen correction factors for force fed and unfed birds, determined in experiment 7, should be applied. To fully understand the implications of this, using the data of experiment 7, the TME and TMEn values

of cracked wheat were calculated (Table 21). The TME value of the cracked wheat was 16.94 MJ/Kg. Applying the nitrogen correction factor 34.39 kJ/g RN proposed by Hill and Anderson (1958), the TMEn value was 16.21 MJ/Kg. Applying the nitrogen correction factor 36.53 kJ/g RN proposed by Titus (1956), the TMEn value was 16.16 MJ/Kg. However, using the correction factor for the force fed birds (35.10 kJ/g RN) and unfed birds (37.70 kJ/g RN), determined in experiment 7, the TMEn value was 16.01 MJ/Kg.

Identical experimental procedures were followed in experiment 7 as in experiment 2. As such, the data of experiments 2 and 7 are directly comparable (Table 21). In both experiments, the test material investigated was cracked wheat originating from the same sample (Tables 4a and 4b). The TME value determined in experiment 2 (15.64 MJ/Kg) differed by 8.3% from that determined in experiment 7 (16.94 MJ/Kg). This difference was due to differences in the energetic value of excreta of both fed and unfed birds. This is supported by Dale and Fuller (1981). It must be assumed that birds of similar treatment in experiments 2 and 7 are at equal nitrogen balance following the TME assay. Therefore, differences in the TME value of the test material are probably due to the preservative preventing any shifts or loss of the energetic nitrogen containing compounds of excreta. Correction of the TME value to zero nitrogen balance tended to increase the variation in results between experiments 2 and 7. Applying the correction factor of Hill and Anderson (1958) produced TMEn values of 14.81 MJ/Kg for experiment 2 and 16.21 MJ/Kg for experiment 7, with a difference of 9.4% between values. Applying the correction factor of Titus (1956) produced TMEn values of 14.76 MJ/Kg for experiment 2 and 16.16 MJ/Kg for experiment 7, with a difference of 9.5% between values. These TMEn values used a correction factor which was unaffected by the errors of nitrogen balance. Therefore, the increase in the variation between the values was

due to differences in the amount of excreta nitrogen to be corrected (Tables 8 and 18). However, as indicated previously, the errors of nitrogen balance trials also significantly affects the nitrogen correction factor which will directly affect the TMEn value. Applying the correction factor for the force fed birds and unfed birds as determined in experiments 2 and 7, resulted in TMEn values of 14.70 MJ/Kg for experiment 2 and 16.01 MJ/Kg for experiment 7. By addition of a preservative to excreta in a standard TMEn assay, the errors associated with nitrogen balance trials are prevented. The result is an 8.9% increase in the determined bioavailable energy value of cracked wheat.

SUMMARY AND CONCLUSIONS

Seven experiments were designed to study some of the errors associated with nitrogen balance trials. The effect of these errors on the standard TMEn assay was evaluated. Three of the seven experiments evaluated the applicability of previously determined nitrogen correction factors. Three of the seven experiments were concerned with the evaluation of two different chemical preservatives which may prevent the errors which occur in nitrogen balance trials. Examination of the data collected from these experiments has led to the following conclusions.

I. Proportion of nitrogen containing compounds in poultry excreta.

1. In the standard TMEn assay, uric acid nitrogen represents the greatest proportion (over 80%) of the total nitrogen excreted, but is not the sole excretory nitrogen compound.

2. The excretion of uric acid nitrogen is dependant on dietary protein intake and feed input level.

3. Use of a uric acid based nitrogen correction factor will not bring the force fed and unfed birds of the TMEn assay to zero nitrogen balance.

4. The nitrogen correction factor proposed by Hill and Anderson (1958), which is based on the uric acid excretion of full fed birds, may not be appropriate for the force fed and unfed birds of the TMEn assay.

5. The relative proportions of nitrogen containing compounds in the excreta total nitrogen of SCLW roosters used in a TMEn assay were:

86.28 - 93.37% uric acid, 9.25 - 12.85% ammonia, 1.38 - 2.40% amino acids, 0.27 - 1.45% urea and 0.21 - 0.22% creatinine.

6. The proportion of excreta nitrogen containing compounds were significantly affected by feed input level. As a result excreta nitrogen make up for the force fed and unfed birds was significantly different from the excreta of full fed birds.

7. The nitrogen correction factor proposed by Titus (1956) was developed by determining the gross energy of a sample of urine of a nondescript full fed bird. This correction factor significantly underestimates the negative nitrogen balance of TMEn assay birds. As such the proposed correction factor may be inappropriate for the force fed and unfed birds of the TMEn assay.

II. Errors of nitrogen balance trials.

1. The TMEn assay, by definition, is a nitrogen balance trial. Therefore, the TMEn assay is subject to the same errors commonly associated with nitrogen balance trials.

2. Extension of the excreta collection period from 1 hour to 48 hours significantly affected the total nitrogen proportion of excreta nitrogen containing compounds. Thirty three to 87.5% of excreta urea nitrogen was degraded, presumably due to bacteria of intestinal or cecal origin. Conversely a significant increase of excreta ammonia nitrogen, 37.14 to 194.4%, was observed. The effect on uric acid was inconclusive and excreta creatinine was not affected.

3. Freeze drying of excreta significantly changed the total nitrogen proportion of nitrogen containing compounds. A loss of 17.1 to 65.7% of ammonia nitrogen occured during freeze drying. The effect on excreta

uric acid and urea was inconclusive. Freeze drying had no effect on excreta creatinine nitrogen.

4. Changes in the excreta total nitrogen proportion of nitrogen containing compounds, which occur over time and as a result of freeze drying, would affect nitrogen balance, the TME nitrogen correction factor and ultimately TMEn values.

III. Use of preservative to prevent the errors of nitrogen balance trials.

1. Two preservatives were evaluated to prevent the errors associated with a standard TME assay. The preservatives were mercuric chloride (0.3 per cent w/v) in glacial acetic acid and boric acid powder plus (0.3 per cent w/w) mercuric chloride.

2. Mercuric chloride (0.3 per cent w/v) in glacial acetic acid failed to prevent the loss of urea nitrogen which occured during extended collection periods and failed to prevent the loss of ammonia as a result of freeze drying.

3. Boric acid powder plus (0.3 per cent w/w) mercuric chloride prevented any change in the proportion of all excreta nitrogen containing compounds under standard TMEn assay procedures.

IV. Proportion of nitrogen containing compounds in poultry excreta with added chemical preservative.

1. Boric acid powder plus (0.3 per cent w/w) mercuric chloride was added to the excreta collected from SCWL roosters. The relative proportion of nitrogen containing compounds in the excreta total nitrogen were: 92.44 - 95.20% uric acid, 3.66 - 5.65% ammonia, 0.27 - 0.36% urea and 0.34 -

0.37% creatinine.

2. Uric acid nitrogen represents the largest, but not the sole portion of excreta total nitrogen. As a result, the uric acid based correction factor proposed by Hill and Anderson (1958) may not be appropriate for the force fed and unfed birds of the TMEn assay.

3. The proportion of excreta nitrogen containing compounds were significantly affected by feed input level. As a result excreta nitrogen make up for the force fed and unfed birds was significantly different from the excreta of full fed birds. Therefore, the nitrogen correction factor developed using full fed birds as proposed by Titus (1956) may be inappropriate for the force fed and unfed birds of the TMEn assay.

4. The proportion of excreta nitrogen containing compounds is significantly affected by feed intake. As a result, nitrogen balance of the full fed, force fed and unfed birds is different. This would suggest that the force fed and unfed birds of the TMEn assay require specific nitrogen correction factors, 35.10kJ/g RN and 37.10kJ/g RN, respectively.

V. TME and TMEn values.

1. In experiment 2, excreta samples did not have preservative added and therefore were subject to the errors of nitrogen balance trials. These errors were prevented in experiment 7 by addition of a preservative to excreta samples. As a result, the TME value of the same sample of cracked wheat was 8.3% greater in experiment 7 than in experiment 2.

2. Errors in nitrogen balance trials cause significant shifts in the contribution of individual nitrogen containing compounds to a nitrogen correction factor for the TMEn assay. The correction factors determined with unpreserved excreta (experiment 2) were greater than those determined with

preserved excreta (experiment 7). As a result, the TMEn value of the same sample of cracked wheat was 8.9% greater in experiment 7 than in experiment 2.

GENERAL CONCLUSIONS

The TMEn assay is subject to the errors of nitrogen balance trials. The errors which occur due to the extended excreta collection period and freeze drying procedure are significant, but can be prevented by use of the chemical preservative, boric acid powder plus (0.3 per cent w/w) mercuric chloride. Preventing the error of nitrogen balance trials in the TMEn assay resulted in an increase in the bioavailable energy of cracked wheat by 8.9%.

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