# AN EXAMINATION OF FACTORS AFFECTING THE NITROGEN CORRECTION FACTOR USED IN THE NITROGEN CORRECTED TRUE METABOLIZABLE ENERGY ASSAY

By

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# ABSTRACT

Six experiments were designed to study factors which may affect the levels of excretory nitrogen compounds and thus the nitrogen correction factor for the true metabolizable energy (TME) assay. The effect of different feedstuffs, cecectomy, and use of a chemical preservative were investigated. The nitrogen factors of 34.39 kJ/gRN proposed by Hill and Anderson (1958) and 36.53 kJ/gRN proposed by Titus (1956) were also evaluated.

In all experiments, uric acid nitrogen represented the largest proportion (over 77.0%) of total excreta nitrogen, but not the sole excretory nitrogen compound. Other nitrogenous compounds include urea, ammonia, creatinine and amino acid nitrogen. In terms of energetic contribution all experiments followed a similar trend. Uric acid nitrogen provided the largest energetic contribution, followed in turn by amino acid nitrogen and ammonia nitrogen. Urea nitrogen and creatinine nitrogen provided only a small contribution.

The results of experiments 1 to 4 showed a lack of effect of a chemical preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the nitrogen correction factor.

Experiments 3 and 4 investigated the effect of different feedstuffs. Experiment 3 involved three samples of wheat (Marshall, Glenlea, and HY320) with similar nutrient compositions. Experiment 4 involved samples of feedstuffs (fish meal, alfalfa meal, and wheat) with varying levels of available amino acids. With the exception of alfalfa meal (presumably due to its fibrous nature), the nitrogen correction factors were similar, thereby suggesting a lack of effect of feedstuff.

Experiment 5 showed a lack of effect of supplemental energy (α D (+) glucose) on the nitrogen correction factor. The values for unfed birds and birds force fed α D (+) glucose were similar.

Experiment 6 showed a lack of effect of cecectomy on the nitrogen correction factor. The values for the intact and cecectomized birds were similar.

The results of these experiments suggest that the values proposed by Hill and Anderson (1958) and Titus (1956) underestimate the metabolic and endogenous nitrogen (and thus energy) losses and therefore may be inappropriate for use in the nitrogen corrected TME assay. Additionally, the correction factors for the unfed birds (which includes those force fed supplemental energy) were lower than the correction factors for the force fed birds, suggesting that specific nitrogen correction factors for unfed and force fed birds be applied.

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Dedicated to my mom...

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ADE | apparent digestible energy |
| Ala | alanine |
| AME | apparent metabolizable energy |
| AMEn | nitrogen corrected apparent metabolizable energy |
| Arg | arginine |
| Asp | aspartic acid |
| BE | bioavailable energy |
| °C | degree celsius |
| cal | calories |
| cm | centimetre |
| $Cr_2O_3$ | chromium sesguioxide |
| Cys | cystine |
| DE | digestible energy |
| GE | gross energy |
| FE | fecal energy |
| FiE | fecal energy of undigested feed residues |
| FmE | fecal energy of metabolic by-products |
| FN | fecal nitrogen |
| g | grams |
| Glu | glutamic acid |
| HCL | hydrochloric acid |
| HeE | heat of basal metabolism |
| His | histidine |
| HjE | heat of activity |
| HrE | heat of product formation |
| J | joules |
| I | feed intake |
| IE | ingested Energy |
| Ile | isoleucine |
| k | nitrogen correction factor |
| kg | kilogram |
| kJ | kilojoule |

| | |
|---|---|
| Leu | leucine |
| Lys | lysine |
| ME | metabolizable energy |
| Met | methionine |
| mg | milligrams |
| MJ | megajoule |
| ml | millilitre |
| NDF | neutral detergent fibre |
| Phe | phenylalanine |
| Pro | proline |
| RN | retained nitrogen |
| RPM | revolutions per minute |
| SCWL | single comb white leghorn |
| Ser | serine |
| TAAA | true available amino acids |
| TAL | true available lipids |
| TAM | true available minerals |
| TDE | true digestible energy |
| Thr | threonine |
| TME | true metabolizable energy |
| TMEn | nitrogen corrected true metabolizable energy |
| Tyr | tyrosine |
| UE | urinary energy |
| UeE | endogenous urinary energy |
| UiE | urinary energy of undigested feed residues |
| UmE | urinary energy of metabolic by-products |
| UN | urinary nitrogen |
| Val | valine |
| wt | weight |
| w/w | weight per weight |

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# INTRODUCTION

It is widely acknowledged that poultry eat to satisfy their energy requirements (Sibbald, 1982). The energy requirement is dependent upon a number of factors, which include maintenance, growth, reproduction, temperature control, and other various metabolic activities. However, it is also important that the levels of energy provided allow the production unit to maximize economic return. As a result, it is important to determine the level of available energy that a diet provides.

Sibbald (1976a) developed the true metabolizable energy (TME) assay as a method of determining available energy. A component of the assay involves test birds which remain unfed for the duration of the experiment in order to measure metabolic and endogenous nitrogen (and thus energy) losses. A potential problem is that endogenous nitrogen losses are increased in the fasted birds in order to satisfy the metabolic demand for energy (Parsons et al., 1982a). This is accomplished through the catabolism of body protein. The resulting energy excretion would therefore overestimate the excretion of endogenous energy of fed birds. Subsequently, a nitrogen correction factor was added to the procedure to avoid overestimation of endogenous energy loss and thus increase the accuracy of the TME assay (Dale and Fuller, 1984b).

The correction factors most commonly used are those proposed by Hill and Anderson (34.39 kJ/gRN) (1958) and Titus (36.53 kJ/gRN) (1956). The value of Hill and Anderson (1958) is based on the energetic value of uric acid nitrogen, assumed to be the sole nitrogen excretory compound. The value of Titus (1956) assumes that there are other excretory nitrogen compounds derived from catabolism of body protein in addition to uric acid nitrogen. The objective of this thesis is to evaluate the nitrogen correction factor and to examine factors which may affect it.

# LITERATURE REVIEW

## I. INGESTED ENERGY - DEFINITION OF TERMS

Lavoisier (in Vohra, 1966) is regarded as the father of the science of nutrition because of his contributions towards the field of animal heat long before the principle of conservation of energy was formulated. He stated that a major part of animal heat originated from the combustion of organic substances in the animal body.

In general, all life processes including maintenance, growth, reproduction, utilization of ingested food, temperature control, and other various activities are dependent upon the energy provided by the diet. However, as not all of the gross energy of a feedstuff is available to a bird, it was necessary to develop assays to measure the bioavailability of energy (Sibbald, 1980a).

The development of several bioassay procedures based on the concept of partitioned ingested energy has led to a number of discrepancies regarding the nomenclature used to describe the results of these bioassays (Pesti and Edwards, 1983). It was with this in mind that in 1981 the National Research Council published a glossary of energy terms allowing for the standardization of terminology for the description of energy utilization by animals. This glossary has gained international approval and has done much to clarify a confusing subject (Sibbald and Wolynetz, 1984a).

GROSS ENERGY (GE) - the heat of combustion of a substance. It is generally measured with an oxygen bomb calorimeter (NRC, 1981).

FEED INTAKE (I) - the weight of the food consumed (NRC, 1981).

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

FECAL ENERGY (FE) - the gross energy of the feces. FE equals the weight of feces times the gross energy of the feces (NRC, 1981). FE consists of two components: an undigested feed residue (FiE), and a metabolic end-product (FmE) comprising cells abraded from the intestinal wall (mucosa), bile, and unabsorbed digestive fluids (Sibbald and Wolynetz, 1984a).

DIGESTIBLE ENERGY (DE) - DE can be divided into two categories. Apparent digestible energy (ADE) is the intake of feed energy less the fecal energy: ADE = IE - FE (NRC, 1981). The term apparent is used because FE consists of both FiE and FmE, i.e. ADE = IE - (FiE + FmE) (Sibbald, 1980a). However, since FmE is a body maintenance cost it should not be charged against the feed (Sibbald, 1980a). Therefore, true digestible energy (TDE) is equal to the intake of feed energy less the fecal energy of feed: TDE = IE - FiE (NRC, 1981). By definition, ADE is less than TDE and IE; however, for completely digestible feedstuffs (such as dextrose or corn oil), TDE = IE because FiE is zero (Sibbald, 1980a).

URINARY ENERGY (UE) - the gross energy of the urine (NRC, 1981). UE consists of three components: absorbed feed that is not utilized and excreted in the urine (UiE), the endogenous component consisting of the nitrogenous by-products of tissue catabolism (UeE), and a metabolic component consisting of the nitrogenous by-products of nutrient utilization (UmE) (Sibbald and Wolynetz, 1984a).

METABOLIZABLE ENERGY (ME) - as with DE, ME can be divided into two categories. Apparent metabolizable energy (AME) is the intake of feed energy less both the fecal energy and urinary energy: AME = IE - FE - UE (NRC, 1981). As with

FmE, since UmE and UeE are a body maintenance loss they also should not be charged against the feed (Sibbald, 1980a). True metabolizable energy (TME), therefore, is a measure of available energy which avoids these penalties: TME = IE - FiE - UiE (Sibbald, 1980a).

RETAINED NITROGEN (RN) - the weight of nitrogen eaten as feed minus the weight of nitrogen excreted as feces and urine. While in birds synthesizing new tissue from dietary energy sources the value is positive, for those birds fed less than maintenance requirements and depending on catabolism of body stores for energy, the value becomes negative (Sibbald and Wolynetz, 1984a).

## II. ASSAYS FOR BIOAVAILABLE ENERGY: AN OVERVIEW

### A. Digestible Energy

As defined previously, the difference between IE and excreta (FE + UE) energy is an estimate of ME not DE. Since DE is the difference between IE and FE (UE excluded), and as feces and urine are voided together by poultry, DE cannot be measured without surgically modifying the digestive tract (Vohra, 1972). Several procedures have been developed.

Non-surgical methods for collecting urine and feces separately have been developed using modified catheters designed to fit into the urodaeum (Davis, 1927; Coulson and Hughes, 1930). Cannulation of the ureters has also been used (Hester et al., 1940). These have been useful for short-term experiments (one half to 24 hours) but have side effects such as increased urine flow (Hester et al., 1940).

Surgical methods involving exteriorization of the ureters (Hester et al., 1940; Newberne et al., 1957) and the colon (colostomy) (Ivy et al., 1968; Paulson, 1969) have been developed. A difficulty here is that birds modified in this manner require careful

maintenance to prevent blockage of the rectum (Ivy et al., 1968). In addition, Emmanuel and Howard (1978) have shown that the metabolism of colostomized birds differ from that of normal birds, while Van Kampen (1981) has observed a higher water intake and lower food intake in colostomized birds.

With the potential problems of the previous methods, this has led to the development of techniques for separating the feces and urine after excretion. In this case the uric acid content of excreta is measured and an energy correction is made (Sibbald 1982). The assumption is that there is a direct relationship between uric acid and urinary energy. O'dell et al. (1960), Richardson et al. (1968) and Waring and Shannon (1969) have shown this to be questionable, observing evidence of a diet effect on urine composition and energy per gram of urinary nitrogen.

Sibbald et al. (1962) have used another technique involving the use of chromium sesquioxide ($Cs_20_3$) as an indicator. Here the birds are fed the test material, killed, and the intestinal and cecal contents are collected for analysis. However, reverse peristalsis moving urine back into the intestinal tract may contaminate the cecal and/or intestinal contents resulting in erroneous results (Sibbald, 1982).

Assays for ADE yield estimates of the proportion of the IE that is available to the bird. Without correcting for FmE losses to obtain a more accurate TDE value, these are at best approximations (Sibbald, 1982). Further, the technical problems involved and assumptions required whether determining ADE or TDE, render these methods impractical.

## B. Metabolizable Energy

For poultry, ME is the simplest practical feeding evaluation system that can be used since poultry void both feces and urine together (Farrell, 1979). There has however been a general lack of agreement on how ME should be measured. The various methods used are the AME (direct or indirect) assay, the TME assay, or the nitrogen corrected ME (AMEn or TMEn) assays.

### 1. Apparent Metabolizable Energy

#### a. Indirect Assays

Indirect biological assays include the use of equations that predict ME from physical and chemical parameters, either singly or in combination, including bulk density, crude protein, ether extract, crude fibre, ash, starch, soluble sugars, and tannins (Sibbald, 1975a). These assays can be done rapidly and are specially suited to situations where a bomb calorimeter is unavailable.

Chemical assays involving equations to predict BE from chemical composition may be divided into two groups: coefficients derived from knowledge (or estimates) of the BE value of specific components, and coefficients derived by regression analysis.

Fraps et al. (1940) stated that the AME could be calculated using proximate analysis of poultry excreta. Titus (1958) derived a series of digestibility coefficients which permit calculation of AME values of individual feeding stuffs. Subsequent general prediction equations have been published by Carpenter and Clegg (1956) and Sibbald et al. (1963). Although BE can be calculated using regression analysis, there are few prediction equations that are generally applicable to several feedstuffs. There

are, however, numerous regression equations which predict the BE values for specific feedingstuffs (Sibbald, 1982).

Equations which predict BE have several limitations, however. First is the assumption that all proteins, carbohydrates, or fats are equally digestible (Sibbald, 1982). Bolton (1957) has shown this to be false. Secondly, prediction of BE of mixed feeds requires that the composition of the feeds be known (Sibbald, 1982). Thirdly, predictions can be misleading unless tested with independent data (Sibbald, et al. 1980).

Nutritional value is thought to increase with bulk density. There are however conflicting results. Lockhart et al. (1961) and Sibbald and Price (1976) have shown ME values to increase with bulk density for oats. For barley, however, Sibbald and Price (1976) and Coates et al. (1977) have found no relationship between ME values and bulk density. Similarly, the AME of wheat did not vary with bulk density (Sibbald and Price, 1976; Coates et al., 1977). As a result bulk density is not a good indicator of AME, thereby limiting its practical value.

A final indirect assay involves a growth comparison between chicks fed a test material and a growth response curve obtained by feeding several levels of a material of known ME content (Squibb, 1971). Growth assays yield highly variable results once again limiting its practical value (Sibbald, 1980a). As a result they are not used by the feed industry.

b. Direct Assays

Direct biological assays are essentially balance experiments in which differences between inputs and outputs are measured (Sibbald, 1982). The most common procedure involves feeding a diet to birds for an acclimization period,

although the need for such a period is not well documented. The feed intake and excreta output are then measured for a period of three or four days, after which the AME value is calculated by measuring the difference between the gross energy of the feed consumed and the gross energy of the excreta (Sibbald, 1980a). The two methods available to quantitatively relate excreta to the food consumed are the total collection technique (i.e. determination of total food intake and quantitative collection of excreta) and the use of an indicator in the diet (Vohra, 1972).

The total collection technique requires measurement of both the feed intake and excreta output and the gross energy of the feed and the excreta. The AME is then calculated as follows: AME = IE - FE - UE (NRC, 1981). There are several objections concerning total collection. The assumption that excreta voided during a period of time corresponds to feed ingested during the same time, is questionable since rates of intake and excretion may vary (Sibbald, 1982). However, Tyler (1958) has shown that possible errors can be reduced by collecting excreta over a period of three or more days. Spilled feed, feathers, down, and scale contamination of excreta make the quantitative collection of excreta difficult (Sibbald, 1980a). A final difficulty is that a variation in moisture can occur over time (Sibbald, 1982).

To avoid the problems inherent in using the total collection technique, an inert indicator may be added to the diet. The amounts of indicator and energy per gram of feed and excreta are measured and calculated as follows (Hill and Anderson, 1958):

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

A number of indicators have been employed in determining AME values. These include chromium sesquioxide ($Cr_2O_3$) (Edin, 1918 in Vohra, 1972), barium sulphate ($BaSO_4$) (Whitson et al., 1943), silica (Gallup, 1929), ferric oxide (Bergheim, 1926), lignin (Kane et al., 1950), crude fibre (Almquist and Halloran, 1971), polyethylene

(Roudybush et al., 1974), and acid insoluble ash (Vogtmann et al., 1975). The most common indicator in AME studies is chromium sesquioxide (Sibbald, 1982).

The use of indicators are not however, without their own share of problems. The indicator must be measured in both the feed and excreta, which can contribute to variability in AME data. $Cr_2O_3$ presents a difficulty in that it can separate out of the excreta during grinding (Vohra, 1972). Indicators are assumed to be dispersed uniformly throughout the feed and excreta, to have similar flow rates through the digestive tract as other feed residues, and to be unabsorbed (Sibbald, 1982). For both $Cr_2O_3$ and $BaSO_4$ this has been shown to be false as they are not completely inert and may be absorbed along the digestive tract (Vohra and Kratzer, 1967). In addition, indicators require additional analytical work resulting in the possibility of additional errors while simultaneously making it difficult to reproduce assay data among laboratories (Sibbald, 1982).

Numerous variations of the direct AME assays have been used. Composition of the reference diet (McIntosh et al., 1962; Sibbald and Slinger, 1963), physical form (McIntosh et al., 1962), and various levels of inclusion of test materials (Hill et al., 1960; Potter et al., 1960; Sibbald et al., 1960; Sibbald and Slinger, 1963) are all important variables which show no consistent patterns. Vohra (1972) has discussed variation of results with regards to age, sex, breed, and species of assay birds. Finally, different methods of feeding and excreta collection have also been reported (March and Biely, 1973; Farrell, 1978; Farrell, 1980; Vohra et al., 1982; Schang and Hamilton, 1982; Parsons et al., 1984; du Preez et al., 1984). According to Sibbald (1980a), the lack of a standardized methodology of determining AME values, leading to variability among AME data, has yielded criticisms and questions as to the validity of AME as an estimate of BE in poultry feedingstuffs.

## 2. True Metabolizable Energy

### a. Development of the TME Bioassay

In studying the variation associated with the AME bioassay, Sibbald and Price (1975) found that the AME value measured with an individual bird, varied from day to day in a 'saw-tooth' manner. That is, on successive days, the AME value was higher and lower than average. The most probable explanation for this was considered a reflection of fluctuation in feed consumption about the maintenance requirement. This assumption led to the confirmation of the hypothesis presented by Guillaume and Summers (1970) that AME values decreased in a curvilinear manner, with feed intake (Sibbald, 1975b). In their study, Guillaume and Summers (1970) had shown that AME values decreased as feed intake was reduced. This was a result of FmE + UeE losses (which are essentially constant) being charged against a smaller quantity of feed. Thus, by definition of AME, as the IE decreases, FmE + UeE (which are subtracted from IE) become proportionately greater and, at a very low IE, can cause negative AME values. The confirmation by Sibbald (1975b) showed that the energy excreted by roosters decreased linearly as feed intake decreased. This relationship led to the development of a bioassay for TME (Sibbald, 1976a).

The bioassay for TME is based on two assumptions (Sibbald, 1981b): 1) in a previously fasted bird, the relationship between energy input as feed (IE) and energy voided as excreta (FE + UE) is linear; and 2) the y-intercept of the regression line is a valid estimate of metabolic and endogenous energy (FmE + UeE) loss. This second assumption implies that there is a single y-intercept at zero IE input for the regression lines of all test materials, and that this is a measure of FmE + UeE. Thus, the measurement of the FE + UE of the unfed birds is believed to be equal to the

FmE + UeE losses of the fed birds (Sibbald, 1982). Although neither assumption can be proven the weight of evidence favours their acceptance (Sibbald, 1982). For example, Tenesaca and Sell (1979) and Shires et al. (1980) have similarly reported a linear relationship. Sibbald (1981b) has demonstrated that a nonlinear relationship will occur with an insufficient excreta collection period.

The initial bioassay of Sibbald (1976a) involved adult single comb white leghorn (SCWL) roosters which were fasted for 21 hours, force (precision) fed 25 grams (g) of test material, and excreta voided during the subsequent 24 hours were collected in plastic trays under the cage. In order to estimate FmE + UeE losses, excreta from fasted birds of similar body weight was also collected. Water was provided ad libitum. These steps were repeated to provide the desired number of replications. The excreta was then collected, frozen, freeze dried, equilibrated with atmospheric moisture, and weighed. The samples were then ground and analyzed for gross energy and total nitrogen. The TME value was calculated as follows:

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

where: IE = intake of feed energy

FE + UE = energy voided by the fed bird

FmE + UeE = energy voided by the unfed bird

Birds used in a TME assay are maintained on the same diet, the composition of which is not important provided the nutrient requirements are satisfied (Sibbald, 1983). However, the birds are generally housed on a maintenance diet containing 15% protein (Sibbald, 1983). The maintenance energy requirement for adult roosters is 489.5 kJ ME per kilogram (kg) body weight (wt) per day as calculated by Guillaume and Summers (1970). The maintenance diet is fed ad-libitum allowing birds to eat according to energy balance.

Some modification of the TME assay has occurred since its inception. The primary modifications have dealt with the length of fasting and excreta collection periods. Since the object of the collection period is to obtain a representative sample of excreta from the test materials fed, all feed residues from the pervious feed must be cleared from the alimentary tract. Thus, the initial modification was the extension of the preliminary fast from 21 to 24 hours in order to reflect the clearance time of the maintenance diet. Sibbald (1979d) found a clearance time of 24 hours was generally sufficient. When cereal grains were fed at levels varying from 10 to 30 g all feed residues were completely cleared within 24 hours. This agrees with Sibbald (1976c) that lengthening the starvation period from 24 to 96 hours by 24 hours intervals had no significant effect on the TME value.

Duration of excreta collection is dependent upon rate of passage which in turn is dependent on a number of factors including temperature, gut microflora, water absorption and diet (Warner, 1981). Of these, diet has shown to have the greatest effect. Feeds of small particle size tend to increase rate of passage whereas feeds high in fibre or with high specific gravities tend to decrease rate of passage (Warner, 1981). The observation that rapeseed hulls were excreted more than 24 hours after force feeding resulted in an extension of the excreta collection period from 24 to 48 hours (Sibbald, 1978a). Sibbald (1979b; 1979d), Mutzar and Slinger (1979b, 1980b, 1980c), and Chami et al. (1980) have reported varying rates of passage (24 to over 36 hours) depending on the test material used. Further support by Kessler and Thomas (1981) has led to the adoption of a 48 hour collection period. This is considered appropriate for most test materials (Sibbald, 1983). Salmon (1983) found that depending upon the feedstuff a collection period of 48 hours may be unnecessary and may result in a

decrease in the TME value. He suggested that it may be preferable to adjust the assay period in accordance with the rate of passage of the material being assayed.

As reported by Sibbald (1976a) there is no implication that the level of feed intake is of importance in the measurement of TME. Sibbald (1977) found further support of this with feed intakes ranging from 10 to 100 g per bird. However, in practice this is not correct as birds fed high levels of feed input often resulted in regurgitation (Sibbald, 1976a) and increased stress (Wehner and Harrold, 1982a). Thus there appears to be an optimal level of input which varies with the size of the bird (Wehner and Harrold, 1982b) and nature of feedingstuff (Sibbald, 1976a). For most feedingstuffs the input level is about 20-25 g per bird, or approximately 1% of body weight (Sibbald, 1976a). However, Mutzar and Slinger (1979b, 1980b) have shown that rate of passage is affected by feed input. They found that for inputs over 30 g a collection period of 36 hours was required to clear the gut. This was indicated by Sibbald (1979d) who found that although 24 hours was sufficient to clear the gut, there was still some effect on rate of passage with varying feed inputs. Sibbald (1982) more recently suggested feeding 30 to 40 g of the test material. This still allows for a collection period of 48 hours.

To increase the level of feed input, Schang et al. (1982b) have suggested a procedure of double force feeding involving a feed input of 30-40 g on two consecutive days and excreta collection only during the 24 hours following the second feeding. The assumption here is that the excreta collected from birds force fed twice and collected over only 24 hours will be equivalent to that voided during the 48 hour period following a single feed input. However, the trend of TME values obtained with double-feeding indicates that method of feeding may influence gut clearance. Further,

increased labour input and possibility of excreta loss during the second force feeding yield further questions as to its applicability.

b. Assay Bird

As mentioned previously, the initial TME bioassay developed by Sibbald (1976a) involved the use of SCWL roosters. Since then, few interspecies comparisons have been made. Sibbald (1976b) found no species differences between adult roosters, laying hens, broiler hens, and turkey hens fed various test materials, with the exception of higher TME values obtained from soybean meal. Similar findings are reported by Parsons and Potter (1980) and Dale and Fuller (1980), in comparing turkeys and chickens and turkey poults and chicks respectively. In all cases the TME values are higher for turkeys. The difference in TME values observed only with soybean meal and not with other feedstuffs maybe due to the cecal microflora of the turkey which are better able to ferment the indigestible carbohydrates of soybeans than the microflora of chicken (Sibbald, 1982). It should be noted, however, that TME values of cecectomized chickens are lower than intact chickens, suggesting that the microflora of chickens are involved to some degree in fermentation of soybean meal (Kessler and Thomas, 1981). Mutzar et al. (1977) deduced differences in TME value between ducks and cockerel were due to incomplete clearance of feed during the 24 hour excreta collection period. Ostrowski-Meissner (1984) found no differences between drakes and cockerels using a 48 hour collection period. Storey and Allen (1982a; 1982b) have determined the TME values of mature, non-laying female Emden geese but have not made comparisons with roosters. Sibbald (1976b) found no genotypic differences between meat-type and egg-type hens while Dale and Fuller (1980) noted an absence of difference between adult leghorn cockerels and 6-week old

broilers. Finally, no differences in TME values have been associated with sex (Sibbald, 1976b) or age (Sibbald, 1978b; Mutzar and Slinger, 1979a; Dale and Fuller, 1980; and Shires et al., 1980). The preferred bird for the TME bioassay is still maintained to be an adult SCWL rooster. Chicks should not be used as they have a limited feed capacity while fasted laying hens are avoided as they often produce shell-less eggs which will often break resulting in excreta contamination (Sibbald, 1983).

c. Force Feeding Techniques

The purpose of force (precision) feeding is to allow for a controlled (known) quantity of feed to be fed to a bird at a specific time. It also avoids the problems encountered with ad libitum feeding including feed spillage, selective feeding, and feed intake variation among birds (Sibbald, 1983). Force feeding techniques are not unique to Sibbald's 1976 bioassay. To conduct experiments regarding feed intake mechanisms, Mather and Ahmad (1974) introduced a technique whereby a cannula was surgically attached to the esophagus of a chicken. However, the requirement for a simple and rapid bioassay renders this technique impractical. The most common and accepted procedure (Sibbald, 1983) involves the insertion of a tube from the beak, via the esophagus into the crop. The initial method involved pushing the feed through a simple glass tube with a glass rod. This was replaced with a glass funnel and metal plunger and subsequently with a stainless steel funnel and aluminum rod. Either glass or stainless steel equipment may be used while plastic is avoided as it may possess electrostatic properties which can cause adherence of feed particles to the equipment. Successful precision feeding also requires control of the bird during the procedure (Sibbald, 1983).

In order to address the animal welfarist concerns mentioned by Farrell (1981) of undue stress accorded to force feeding dry pellets of ground feed, Wehner and Harrold (1982a) tested a variety of feeding techniques. These involved delivery, through a tube inserted into the crop, of a dog feed via a glass rod and of a moist pellet, slurry, and paste via a syringe. They found that use of a slurry or paste-feeding alleviated the stressful conditions of force feeding dry preparations (i.e. retracted head and neck, crouched posture, and regurgitation). In response, Fraser and Sibbald (1983) found the behavioural effects indicative of stress were fleeting and that the stressful conditions noted by Wehner and Harrold (1982a) were likely due to the increased time taken to complete the force feeding (3-13 minutes vs 30 seconds). It was concluded that rapid completion of force feeding should essentially eliminate these problems.

Teeter et al. (1984) have developed a force feeding gun in order to decrease the time required for force feeding. Each bird was able to be fed in less than 30 seconds, and in addition, no signs of discomfort or regurgitation were exhibited. However, there are several disadvantages. Large particles may become trapped at the neck of the gun requiring increased pressure to force the feed out. All test materials must be fed in combination with water which can result in feed-water separation. Also, because feed particle size and diet water binding capacity vary with experimental rations, individual optimal feed/water ratios are required for diets.

d. Methods of Collection

The quantitative collection of excreta is an extremely important aspect of the TME bioassay. It is necessary that the collection be as precise as possible. Blakely (1963) developed a technique involving surgical attachment of a plastic bag to the

cloaca of turkeys. Similar practices are common in experiments using colostomized birds for the separate collection of urine and feces (Newberne et al., 1957; Paulson, 1969). Hayes and Austic (1982) have also described a method which is similar to that of Blakely (1963). Again, a plastic tube is sutured to the cloaca of adult roosters to which plastic bags may be attached for collection of excreta.

Generally, excreta is collected from trays which are placed beneath the cages housing the birds (Sibbald, 1982). They are made of smooth plastic and are larger than the bottom of cages to prevent loss of excreta (Sibbald, 1983). However, trays possess the disadvantage that scales and feathers may become trapped in the excreta. There also remains the possibility of excreta falling beyond the edges of the trays. Terpstra and Janssen (1975) housed birds in narrow cages and restrained them using neck-tethers. This permits excreta to drop over only a small area thereby reducing scale and feather contamination as well as excreta loss. Wehner and Harold (1982a) used a blower unit placed over the collection pans to keep air flowing and minimize scale and feather collection.

A procedure whereby human colostomy bags are attached to birds to collect excreta is described by Sibbald (1983). The feathers surrounding the cloaca are trimmed and colostomy bags are attached to the bird with a strong adhesive following force feeding. Following excreta collection the colostomy bags are removed, sealed, frozen, and stored. The method allows for precise collection of excreta while eliminating scale and feather contamination. Problems with adhesive failure and loss of collection bags do exist however. Recently, Almeida and Baptista (1984) designed a harness to hold an excreta collection bag over the cloaca. This procedure avoids the use of an adhesive and reduces loss of collection bags due to adhesive failure. Humphreys (1985) investigated the addition of a preservative in the collection bags

to prevent loss of nitrogen over time. Boric acid powder plus mercuric chloride was successful in preventing any change in proportion of excreta nitrogen compounds.

e. Assay Frequency

Assay frequency is dependent upon the length of rest period required to allow recovery of body weight. Sibbald (1978a) found that a rest period of 12 days is a sufficient time period to allow birds to recover their initial body weight, if a 24 hour collection period is used. Similar findings have been reported by both Mutzar and Slinger (1980b) and Schang et al. (1982a). Mutzar and Slinger (1980b) however have suggested that for feedstuffs requiring a 48 hour collection period, a longer rest period may be required. Further, Mutzar and Slinger (1980a) observed that for birds fasted over a period of 72 hours only 63% had regained their lost weight by the 32nd day after being returned to feed. Mutzar and Slinger (1980a) report that a recovery period of 4 weeks is required to return to initial body weight. Salmon (1983) states that the duration of the rest period is dependent upon the length of the excreta collection period. By conducting a collection period in accordance with the rate of passage of a test material used, the rest period may also be adjusted.

## III. THE NITROGEN CORRECTION FACTOR

Dietary nitrogen deposited in the body, if catabolized, is excreted as energy containing compounds such as uric acid, urea, ammonia, and creatinine. Nitrogen balance is affected by variables such as feed intake and composition and can be either positive or negative during a BE bioassay (Sibbald, 1982). Traditional bioassays for metabolizable energy such as those described by Hill et al. (1960) and Sibbald and Slinger (1963) yielded AME values which included a correction for retained nitrogen

(AMEn). This was done to account for protein stored in the carcass during the experimental period. In contrast, the TME assay described by Sibbald (1976a) included test animals which were in negative nitrogen balance and as such did not include a nitrogen correction (Dale and Fuller, 1984b).

Parsons et al. (1982a) have noted that a potential problem with the TME bioassay is that it involves differences in energy associated with loss of nitrogen when comparing fed versus fasted birds. Endogenous nitrogen excretion should be greater in fasted birds due to degradation of body protein to meet energy requirements. As a result, energy excreted by the fasted birds overestimates the excretion of endogenous energy by fed birds. Energy and nitrogen excretion between fasted birds can also vary depending on the extent of tissue catabolism and metabolic body size (Sibbald and Wolynetz, 1984b). In light of this, a nitrogen correction is essential to avoid overestimation of endogenous energy (Dale and Fuller, 1984b). Reports such as those by Parsons et al. (1982a) and Sibbald and Morse (1983b) support the use of the nitrogen correction in TME assays. Sibbald and Morse (1983b) have shown that the correction of excreta energy output to zero nitrogen balance improved the estimates of FmE and UeE in addition to reducing random variation.

In practice, TME values are corrected to zero nitrogen balance as follows (Dale and Fuller, 1984b):

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

where:

| | | |
|---|---|---|
| IE | = | intake of feed energy |
| FE + UE | = | energy voided by the fed bird |
| FmE + UeE | = | energy voided by the unfed bird |
| Ni - Ne | = | nitrogen balance |
| k | = | nitrogen correction factor |

Generally, one of two nitrogen correction factors have been used when applying the nitrogen correction. Hill and Anderson (1958) proposed a value of 34.39 kJ per gram of RN based on the energetic value of uric acid. Here uric acid was considered to be the sole nitrogen excretory product in the chicken. On the other hand, Titus (1956) proposed a value of 36.53 kJ per gram RN, based on the fact that in addition to uric acid, excretory products of protein metabolism also include urea, ammonia, creatinine and other nitrogenous compounds. This value was obtained from the energetic determination of a sample of nondescript chicken urine using full fed birds. Using a regression equation to estimate the coefficients of feed and urinary nitrogen (FN + UN), Sibbald and Wolynetz (1984b) obtained values of 34.68 ± 1.57 kJ for SCWL cockerels and 38.13 ± .71 kJ for meat-type birds. In a subsequent study, Sibbald and Wolynetz (1985) obtained a slope estimate of 33.54 ± 5.12 kJ after adjusting the regression of FmE + UeE on FN + UN for birds and days. They also found, however, that the coefficient of FN + UN varied among birds and over time and suggested that the E/g of nitrogen may not be constant and that the proportions of energy containing nitrogenous end products of tissue catabolism may vary. This will be discussed in more detail in the following section concerning metabolic and endogenous energy and nitrogen losses.

Studies have been conducted to assess the affect of the nitrogen correction. Shires et al (1980) measured TME and TMEn values using chicks and adult cockerels and obtained reductions of 8% and 6% respectively when using the correction. Mutzar and Slinger (1981c) obtained values of TMEn which were consistently higher than its TME counterpart. However, Sibbald and Morse (1983b) note that Mutzar and Slinger (1981c) failed to correct FmE + UeE loss to zero nitrogen balance. Their correction was based solely on the nitrogen balance of the fed bird and ignored the nitrogen loss

of the fasted bird (Sibbald, 1982). Sibbald and Morse (1983a; 1983b) found that the estimated error variance of energy voided, and hence the TME, was reduced by more than 40% when a correction to zero nitrogen balance was applied. For unfed birds, the energy loss was reduced from 127.3 to 56 kJ per bird per 72 hours (Sibbald and Morse, 1983a). The effects of the nitrogen correction have been explained by Dale and Fuller (1984b). The first effect is provided by the adjustment made in FnE + UeE loss. They found that the large negative nitrogen balance of fasted birds, when multiplied by the factor 36.53 kJ/g RN, reduced the estimate of FnE + UeE loss by more than 50%. Since this lower value is subtracted from the excreta energy of fed birds, the values for TMEn are lower than those for TME. The second effect is provided by the direct adjustment made in the excreta energy of fed birds (which is less than seen with fasted birds due to a less negative nitrogen balance). Here the overall effect is reversed. The TMEn value is raised since the excreta energy of fed birds is decreased by the nitrogen correction. Therefore, the larger the reduction in excreta energy (due to the more severely negative nitrogen balance), the higher the TMEn will be. The magnitude of the correction decreases as the fatness of the assay bird increases and also varies according to the ability of the feed tested to conserve body nitrogen (Sibbald, 1982).

Wolynetz and Sibbald (1984) studied the relationship between AME and TME and the effect of a nitrogen correction. The accuracies of AME and AMEn are affected by feed intake. When feed intake is ad-libitum, AME<AMEn when RN<0, and AME>AMEn when RN>0. At low feed intakes both AME and AMEn underestimate BE. TME, on the other hand, overestimates BE at low intakes. However, the difference is less than the underestimation of BE using AME or AMEn. TMEn is not as greatly affected by feed intakes. Variances of AMEn and TMEn are generally less

than those of AME and TME, respectively, with the differences decreasing as feed input increases. Due to variability associated with the energy losses of fasted birds, variances of TME and TMEn are larger than those of AME and AMEn. However, in the TME bioassay (Sibbald, 1976a) where the feed input is 30-40 g, these differences are small. A further disadvantage of AME and AMEn is that a reduction in intake associated with unpalatable ingredients can result in misleading BE estimates.

Dale and Fuller (1984a) conducted a study to determine the effect of the nitrogen correction on the repeatability of TME and TMEn values. Between assays, differences in determined TME and TMEn values were reduced by more than half when the nitrogen correction was applied. They concluded that both values are highly reproducible.

Mutzar and Slinger (1981c) reported that the nitrogen correction was affected by the type and level of protein fed. However, only 46 to 48% of the variation in TMEn was due to the nitrogen input, with the rest being attributed to unknown factors. Dale and Fuller (1984b) also noted a positive correlation between the level of protein fed and the difference between the TME and TMEn content. As the percent protein increased, the percent difference between TME and TMEn also increased. This was due to the fact that with an increase in nitrogen intake the negative nitrogen balance becomes less severe. Sibbald and Morse (1983a) remark though, that since the nitrogen represents a high proportion of the FmE + UeE at any input level, correction to zero nitrogen balance will reduce any existing bias.

To minimize tissue nitrogen loss and improve the estimate of FmE + UeE Dale and Fuller (1981), du Preez et al. (1981), and Campbell et al. (1983) have suggested using a highly digestible energy source. In all cases both energy and nitrogen excretion were reduced. On the other hand, Sibbald (1975b; 1976a) reported that

FmE + UeE remained unchanged. Sibbald (1982) suggested that the apparent differences may reflect differences in degree in fatness of the birds. Further, Sibbald and Morse (1983b) suggest that the procedure is unnecessary as the nitrogen correction reduces the variation of TME estimates.

## IV. METABOLIC PLUS ENDOGENOUS ENERGY AND NITROGEN

Measurement of metabolic and endogenous energy (FmE + UeE) is an important part of the bioassay for TME developed by Sibbald (1976a). In the bioassay starved birds are used to measure the FmE + UeE losses of fed birds. The basis for this is that FmE + UeE losses are considered a body maintenance cost, not derived directly from the feed, and therefore should not be charged against the feed. Further, Sibbald (1975b) suggested that the FmE + UeE losses in fasted birds are relatively constant and equal to the FmE + UeE losses of fed birds.

Both Dale and Fuller (1982) and Parsons et al. (1982a) have stated that the use of fasted roosters may overestimate the endogenous energy loss of fed birds due to the physiological stress derived from the starvation period. During the initial 24 hour fast (to clear feed residues from the gastrointestinal tract) the roosters enter a postabsorptive state where presumably the supplies of stored glycogen are exhausted. Over the following 24 to 48 hour collection period the fasted birds must then rely on catabolized tissue to meet basal energy needs (Dale and Fuller, 1982). The FmE + UeE losses of the fasted bird are dependent upon the nature of the materials being catabolized to meet the energy requirements (Sibbald and Morse, 1983b). Catabolism of glycogen and fat yields the end products, water and carbon dioxide, which are completely oxidized resulting in little direct effect on FmE + UeE (Sibbald, 1981c). However, when amino acids and proteins are degraded, energy-containing nitrogenous

compounds are excreted (Sibbald and Morse, 1983b). In fed roosters, tissue catabolism is minimized to the extent that the feedstuff being tested is able to supply the required energy (Dale and Fuller, 1982). Dale and Fuller (1982) have shown that FmE + UeE losses are inversely proportional to caloric intake in roosters that are in a negative energy balance. A similar effect on nitrogen excretion is also shown. Since the assay birds are in different stages of energy balance, it cannot be assumed that for both fed and fasted birds the FmE + UeE losses are equal. In addition, non-feed related factors such as body size, physiological state, and environmental temperature may play a role in contributing to the amount of body nitrogen catabolized, thus contributing to variation in FmE + UeE (Sibbald and Wolynetz, 1984b).

Although variation in FmE + UeE among birds of the same population is expected, what is surprising is the magnitude with which that variation occurs. Using adult roosters, Guillaume and Summers (1970) first reported FmE + UeE losses to be 2.76 kJ/kg/day. In a set of experiments, Sibbald (1975b) predicted FmE + UeE using a regression line of gross energy of excreta voided and weight of wheat consumed. The intercept of the regression line provided estimates of 35.56 kJ/bird/day and 46.82 kJ/bird/day. No specific reasons could be given for the differences between these two experiments. Patchell and Edmundson (1977a, b) have reported values ranging from 33.9 to 101.2 kJ/bird/day with a mean of 57.0 kJ for 6 birds and 25.8 to 62.9 kJ/bird/day with a mean of 41.5 kJ for 48 birds, respectively. Sibbald (1982) suggested that these large differences may be due to the inadequate clearance of feed residues from the digestive tract. Farrell (1978) however has also reported a wide variation with values ranging from 32.5 to 82.0 kJ/bird/day. In a study using 300 birds through 38 experiments over a period of 3 years, Sibbald and Price (1978) observed a range of 25.0 to 69.3 kJ/bird/day with a mean of 43.7 kJ. Much of the

variation noted was attributed to variation between assays. Bilgili and Arscott (1982) obtained values ranging from 18.9 to 44.6 kJ/bird/day while Campbell et al. (1983) report a range of 40.85 to 66.44 kJ/bird/day. There are, however, several studies which report low variations in FmE + UeE losses. Ranaweera and Nano (1981) obtained a mean value of 7.04 ± 2.94 kJ/bird/day for 22 birds. Low variability has also been reported by Miski and Quazi (1981), Sibbald and Price (1980) and Sibbald (1981a). A mean value of 43.73 ± 6.94 kJ/bird/day was observed upon examination of 808 FmE + UeE values by Sibbald (1981c). The wide range of values were primarily attributed to differences among birds and experiments.

Sibbald and Morse (1983b) studied the variation in both FmE + UeE and fecal and urinary nitrogen (FN + UN) losses. They found that FnE + UeE varied from 36.9 to 44.7 kJ/bird/day and FN + UN varied from .659 to .717 g/bird/day over a series of 4 experiments. Similar variations of 34.1 – 45.9 kJ/bird/day for FmE + UeE and .550 – .755 g/bird/day for FN + UN, over a series of 14 experiments, were reported by Sibbald and Wolynetz (1984b). In both studies the variation in FmE + UeE was significantly correlated with FN + UN excreted. These support an earlier study by Dale and Fuller (1982) which also showed an association between FN + UN excreted and FmE + UeE. In this case FmE + UeE varied from 44.18 - 67.78 kJ/bird/day and FN + UN varied from .650 - 1.42 g/bird/day. As mentioned previously, the observation that FN + UN varies among birds suggests that the E/g of nitrogen may not be constant which leads to the implication that the proportion of energy containing nitrogenous compounds voided as a result of tissue catabolism, varies (Sibbald and Wolynetz, 1984b).

The content and variation of nitrogenous compounds in chickens has been investigated by several scientific groups. Using four different dietary protein-water

availability regimes, McNabb and McNabb (1975) found that the proportions of nitrogen compounds varied as follows: uric acid 55-72%, ammonia 11-21%, and urea 2-11%. These are in agreement with results previously obtained by O'dell et al. (1960), which also showed a variation of 1.7-2.2% for amino acids and 0.2-0.9% for creatine and creatinine. Krogdahl and Dalsgard (1981), feeding a commercial diet containing 15% protein, obtained values for urinary nitrogen of 88%, 7%, 3% for uric acid, ammonia, and urea respectively with 2% unidentified. Here urinary ammonia constituted 90% of ammonia in total excreta and urinary urea 86% of the urea in excreta. Starvation has also been shown to significantly affect the proportion of nitrogenous compounds. Waring and Brown (1965) found that in the case of starved birds the proportion of uric acid nitrogen to total nitrogen was 50-60% but rose to 85-95% on feeding diets containing 15-18% protein. They surmised that the proportions of the various nitrogenous constituents of urine must vary considerably according to whether the protein catabolized was of endogenous or exogenous origin. Comparing fed and unfed birds, Sykes (1971) found that starvation decreased the amount of uric acid nitrogen (84.1-57.8%) and urea nitrogen (5.2-2.9%) and increased the amount of ammonia nitrogen (6.8-23.0%), amino acid nitrogen (1.7-2.8%) and creatinine nitrogen (0.5-4.3%). Mutzar and Slinger (1980e) observed that the excretion of ten of sixteen amino acids increased significantly for birds fed a nitrogen-free diet. When comparing values obtained for levels of amino acids excreted, Mutzar and Slinger (1980e, 1981a) reported levels of 241.5 mg/bird and 272.8 mg/bird respectively, for samples collected over a 30 hour period following an initial 24 hour starvation period. Sibbald (1979c) obtained a level of 309.4 mg/bird of amino acids excreted over a collection period of 24 hours following an initial 24 hour starvation period. The levels of amino acids reported here are for those amino acids which were similarly measured.

The FmE + UeE losses of a bird have been shown to vary with the duration of starvation. Following an initial 24 hour fast, Sibbald (1976c) observed the mean FmE + UeE losses of adult cockerels in each of four subsequent 24 hour collection periods. Losses were recorded as 45.03 ± 2.05, 37.03 ± 1.63, 34.93 ± 1.84, and 34.43 ± 2.93 kJ/bird respectively. Shires et al. (1979) obtained values of 45.86, 37.53, and 32.96 kJ/bird for FmE + UeE losses and .662, .577, and .646 g/bird for FN + UN losses measured over 12, 24, and 48 hours respectively. Additional evidence of a decrease in FmE + UeE with the duration of starvation have been reported by Mutzar and Slinger (1980a), Sibbald (1981c) and Sibbald and Morse (1982). In order to observe FmE + UeE and FN + UN losses over a longer period of fasting, Sibbald and Wolynetz (1985) starved birds for 48 hours to clear feed residues from the alimentary canals, and then obtained values at 24 hour intervals over a period of 11 days. They observed a steady decrease in FmE + UeE from 36.7 to 30.5 kJ/bird over the first five days after which the values remained essentially constant ranging between 26.7 and 29.8 kJ/bird through days 6 to 11. Similar observations were noted for FN + UN; a steady decrease from .550 to .489 g/bird from days 1 through 5 with values fluctuating between .471 and .543 g/bird through days 6 to 11. It should be noted that an increase or decrease in FN + FU was followed by a corresponding increase or decrease in FmE + UeE. A similar relationship was identified by Sibbald and Wolynetz (1984b) and Sibbald and Morse (1983a,b). These results agree with a similar long-term fasting study with fasting geese by Maho et al. (1981). Essentially three phases were identified: first a rapid decrease in the rates of nitrogen loss; next a slow steady decline in the rates of loss; then a critical period in which rates of loss increased rapidly.

Studies have indicated that birds tend to have relatively the same amounts of FmE + UeE and FN + UN from period to period. Sibbald and Price (1978) found no evidence of any relationship between different times and the magnitude of energy loss over a period of three years during which 38 experiments were conducted. Mutzar and Slinger (1980a) found that the average FmE + UeE of 15 birds for any particular period of collection (over a two month period) at two different times were essentially equal; for 24 hour collection period, 31.55 vs. 32.09 kJ/bird and for 48 hour collection period, 58.24 vs. 59.25 kJ/bird. Excretion values for amino acids were also found not to vary significantly over different collection periods (Mutzar and Slinger 1981a). Mutzar and Slinger (1980a) have suggested that the FmE + UeE value, once measured for a bird could be used to correct for the same bird for at least two months. Results from Sibbald and Wolynetz (1984b) have also indicated a relative consistency for both FmE + UeE and FN + UN over a considerable period of time. However, they caution that a measurement of FmE + UeE or FN + UN taken at a particular time should not be used to accurately predict the same quantity for the same bird even 28 days later. They found that approximately 41 to 58% of the variability in a second measurement could be accounted for by the first measurement. For FN + UN, only 10 to 14% of the variability in a subsequent measurement could be explained by a prior measurement. Therefore, the use of constants for FmE + UeE and FN + UN are, at best, questionable.

Observations that FmE + UeE losses varied among birds within a population, led Sibbald (1976a) to suggest that birds within a treatment should be selected according to similar body weights. Shires et al. (1979), Campbell et al. (1983) and Sibbald and Wolynetz (1984b) have all shown a body weight effect on FmE + UeE excretion. Sibbald and Wolynetz (1984b) and Sibbald and Wolynetz (1985) showed an

effect of body weight on FN + UN excretion while Mutzar and Slinger (1981b) found that approximately 20% of the variation in amino acids excreted could be associated with changes in body weight. Miski and Quazi (1981) observed age-related differences which were explained as a body weight: age effect by Sibbald (1982). A similar body weight-age effect had also been shown by Sibbald (1981a). Sibbald (1981c) states that the effect of body weight is a result of lighter birds having a smaller fat reserve, and therefore when fasted, catabolize more tissue protein per unit of body weight. When tissue protein is utilized as a source of energy, uric acid is excreted which contributes energy to FmE + UeE whereas another bird may degrade fat and void carbon dioxide which does not contribute to FmE + UeE. On the other hand, the absence of a close relationship between body weight and FmE + UeE has been confirmed by a number of authors including Farrell (1978), Sibbald and Price (1978), Mutzar and Slinger (1980a), Ranaweera and Nano (1981), Bilgili and Arscott (1982) and Sibbald and Wolynetz (1985). An absence of a relationship between body weight and FmE + UeE was also noted in studies involving geese (Storey and Allen, 1982a). The recognition that FmE + UeE losses vary among birds within a population led Edmundsen et al. (1978) to suggest that each bird be used as its own estimator of FmE + UeE. This would act to negate the effect of bird variation associated with body weight. Variation in FmE + UeE has been shown to be largely characteristic of the bird (Mutzar and Slinger, 1980a; Sibbald and Price, 1980). With the absence of a body weight effect on FmE + UeE, Sibbald and Wolynetz (1985) theorize that the composition of the change in body weight may have varied among birds and days. With a loss in weight, the energy required for maintenance also decreases, which should result in a decrease in the catabolism of tissues necessary to provide such energy. Changes in body

composition and the nature of tissues catabolized, in all likelihood complicate the relationship between body weight and FmE + UeE excretion.

Both FmE + UeE and FN + UN appear to be influenced by breed, strain, and sex of the bird. Comparing single comb white leghorn (SCWL) roosters with broiler chicken pullets, Miski and Quazi (1981) found that SCWL FmE + UeE losses were significantly higher (3.25 ± .2 vs. 2.60 ± .14 kJ/100 g body weight/24 hr.). The effect was mainly attributed to differences in body composition and higher basal metabolic rate energy needs per unit body weight in the SCWL roosters. Both Sibbald (1981c) and Sibbald and Wolynetz (1984b) have shown a strain effect when comparisons among different strains of SCWL roosters were made. It should be noted however that Sibbald and Wolynetz (1984b) failed to identify a strain effect between different strains of meat-type birds. Sibbald and Wolynetz (1985) also failed to show a significant difference among different strains of SCWL cockerels. Miski and Quazi (1981) detected no significant differences when comparing the average FmE + UeE losses per unit body weight between male and female broiler chicks. The lack of significance was attributed to an insufficient number of male chicks. Parsons et al. (1982a), upon comparing FmE + UeE and FN + UN values between SCWL males and females, obtained values of 1.049 and .453 gN/bird and 62.01 and 44.94 kJ/bird, respectively, from samples collected over a 30 hour period. The greater nitrogen loss, and thus lower energy excretion, for females versus males was believed to be due to the deposition of nitrogen in the eggs laid by the females. Body weight was not a factor in the differences in the energy excreted as both the males and females were of similar weights.

Environmental temperature has been shown to have a direct effect on both FmE + UeE and FN + UN losses. Dale and Fuller (1981, 1982) observed evidence of

a seasonal change in which FmE + UeE losses tended to be higher in winter and lowest in the summer. Yamazaki and Zi-Yi (1982), upon comparing the FmE + UeE and FN + UN losses at hot (25-35°C) and cool (5-15°C) temperatures, obtained values of 64.6 and 126.5 kJ/bird/48 hr. for FmE + UeE, and 1.33 and 2.90 gN/bird/48 hr. for FN + UN, respectively. Resulting TME values were also significantly higher, further indicating a direct effect of environmental temperature. Sibbald and Wolynetz (1984b), observing similar affects on FmE + UeE and FN + UN, explain that within limits, the maintenance energy requirement decreases as the environmental temperature increases. Therefore, the catabolism of body tissue and the excretion of energy-containing compounds by fasted birds would be expected to decrease with an increase in the environmental temperature. It should be noted, however, that an observed increase in FmE + UeN at the highest temperature supports previous reports (NRC, 1981) that an increase in the energy requirement is necessary for thermal regulation. Interestingly, Sibbald and Wolynetz (1985) found little affect of temperature on both FmE + UeE and FN + UN losses.

It has been suggested that FmE + UeE and FN + UN losses vary with both the nature and quantity of the feed consumed (Sibbald, 1982). The belief appears to originate from a study by Hallsworth and Coates (1962) suggesting that the severity of the erosion of the intestinal mucosa increased with the intake of dietary fibre, thereby increasing FmE, and thus FmE + UeE. It should be pointed out, however, that the report provided no evidence of any effect of fibre intake of FmE + UeE or FN + UN. Rolls et al. (1978) on the other hand found that additional fibre had no effect on the rate of removal of the intestinal epithelium.

Studies by Farrell (1981) indicate that there is a correlation between neutral detergent fibre (NDF) and endogenous energy output. FmE + UeE losses were

reported to increase linearly as the NDF content of the feedingstuff increased. However, at NDF levels greater than 12%, FmE + UeE losses remained constant at approximately 87.86 kJ/bird/32 hours. Sibbald (1982) argues however that clearance time through the gut increases with the increase of NDF content of the feedingstuff, and therefore the 32 hour excreta collection period used by Farrell (1981) was insufficient for medium to high NDF contents. Tenesaca and Sell (1981), feeding silica gel either alone or in combination with corn, found that FmE + UeE increased linearly with each increment of silica gel. They reasoned that silica gel was representative of the indigestible portion of feedstuffs, and that a similar effect of poorly digested materials would be expected. Sibbald (1982) disputes this and suggests that further investigation is required to determine why silica gel behaves in this manner. There is indication that FN + UN excretion is affected by dietary fibre levels. Mutzar and Slinger (1980e) report a significant loss of FN + UN when alfafloc was fed at levels of 20 g verses 0 and 10 g/bird. A later study (Mutzar and Slinger, 1980d), while not significant, also indicates an increase in FN + UN excretion of birds fed diets containing up to 15% alfafloc. Endogenous amino acid excretion also appears to be affected by the level of dietary fibre. Mutzar and Slinger (1980e) observed that significant differences among amino acids increased as the level of alfafloc fed was also increased. A similar effect of high fibre versus low fibre diets has been shown by Parsons et al. (1983) and Parsons (1984). Speculation that the type and source of dietary fibre may affect both amino acid and nitrogen excretions (Mutzar and Slinger, 1980d) has been confirmed by both Parsons et al. (1983) and Parsons (1984). Mutzar and Slinger (1980e) caution, however, that the feeding of crude fibre as the sole dietary ingredient may not have the same effect on FN + UN losses as when it is fed mixed with a diet at practical levels. For example, crude fibre in pea hulls versus

rapeseed meal are generally different physico-chemically than pure cellulose such as alfafloc and would be expected to have a greater abrasive effect on the digestive tract (Mutzar and Slinger, 1980d). This in combination with released endogenous nitrogen (eg. digestive enzymes) which have not been completely reabsorbed could result in FN + UN values which substantially exceed or misrepresent those of fasted birds.

Similar studies involving dietary fibre to those discussed above have produced very different results. In a study similar to Farrell (1981), Sibbald (1976a) failed to obtain a correlation between dietary fibre and FmE + UeE. Sibbald (1980b), upon addition of supplemental cellulose or sand (ranging from 4 to 16%) in combination with various test materials, concluded that there was no effect on the FmE + UeE or amino acid excretion. Farrell (1981) explains this as an affect of sand retention in the gizzard. In a further study using graded levels of a cellulose:carboxymethyl cellulose mixture or sawdust as the sole test material, Sibbald (1981a) again reports no effect on either FmE + UeE and FN + UN excretion. Mutzar and Slinger (1980d) were unable to measure the effects of either cellulose or corn cob meal on FmE + UeE due to the incomplete clearance of residue over the 30 hour collection period. Similarly Mutzar and Slinger (1980d) observed the absence of a trend toward an increase or decrease in the various amino acids in relation to increases in the dietary alfafloc content over a 0 to 20% inclusion level.

In an attempt to examine the effects of dietary protein on urinary nitrogen components, Teekell et al. (1968) measured the changes in the urinary nitrogen components from diets ranging from 0 to 14.5% protein. Overall, urinary nitrogen was shown to decrease as the level of dietary protein decreased. More specifically both uric acid and ammonia decreased as dietary protein decreased. Urea nitrogen excretion varied considerably on a daily basis with a trend towards an overall

decrease. Creatinine nitrogen excretion was extremely variable on a daily basis with a tendency to maintain a constant level over the trial while amino acid nitrogen remained extremely constant regardless of protein intake. Similar results were obtained by McNabb and McNabb (1975). While Ward et al. (1975) found that an increase in dietary protein (11 to 33%) also led to a corresponding increase in uric acid, ammonia levels however were shown to remain constant. A study by Shires et al. (1979) was conducted to examine the effects of dietary protein on both FmE + UeE and FN + UN. Here birds were allowed a period of 10 days to adapt to one of three diets containing either 10, 20, or 30% crude protein before the start of the assay. Fritz et al. (1936) in earlier work found that a period of 3 days is sufficient to overcome any effect of the previous diet on FN + UN excretion of the rooster. The birds were then either force-fed one of each of the three diets or remained unfed. The FmE + UeE output for all three levels of protein were significantly higher for the unfed bird as compared to the fed bird. Also the FmE + UeE, although significant only for unfed birds at protein levels of 30%, did show a trend to increase as protein level increased. The levels were 75.06, 78.86, and 80.33 kJ/bird/24 hr. for the fed birds and 33.22, 36.19, and 43.14 kJ/bird/24 hr. for the unfed birds for diets containing 10, 20, and 30% protein, respectively. The FN + UN outputs between fed and unfed birds were not significantly different at any of the three protein levels fed. However, the differences were significant for each of the three levels of dietary protein. The levels were .599, .708, and .824 gN/bird/24 hr. for the fed birds and .516, .623, and .745 gN/bird/24 hr. for the unfed birds for diets containing 10, 20, and 30% protein, respectively. That nitrogen excretion during a fast varies according to the prior nitrogen intake has also been confirmed by Okumura et al. (1981). Parsons et al. (1982b) examined the effect of source of dietary protein on the amino acid composition

of excreta. They compared casein, gelatin, corn gluten meal, and feather meal and found a significant influence of protein source on amino acid composition to the excreta. Sibbald (1982) concludes that variability in FmE + UeE associated with the previous diet should have no effect on BE bioassays as long as all the birds have been treated in a similar fashion.

Dietary energy levels also appear to have an effect on both FmE + UeE and FN + UN. Richardson et al. (1968) found that lower levels of nitrogen were excreted from a ration containing 15% protein with 1.998 kcal/kg as opposed to an isoprotein ration with 1.776 kcal/kg. Analysis of the urine showed that the higher energy level resulted in higher levels of ammoniacal and amino acid nitrogen (18.5 vs 7.0% and 1.0 vs .7% of total urinary nitrogen respectively) and lower levels of uric acid and urea nitrogen (74.3 vs 78.9% and 2.5 vs 7.5% of total urinary nitrogen respectively). Creatinine excretion was relatively constant regardless of the energy intake. Okumura et al. (1981) also observed a trend towards decreased nitrogen excretion as the energy level of the diet was increased. Sibbald (1979a) on the other hand observed no effect on the excretion of amino acids upon administration of levels of glucose ranging from 0 to 30 g. With the administration of a completely digestible energy source, FmE + UeE and FN + UN excretion tends to vary with the level fed. Dale and Fuller (1982) found that both FmE + UeE and FN + UN decreased significantly as increased levels of a 50% glucose-corn starch were fed (0, 12.5, and 25.0 g). Similar results were obtained upon comparing the effects of practical feed ingredients such as corn and soybean meal supplemented with or without glucose. Campbell et al. (1983) fed varying levels of dextrose (7.2 to 51.5 g) and observed that FmE + UeE, while lacking a trend towards an increase or decrease, did show variation in response to the levels fed. Sibbald and Morse (1983b) force fed a variety of ingredients as a source of

supplemental energy and observed a reduction, not prevention, in FN + UN and therefore FmE + UeE losses. This is contradictory to findings by Sibbald (1975b; 1976a) that the slopes of the regressions of FmE + UeE on feed intake of graded levels of dextrose and corn oil did not differ, implying that FmE + UeE was independent of energy intake. Sibbald and Morse (1983b) also noted that a bias was introduced if the source was not completely digestible, but could be overcome by providing the same amount of the energy source to all the birds. Free-choice feeding was offered, however feed spillage, excreta contamination, and variable intakes made accurate measurements difficult. The results of these experiments agree with a statement by Dale and Fuller (1982) that FmE + UeE excretion is inversely proportional to the caloric intake of roosters in a negative energy balance. A similar effect on FN + UN suggests that those FN + UN losses due to degradation of body protein to provide energy to meet the basal energetic needs can be reduced, if not eliminated, by the provision of dietary energy. However, Sibbald and Morse (1983b) state that "there is an inevitable metabolic fecal plus endogenous urinary N loss that cannot be reduced by the provision of supplementary E." Sibbald and Morse (1982, 1983b) suggest a preferred method of determining FmE + UeE would be to force feed each test material at various levels without supplemental energy and then calculate the regression FmE + UeE on feed input.

The effect of feed input on FmE + UeE losses has been investigated by few researchers. Kussaibati et al. (1982) examined the FmE + UeE losses of starved chicks previously fed ad-libitum or one-third ad-libitum. They found that higher FmE + UeE losses occurred with those chicks previously fed ad-libitum. Sibbald and Morse (1982) measured the cumulative FmE + UeE losses of adult cockerels force fed single test materials at various input levels. They observed that a linear relationship

between energy input and output existed over all input levels so long as sufficient time was provided for complete clearance of the feed residues. The FmE + UeE losses were determined by the regression to zero intakes and were found to be similar to those of the fasted birds. The mean intercept was essentially the same as the mean energy voided by the unfed birds, 137.0 kJ and 135.2 kJ, respectively. These agree with results previously obtained by Sibbald (1976a) in a similar set of experiments. Sibbald and Morse (1982) conclude that feed input does not affect the FmE + UeE losses.

A possible error associated with measuring metabolic and endogenous energy and nitrogen excretion may be losses incurred during the time between the voiding of the excreta and the analysis for the energy and nitrogen content. These losses can occur either through nitrogen lost to the atmosphere as ammonia or as a result of changes due to microbial fermentation. The problem is potentiated due to the fact that excreta collected in bags remain moist and at room temperature for up to 48 hours, promoting conditions which allow for increased microbial activity. In ruminants several studies have been conducted to prevent such losses. Flatt (1957) compared energy losses of Jersey cows from samples analyzed fresh or stored in cans and observed no appreciable difference between the two. In a similar study, Jacobson et al. (1959) found no significant differences between analysis on samples canned and stored for varying periods of time up to six months and analysis on fresh samples. It was concluded that canning prevented any apparent loss of energy or nitrogen during storage, thereby providing an appropriate method of preserving fresh excreta until samples were required for analysis. Martin (1966) noted that boric acid (2% w/w) appeared to be effective in preventing ammonia loss from the urine of sheep providing the pH was maintained below 2. However, he cautioned that acid hydrolysis of urea

may occur and therefore effect total nitrogen. Using a potassium dichromate-mercuric chloride mixture, Martin (1966) attributed a rise in the pH of the urine during a 24 hour storage to urea hydrolysis, and recorded losses as ammonia from 0.7 to 9.7% of the total urinary nitrogen. He also reported low ammonia losses from urine preserved with sulphuric acid. In light of this, Rocks (1977) suggests that prevention of nitrogen losses and urea degradation could be accomplished using a weak acid to buffer the urine at a low pH and an inorganic bacteriostat. Of six preservatives tested, only mercuric chloride (0.3% w/w) in glacial acetic acid and boric acid powder plus 0.3% w/w mercuric chloride proved effective in preventing changes in total nitrogen, urea nitrogen, and ammoniacal nitrogen. Refrigeration of unpreserved urine also maintained the levels of nitrogenous constituents at the initial levels, however only for a period of 14 days thereby rendering this method somewhat impractical. Few studies have been conducted on poultry excreta. Smith et al. (1978) evaluated the effectiveness of several preservatives in reducing microbial populations and nitrogen loss from wet cage layer excreta. An acetic-propionic acid mixture, formaldehyde, and an acetic-propionic acid-formaldehyde mixture were tested at rates of 0.25, 0.50, and 1.0% w/w. All three chemicals were equally effective in preventing spoilage and nitrogen losses at treatment rates of 0.50% and 1.0% w/w. The treatment rate of 0.25% w/w was generally ineffective. Narasimhalu et al. (1981) found that sodium metabisulfite, sodium hypochlorite, and formaldehyde were suitable for reducing microbial population in broiler and layer excreta. Rates of 0.01, 0.05, 0.1, and 0.5% w/w were tested and shown to be effective. They also suggested that propionic acid or tannic acid in combination with either sodium hypochlorite or formaldehyde may also be effective. However, neither this nor the effects of any of the preservatives on energy and nitrogen losses were investigated. Muramatsu and Okumura (1979) site

the use of 2.5 N sulfuric acid to prevent microbial action and ammonia loss but offer no comparative analysis as to its effectiveness. Humphreys (1985) compared mercuric chloride (0.3% w/w) in glacial acetic acid and boric acid powder plus 0.3% w/w mercuric chloride and found that only boric acid plus 0.3% w/w mercuric chloride prevented any change in the proportion of uric acid, urea, creatinine, and ammonia nitrogen.

The function of the microflora in the gastrointestinal tract, in relation to energy digestion and absorption, is considered important but controversial. Although microbes are found throughout the tract, one of the major sites is in the ceca where fermentation is the principal activity (Sibbald, 1982). The function of the ceca, which has been thoroughly reviewed by McNab (1973), includes both carbohydrate digestion or microbial decomposition of cellulose and protein digestion or non-protein nitrogen absorption.

An early study by Nitsom and Alumot (1963), in which raw and heated soybean meals were fed to intact and cecectomized birds, showed that the intact birds retained more nitrogen from raw soybean meal. The suggestion was made that cecal proteolysis was compensating for inhibition of proteolysis in the small intestine. Payne et al. (1971) observed an effect on apparent amino acid digestibility of fish meal amino acids containing 5 and 11% protein. However, the differences between the intact and cecectomized birds were not statistically significant. They also cautioned that the excretion of amino acids, following the feeding of the poor quality fish meal containing 5% protein, may have been of endogenous rather than dietary origin. They suggested though that the amino acids that were digested within the ceca were probably absorbed from the ceca, indicating that cecectomized birds could not recover as much of the endogenous secretions as otherwise recovered by the intact birds. This

agrees with an earlier study by Payne et al. (1968) that cecectomy was associated with a substantial reduction in the digestibility of amino acids. Kessler et al. (1981) observed the amino acid excretion pattern of fasted, intact and cecectomized roosters and found that the cecectomized roosters excreted more amino acids than did the intact roosters. Similar findings by Thomas (1980) showed a consistent increase in the quantity of endogenous amino acids voided each day by cecectomized birds as compared to intact birds. Parsons et al. (1983) found that the levels of alanine and aspartic acid were significantly greater and the level of proline significantly less in digesta from intact roosters as compared to cecectomized roosters upon force feeding a high-fibre diet. In contrast, amino acid compositions of excreta from intact roosters fed a low-fibre diet were similar to that of digesta from cecectomized roosters. Parsons (1984) observed that fasted, cecectomized hens excreted significantly higher levels of most analyzed amino acids than fasted, intact hens, with the differences being greatest for threonine, proline, and leucine. Total amino acid excretion was also significantly different, with cecectomized hens excreting a 36% higher level of amino acids than intact hens. On comparing hens force-fed a fibre (cellulose) diet, it was found that cecectomized hens excreted larger amounts of amino acids than intact hens, with differences being significant for threonine, proline, and isoleucine. However, for hens force-fed a starch (potato starch/pectin) diet, amino acid excretions for both cecectomized and intact hens were similar (Parsons, 1984). In a further study, Parsons (1985) showed that cecectomy increased amino acid excretion by 29%. In addition the fasted, cecectomized roosters excreted significantly more energy (71.6 kJ/48 hr.) than did the fasted, intact roosters (63.6 kJ/48 hr.). Total nitrogen excreted was 0.93 g/48 hr. for the intact roosters and 0.97 g/48 hr. for the cecectomized roosters. These differences were not significant. Kessler and Thomas (1981) have also

reported higher energy excretions by fasted, cecectomized birds, however these differences were not significant. Kese and March (1975) observed no effect of cecectomy on uric acid excretion although uric acid decomposing anaerobic bacteria have been found in the ceca of chickens (Barnes and Impey, 1974).

Both Nitsom and Alumot (1963) and Nesheim and Carpenter (1967) have demonstrated that substantial proteolysis occurs in the ceca of growing chicks. The results of the aforementioned studies suggest that microbial metabolism in the ceca significantly affects amino acid excretion in chickens. In the fasted bird, proteolysis and deamination of amino acids are in all likelihood the primary mode of microbial action as a result of a low level of fermentable carbohydrate present in the lower gut (Rérat, 1978). This is supported by Parsons (1984) in which a regression analysis of excreta amino acid profiles suggested that microbial protein had a larger effect on excreta from intact hens than on that from cecectomized hens. In addition, the largest differences observed between fasted, cecectomized and intact chickens are often between threonine and proline, which are abundant in intestinal mucin, pancreatic juices, and other endogenous protein (Holmes et al. 1974; Rérat, 1978). Therefore, microbial proteolysis and deamination could explain the reduction of amino acids excreted by fasted, intact hens as compared to fasted, cecectomized hens. The present studies all indicate that the ceca in some manner does affect the amino acid excretion pattern.

To date, little or no work has been done on the preservation of poultry excreta and its application to the nitrogen corrected true metabolizable energy assay. Additionally, the proposed values for the nitrogen correction factor have failed to consider all of the excretory nitrogen compounds of endogenous and metabolic origin. As a result, an examination of their individual energetic contributions to a value for

the nitrogen correction factor has not been conducted. With this in mind, a series of experiments were designed to examine and quantify the excretory nitrogen compounds and their energetic contribution to a nitrogen correction factor under various conditions.

# MATERIALS AND METHODS

## I. GENERAL

### A. Experimental Objectives

Six experiments were designed to examine and quantify the variation of nitrogen compounds in the excreta of adult SCWL cockerels and their contribution to the nitrogen correction factor used in the true metabolizable energy assay. Experiments 1 to 4 were conducted to study the use of a preservative to prevent the loss of excreta nitrogen over time or due to microbial activity. The effects of different feedstuffs (experiments 3 and 4), administration of supplemental energy (experiment 5), and cecectomy (experiment 6) were also investigated.

### B. Experimental Birds and Management

All experiments involved adult SCWL cockerels (Shaver 288) which ranged between 386 and 742 days of age, with all the birds used within an experiment of similar age. The birds were obtained from a commercial hatchery and were fed typical chick starter and grower diets until maturity, after which a rooster maintenance diet (Table 1), formulated to prevent weight loss or gain, in mash form was provided. The vitamin/mineral premix is presented in Table 2. The feed was provided ad libitum from tube type feeders (40 cm diameter) and water was provided ad libitum from dish cup (15 cm diameter) waterers. At maturity the birds were placed in floor pens in an environmentally controllable barn which provided 14 hours of light per day and a temperature of 20°C. Experimental birds were randomly selected from the spare birds housed in the floor pens.

**Table 1. Composition of rooster maintenance diet**

| Ingredients | kg |
|---|---|
| Barley | 577.50 |
| Wheat | 300.00 |
| Soybean meal (48.5%) | 85.00 |
| Calcium Carbonate | 14.00 |
| Biofos | 11.00 |
| Iodized Salt | 2.50 |
| Vitamin/Mineral premix[1] | 10.00 |
| | 1000.00 |
| Protein (%) | 14.95 |
| Metabolizable energy (MJ/kg) | 11.55 |

[1]Vitamin/Mineral Premix (Table 2).

**Table 2. Composition of vitamin/mineral premix**

| Ingredients | g/kg Premix |
|---|---|
| Vitamin A (500,000 I.U./g) | 0.80 |
| Vitamin D (200,000 I.C.U./g) | 0.25 |
| Vitamin E (40,000 I.U./kg) | 12.50 |
| Vitamin K | 0.10 |
| Vitamin $B_{12}$ (27.2 mg/kg) | 11.00 |
| Vitamin $B_{58}$(2-4-6-100)[1] | 76.00 |
| Copper Sulfate (25.2% Cu) | 1.19 |
| Manganous Oxide (60% Mn) | 4.17 |
| Zinc Oxide (72% Zn) | 6.94 |
| Wheat Middlings | 888.00 |

[1]Vitamin $B_{58}$ contains per kg:

| | |
|---|---|
| Riboflavin | 4400 mg |
| Ca Pentothenate | 8800 mg |
| Niacin | 13200 mg |
| Choline Chloride | 220000 mg |

The experimental birds were housed in individual wire cages (60×45×40 cm) raised off the floor and located in an environmentally controllable barn which provided 24 hours continuous lighting and an approximate temperature of 20°C. The continuous lighting was provided to prevent any diurnal effects on excretion patterns. The birds, when not on test, were fed a rooster maintenance diet (Table 1) ad libitum from feeders (8×10×15 cm) with water provided ad libitum from an automatic watering cup system. Birds were not used in any experiments until they were acclimatized to the cage environment for a period of not less than 4 weeks. Culled birds were replaced with birds selected at random from the spare group.

The daily management of the spare birds involved cleaning soiled litter, ensuring that both the feeders and waterers were clean, providing feed and water, and culling birds. Similarly, daily management of the caged (experimental) birds involved feeding, cleaning feeders and water cups, manure handling, and culling birds.

C. Chemical Analysis

Gross energy of the feed and excreta was determined using a Parr Oxygen Bomb Calorimeter (Parr Instrument Company, Moline, IL). The gross energy values of the nitrogen compounds (urea, creatinine, uric acid, ammonia, and amino acids) used for the calculation of the nitrogen correction factor were also determined using a Parr Oxygen Bomb Calorimeter. The gross energy of ammonia was obtained from using ammonium benzoate and correcting for the benzoic acid component. To overcome the difficulty of burning the amino acid samples in oxygen bomb calorimeter, benzoic acid was added to the sample in a 1:1 ratio. The gross energies were then determined by correcting for the benzoic acid component. The determined energetic values are shown in Table 7.

Total nitrogen (macro Kjeldahl) and moisture of feed and excreta samples were determined according to A.O.A.C. (1980) methods. Similarly, both excreta urea and ammonia nitrogen were determined according to A.O.A.C. (1980) methods. However, due to small sample size, only 1 gm (not 2 gm) of finely ground excreta was used per replicate analyzed. Excreta uric acid was determined using the spectrophotometric method developed by Marquardt (1983).

Excreta creatinine was determined by a modification of the procedure outlined in the Sigma Technical Bulletin No. 555 (1982). The modification involved weighing into a 125 ml flask 0.5 gm of finely ground excreta followed by the addition of 50 ml of 0.02N HCL. The flask was stoppered tightly and shaken continually for 1 hour at room temperature at 250 RPM. The sample was then centrifuged for 10 minutes at 10,000 RPM, the precipitate discarded, and remaining solution analyzed spectrophotometrically as described in the Sigma Technical Bulletin No. 555 (1982).

Excreta amino acids were analyzed using a Biochrom LKB 4151 Alpha Plus Amino Acid Analyzer (LKB Biochrom Ltd., Cambridge, England). Sample preparation was according to Andrews and Baldar (1985).

D. Statistical Analysis

For all experiments, analysis of variance and test of treatment differences were computed using S.A.S. - General Linear Model Procedure (SAS ,1990). Comparison of treatment differences were conducted using Tukey's Test (Steel and Torrie, 1980). For experiments 1, 2, 5, and 6 treatment means were analyzed by one way analysis of variance. For experiments 3 and 4 treatment means were analyzed by orthogonal contrasts.

## II. EXPERIMENT 1. Examination and quantification of nitrogen compounds in poultry excreta collected with preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) and the contribution to a nitrogen correction factor for the T.M.E. assay.

Experiment 1 was designed to determine the proportion of excretory nitrogen compounds in poultry excreta collected in a preservative – boric acid powder plus 0.3 percent w/w mercuric chloride. Their contribution to a nitrogen correction factor for the TME assay was also determined.

The preservative used was made by dissolving 1 g of boric acid powder plus 0.3% w/w mercuric chloride in 20 ml of water. The boric acid powder was mixed on a hot plate until brought completely into solution.

Twenty-four birds were randomly selected and distributed among 24 wire cages. Following a 24 hour fast, a human colostomy bag containing 60 ml of the boric acid solution was attached around the cloaca to collect excreta according to the procedure outlined by Sibbald (1983). The collection bags were attached using a strong adhesive (3M–EC847) and duct tape was applied to the edges of the bags to prevent damage, and thus loss of sample, due to pecking. The birds remained unfed, and exactly 48 hours later the bags were removed and the samples were frozen. In all, 7 bags damaged due to pecking were eliminated, resulting in 17 samples. Excreta samples were then freeze dried, equilibrated with atmospheric moisture, weighed, ground, and stored in a freezer until analyzed. All excreta samples were analyzed for moisture, total nitrogen, urea, ammonia, uric acid, creatinine, and individual amino acids.

III. EXPERIMENT 2. Examination and quantification of nitrogen compounds in poultry excreta collected with and without preservative and the contribution to a nitrogen correction factor for the T.M.E. Assay.

Experiment 2 was designed to determine the direct effect of a preservative to prevent changes in excretory nitrogen compounds over time (through ammonia loss and urea hydrolysis) or as a result of microbial activity. The preservative investigated was boric acid powder plus 0.3 percent w/w mercuric chloride. The contribution of the excretory nitrogen compounds to a nitrogen correction factor for the T.M.E. assay was also determined.

Twenty-two birds were randomly selected and distributed among 22 wire cages. The birds were then divided, at random, into 2 groups of 11 birds per treatment: 1) excreta collection with preservative; 2) excreta collection without preservative. The preservative was the same as that used in experiment 1. Following a 24 hour fast, a human colostomy bag containing either 0 ml or 60 ml of the boric acid solution was attached around the cloaca of the appropriate treatment bird as described in experiment 1. The birds remained unfed, and exactly 48 hours later the bags were removed and the samples frozen. In both treatments, with and without preservative, several bags were damaged due to pecking resulting in 9 and 10 replicates per treatment respectively. Excreta samples were then freeze dried, equilibrated with atmospheric moisture, weighed, ground, and stored in a freezer until analyzed. All excreta samples were analyzed for total nitrogen, moisture, urea, ammonia, uric acid, creatinine, and individual amino acids.

IV. EXPERIMENT 3. Effect of different feedstuffs, and of poultry excreta collected with and without preservative, on the proportion of excretory nitrogen compounds and the contribution to a nitrogen correction factor for the TME assay.

This experiment was designed to determine the effect of different samples of wheat (Marshall, Glenlea, and HY320), in conjunction with the effect of excreta collected with and without preservative, on the proportion of excretory nitrogen compounds. The preservative used was the same as that used in experiment 1 (boric acid powder plus 0.3 percent w/w mercuric chloride) and as in experiment 2 was investigated to determine a direct effect on the nitrogen excretory compounds. The effect of type of feedstuff, and of a preservative, on the proportion of nitrogen compounds and their resulting contribution to a nitrogen correction factor, was determined.

Thirty-six birds were randomly selected and distributed among 36 wire cages. The birds were then randomly allocated to 1 of 6 treatments, 6 birds per treatment: 1) precision fed 25 g of Marshall wheat (Table 3a) - excreta collection with preservative; 2) precision fed 25 g of Marshall wheat - excreta collection without preservative; 3) precision fed 25 g of Glenlea wheat (Table 3b) - excreta collection with preservative; 4) precision fed 25 g of Glenlea wheat - excreta collection without preservative; 5) precision fed 25 g of HY320 wheat (Table 3c) - excreta collection with preservative; 6) precision fed 25 g of HY320 wheat - excreta collection without preservative. All birds were starved for 24 hours prior to the administration of the treatment. Following the 24 hour fast, the birds were force fed 25 g of the appropriate variety of wheat as per Sibbald (1983). Immediately following force feeding, a human colostomy bag containing either 0 ml or 60 ml of the boric acid solution was attached

**Table 3a. Marshall Wheat - Experiment 3**

| Chemical Analysis | Air Dry Basis | Dry Matter Basis |
|---|---|---|
| Dry Matter (%) | 87.68 | 100.00 |
| Total Nitrogen (%) | 2.28 | 2.60 |
| Crude Protein (%) | 14.23 | 16.23 |
| Gross Energy (MJ/kg) | 16.39 | 18.69 |

**Table 3b. Glenlea Wheat - Experiment 3**

| Chemical Analysis | Air Dry Basis | Dry Matter Basis |
|---|---|---|
| Dry Matter (%) | 88.08 | 100.00 |
| Total Nitrogen (%) | 2.43 | 2.76 |
| Crude Protein (%) | 15.21 | 17.27 |
| Gross Energy (MJ/kg) | 16.55 | 18.80 |

**Table 3c. HY320 - Experiment 3 and 4**

| Chemical Analysis | Air Dry Basis | Dry Matter Basis |
|---|---|---|
| Dry Matter (%) | 88.01 | 100.00 |
| Total Nitrogen (%) | 2.42 | 2.75 |
| Crude Protein (%) | 15.14 | 17.20 |
| Gross Energy (MJ/kg) | 16.51 | 18.76 |

around the cloaca of the appropriate treatment bird. The collection bags were attached as outlined in experiment 1. Exactly 48 hours later the bags were removed and the samples frozen. Excreta samples were then freeze dried, equilibrated with atmospheric moisture, weighed, ground, and stored in a freezer. Prior to chemical analysis, excreta samples within each treatment were randomly pooled into 2 groups of 3 resulting in 2 replicates per treatment for analysis. In treatment 4 however, 2 bags were damaged due to pecking, and as a result the samples were pooled into 2 groups of 2 to provide for 2 replicates within the treatment. The excreta was analyzed for total nitrogen, moisture, urea, ammonia, uric acid, creatinine, and individual amino acids. Feed samples were analyzed for moisture, gross energy, total nitrogen.

V. EXPERIMENT 4. Effect of different feedstuffs, and of poultry excreta collected with and without preservative, on the proportion of excretory nitrogen compounds and the contribution to a nitrogen correction factor for the TME assay.

Experiment 4 was designed to determine the effect of a feedstuff containing low (alfalfa meal), medium (wheat), and high (fish meal) levels of available amino acids, in conjunction with the effect of excreta collected with and without preservative, on the proportion of excretory nitrogen compounds. The preservative used was the same as in experiment 1 (boric acid powder plus 0.3 percent w/w mercuric chloride) and as in experiments 2 and 3 was investigated to determine a direct effect on the nitrogen excretory compounds. The effect of type of feedstuff, and of a preservative, on the proportion of nitrogen compounds and their resulting contribution to a nitrogen correction factor, was determined.

This experiment was designed and implemented identical to experiment 3, except that the treatment groups were as follows: 1) precision fed 25 g of fish meal (Table 4a) – excreta collected with preservative; 2) precision fed 25 g of fish meal – excreta collected without preservative; 3) precision fed 25 g of alfalfa meal (Table 4b) – excreta collected with preservative; 4) precision fed 25 g of alfalfa meal – excreta collected without preservative; 5) precision fed 25 g of wheat (Table 3c) – excreta collected with preservative; 6) precision fed 25 g of wheat – excreta collected without preservative. The wheat that was force fed was from the same sample that was used in experiment 3. Treatments 1 and 5 each lost 1 bag while treatment 3 lost 2 bags due to damage from pecking. As a result, samples from treatments 1 and 5 were pooled into a group of 3 and a group of 2, while treatment 3 samples were pooled into 2 groups of 2, in order to provide 2 replicates within each treatment. The excreta was analyzed for total nitrogen, moisture, urea, ammonia, uric acid, creatinine, and individual amino acids. Feed samples were analyzed for gross energy, total nitrogen, and moisture.

VI. EXPERIMENT 5. Effect of supplemental energy on the proportion of excretory nitrogen compounds and the contribution to a nitrogen correction factor for the TME assay.

Experiment 5 was designed to determine the effect of supplemental energy (α D (+) glucose) on the proportion of nitrogen compounds in poultry excreta. The contribution of the excretory nitrogen compounds to a nitrogen correction factor for the TME assay was also determined.

Twenty-eight birds were randomly selected and distributed among 28 wire cages. They were then divided, at random, into 2 groups of 14 birds per treatment:

**Table 4a. Fish Meal - Experiment 4**

| Chemical Analysis | Air Dry Basis | Dry Matter Basis |
|---|---|---|
| Dry Matter (%) | 97.28 | 100.00 |
| Total Nitrogen (%) | 10.53 | 10.82 |
| Crude Protein (%) | 65.80 | 67.64 |
| Gross Energy (MJ/kg) | 19.76 | 20.31 |

**Table 4b. Alfalfa Meal - Experiment 4**

| Chemical Analysis | Air Dry Basis | Dry Matter Basis |
|---|---|---|
| Dry Matter (%) | 85.62 | 100.00 |
| Total Nitrogen (%) | 2.80 | 3.27 |
| Crude Protein (%) | 17.47 | 20.40 |
| Gross Energy (MJ/kg) | 17.04 | 19.90 |

1) force fed 30 g of α D (+) glucose; 2) unfed. Both the force fed and unfed birds were starved for 24 hours. Following the 24 hour fast, the treatment 1 birds were force fed 30 g of α D (+) glucose as per Sibbald (1983). Immediately after force feeding, a human colostomy bag was attached around the cloaca to collect excreta (Sibbald 1983). The collection bags were attached as described in experiment 1. Collection bags were also attached to the unfed birds. Exactly 48 hours later the bags were removed and the samples were frozen. Excreta samples were then freeze dried, equilibrated with atmospheric moisture, weighed, ground, and stored in a freezer. Prior to chemical analysis, excreta samples within each treatment were randomly pooled in pairs to provide 7 replicate excreta samples per treatment. However, four bags in treatment 1, and 2 bags in treatment 2 damaged through pecking were eliminated, resulting in 5 and 6 replicates per treatment respectively. The excreta was analyzed for total nitrogen, moisture, urea, ammonia, uric acid, creatinine, and individual amino acids.

VII. EXPERIMENT 6. Effect of cecectomy on the proportion of excretory nitrogen compounds and the contribution to a nitrogen correction factor for the TME assay.

Experiment 6 was designed to determine the effect of cecectomy on the proportion of excretory nitrogen compounds and theier contribution to a nitrogen correction factor for the TME assay.

Twenty-four birds were randomly selected and distributed among 24 wire cages. They were then divided, at random, into 2 groups of 12 birds per treatment: 1) cecectomized–unfed; 2) intact–unfed. The birds designated to treatment 1 were then cecectomized and provided with a 4 week period to recuperate following surgery. Both the cecectomized and intact birds were starved for 24 hours. After the 24 hour

fast, a human colostomy bag was attached around the cloaca to collect excreta (Sibbald 1983). The collection bags were attached using a strong adhesive (3M–EC847) with duct tape applied to edges of the bags to prevent damage and sample loss due to pecking. The birds remained unfed, and exactly 48 hours later the bags were removed and the samples were frozen. Excreta samples were then freeze dried, equilibrated with atmospheric moisture, weighed, ground, and stored in a freezer. Prior to chemical analysis, excreta samples within each treatment were randomly pooled in pairs to provide 6 replicate excreta samples per treatment. The excreta was analyzed for total nitrogen, moisture, urea, ammonia, uric acid, and individual amino acids.

# RESULTS

I. EXPERIMENT 1. Examination and quantification of nitrogen compounds in poultry excreta collected with preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) and the contribution to a nitrogen correction factor for the TME assay.

Excreta total nitgrogen and nitrogen levels from the excretory nitrogen compounds are presented in Table 5, and expressed as milligrams of nitrogen per gram of excreta. Excreta total nitrogen was 167.96 mg with excreta uric acid nitrogen comprising the majority of the total nitrogen with a value of 135.37 mg. The proportion of excreta total nitrogen as excreta urea nitrogen was 1.22 mg and as excreta ammonia nitrogen was 12.68 mg. Excreta creatinine nitrogen contributed 0.36 mg of excreta total nitrogen. Collectively, excreta amino acid nitrogen contributed 9.31 mg toward the total excretion of nitrogen.

From the data shown in Table 5, uric acid nitrogen represents the greatest proportion (80.60%), but not the sole nitrogen excretory product. Ammonia nitrogen provides the second largest contribution (7.55%) of nitrogen to excreta total nitrogen. Total amino acid nitrogen comprises 5.54% of excreta total nitrogen, making it the third largest component (the individual amino acid component of excreta total amino acid nitrogen is also shown in Table 5). The remaining nitrogen excretory products consist of urea nitrogen (0.73% of excreta total nitrogen) and creatinine nitrogen (0.21% of excreta total nitrogen).

The contribution of each excretory nitrogen compound to the nitrogen correction factor for the TME assay was determined and are presented in Table 6. The values were derived using the energetic value of each component which are shown in Table 7.

**Table 5.** Levels of excretory nitrogen compounds in poultry excreta[1] with preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) – Experiment 1.

Milligrams of nitrogen per gram of excreta

| Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|
| 167.96 | 135.37 (80.60)[2] | 1.22 (0.73) | 12.68 (7.55) | 0.36 (0.21) | 9.31 (5.54) |

* Individual amino acid component

| Amino Acid | | Amino Acid | |
|---|---|---|---|
| Lys | 1.34 (0.80) | Ala | 0.58 (0.35) |
| His | 0.83 (0.50) | Cys | 0.33 (0.20) |
| Arg | 1.22 (0.73) | Val | 0.44 (0.26) |
| Asp | 0.69 (0.41) | Met | 0.10 (0.06) |
| Thr | 0.51 (0.30) | Ile | 0.27 (0.16) |
| Ser | 0.51 (0.30) | Leu | 0.43 (0.26) |
| Glu | 1.04 (0.62) | Tyr | 0.15 (0.09) |
| Pro | 0.65 (0.39) | Phe | 0.22 (0.13) |

[1] excreta wt = 9.71 g.
[2] ( ) represents proportion (as a %) of total nitrogen.

**Table 6.** Energetic contributions[1] of individual excretory nitrogen compounds to a nitrogen correction factor for the TME assay – Experiment 1.

| Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids | Correction Factor[2] |
|---|---|---|---|---|---|
| kJ/gN | | | | | |
| 27.62 | 0.16 | 1.71 | 0.12 | 7.96* | 37.57 |

*Energetic contribution of individual amino acids component

| Amino Acid | kJ/gN | Amino Acid | kJ/gN |
|---|---|---|---|
| Lys | 0.97 | Ala | 0.40 |
| His | 0.37 | Cys | 0.28 |
| Arg | 0.49 | Val | 0.55 |
| Asp | 0.47 | Met | 0.15 |
| Thr | 0.45 | Ile | 0.41 |
| Ser | 0.32 | Leu | 0.64 |
| Glu | 1.00 | Tyr | 0.28 |
| Pro | 0.75 | Phe | 0.43 |

[1] energetic contribution = % of total nitrogen (Table 5) × energetic value (kJ/gN) (Table 7).
[2] correction factor = sum of the energetic contributions of the excretory nitrogen compounds.

**Table 7.** Energetic value of excretory nitrogen compounds

| Compound | kJ/g | kJ/gN |
|---|---|---|
| Uric Acid | 11.42 | 34.27 |
| Urea | 10.46 | 22.43 |
| Ammonia | 18.62 | 22.64 |
| Creatinine | 20.71 | 55.77 |
| Lys | 23.18 | 120.96 |
| His | 20.46 | 75.56 |
| Arg | 21.47 | 66.74 |
| Asp | 11.92 | 113.30 |
| Thr | 17.57 | 149.45 |
| Ser | 13.81 | 103.60 |
| Glu | 15.32 | 160.88 |
| Pro | 23.56 | 193.64 |
| Ala | 18.24 | 116.02 |
| Cys | 16.40 | 140.71 |
| Val | 24.90 | 208.24 |
| Met | 22.80 | 242.92 |
| Ile | 27.03 | 253.13 |
| Leu | 26.53 | 248.45 |
| Tyr | 24.23 | 313.38 |
| Phe | 27.74 | 327.15 |

Uric acid nitrogen represents the largest portion of the nitrogen correction factor at 27.62 kJ/gN. Excreta total amino acid nitrogen (7.96 kJ/gN) was also shown to make a considerable contribution to the correction factor. Ammonia nitrogen contributed 1.71 kJ/gN while both urea nitrogen (0.16 kJ/gN) and creatinine nitrogen (0.12 kJ/gN) provided only a small contribution. The correction factor determined was 37.57 kJ/gN.

II. EXPERIMENT 2. Examination and quantification of nitrogen compounds in poultry excreta collected collected with and without preservative and the contribution to a nitrogen correction factor for the TME assay.

Excreta total nitrogen and nitrogen levels from the excretory nitrogen compounds are presented in Table 8. The treatment means for total uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta. In the excreta collected without preservative total nitrogen (220.56 mg) was significantly ($P<0.05$) higher from that of the excreta collected with preservative (169.28 mg).

The treatment means of excreta uric acid, ammonia, and creatinine nitrogen followed similar patterns. Uric acid nitrogen from excreta collected without preservative (179.84 mg) was significantly ($P<0.05$) higher than from excreta collected with preservative (137.49 mg). Both excreta ammonia nitrogen and excreta creatinine nitrogen levels were also significantly ($P<0.05$) higher from excreta collected without preservative than with preservative. The levels for excreta ammonia nitrogen were 24.13 mg from excreta without preservative and 14.80 mg from excreta collected with preservative. The levels for excreta creatinine nitrogen were 0.49 mg from excreta collected without preservative and 0.31 mg from excreta collected with preservative.

**Table 8.** Levels of excretory nitrogen compounds in poultry excreta[1] with (+) and without (–) preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) – Experiment 2.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Unfed (–)Preservative | 220.56$^{A2}$ | 179.84$^{A}$ (81.53)[3] | 2.57$^{A}$ (1.17) | 24.13$^{A}$ (10.94) | 0.49$^{A}$ (0.22) | 10.06$^{A}$ (4.56) |
| Unfed (+)Preservative | 169.28$^{B}$ | 137.49$^{B}$ (81.22) | 1.26$^{A}$ (0.74) | 14.80$^{B}$ (8.74) | 0.31$^{B}$ (0.18) | 8.90$^{A}$ (5.26) |
| Root MSE | 14.86 | 16.23 | 1.74 | 3.99 | 0.06 | 1.91 |

* Individual amino acid component

| Amino Acid | (-) preservative | (+) preservative | Amino Acid | (-) preservative | (+) preservative |
|---|---|---|---|---|---|
| Lys | 1.38$^{A}$ (0.63) | 1.21$^{A}$ (0.71) | Ala | 0.79$^{A}$ (0.36) | 0.62$^{A}$ (0.37) |
| His | 0.93$^{A}$ (0.42) | 0.79$^{A}$ (0.47) | Cys | 0.41$^{A}$ (0.19) | 0.37$^{A}$ (0.22) |
| Arg | 1.35$^{A}$ (0.61) | 1.14$^{A}$ (0.67) | Val | 0.50$^{A}$ (0.23) | 0.45$^{A}$ (0.27) |
| Asp | 0.72$^{A}$ (0.33) | 0.67$^{A}$ (0.40) | Met | 0.20$^{A}$ (0.09) | 0.14$^{B}$ (0.08) |
| Thr | 0.47$^{A}$ (0.21) | 0.47$^{A}$ (0.28) | Ile | 0.27$^{A}$ (0.12) | 0.25$^{A}$ (0.15) |
| Ser | 0.50$^{A}$ (0.23) | 0.48$^{A}$ (0.28) | Leu | 0.47$^{A}$ (0.21) | 0.42$^{A}$ (0.25) |
| Glu | 0.99$^{A}$ (0.45) | 0.89$^{A}$ (0.53) | Tyr | 0.15$^{A}$ (0.07) | 0.14$^{A}$ (0.08) |
| Pro | 0.69$^{A}$ (0.31) | 0.65$^{A}$ (0.38) | Phe | 0.24$^{A}$ (0.11) | 0.22$^{A}$ (0.13) |

[1] excreta weight: (–) preservative – 6.75 g; (+) preservative – 9.03 g

[2] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[3] ( ) represents proportion (as a %) of total nitrogen.

Urea nitrogen levels were not significantly different (P>0.05) between the excreta collected without preservative (2.57 mg) and the excreted collected with preservative (1.26 mg).

Among excreta individual amino acid nitrogen, only methionine levels were significantly (P<0.05) different. Excreta collected without preservative contained 0.20 mg while excreta collected with preservative contained 0.14 mg. Among all other excreta individual amino acid nitrogen levels, with the exception of threonine (the values are equal), levels from excreta collected without preservative tended to be higher than from excreta collected with preservative. Excreta total amino acid nitrogen between the two treatments did not differ significantly (P>0.05).

In both treatments, the percentage of excreta total nitrogen (Table 8) that each of the excretory nitrogen compounds represents followed the same pattern as Experiment 1. From the excreta collected without preservative, uric acid nitrogen represents the largest portion (81.53%) of excreta total nitrogen, followed by excreta ammonia (10.94%), total amino acid (4.56%), urea (1.17%), and creatinine (0.22%) nitrogen. Similarly, from the excreta collected with preservative, uric acid represents the largest portion (81.22%) of excreta total nitrogen, followed by excreta ammonia (8.74%), total amino acid (5.26%), urea (0.74%), and creatinine (0.18%) nitrogen.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in Table 7) and treatment values are presented in Table 9. In both treatments uric acid nitrogen represented the largest contribution to the nitrogen correction factor. Excreta total amino acid nitrogen was also shown to make a considerable contribution to the correction factor. Ammonia nitrogen was the third largest contributor to the correction factor while both urea nitrogen and creatinine nitrogen provided only a

**Table 9.** Energetic contributions of individual excretory nitrogen compounds to a nitrogen correction factor for the TME assay - Experiment 2.

| Treatment | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* | Correction Factor |
|---|---|---|---|---|---|---|
| | | | kJ/gN | | | |
| Unfed (-)Preservative | 27.94 | 0.26 | 2.48 | 0.12 | 6.53 | 37.33 |
| Unfed (+)Preservative | 27.83 | 0.17 | 1.98 | 0.10 | 7.59 | 37.67 |

* Energetic contribution of individual amino acid component

| Amino Acid | (-) preservative | (+) preservative | Amino Acid | (-) preservative | (+) preservative |
|---|---|---|---|---|---|
| | | kJ/gN | | | |
| Lys | 0.76 | 0.87 | Ala | 0.42 | 0.43 |
| His | 0.32 | 0.35 | Cys | 0.26 | 0.31 |
| Arg | 0.41 | 0.45 | Val | 0.47 | 0.55 |
| Asp | 0.37 | 0.45 | Met | 0.22 | 0.20 |
| Thr | 0.32 | 0.42 | Ile | 0.31 | 0.37 |
| Ser | 0.24 | 0.29 | Leu | 0.53 | 0.62 |
| Glu | 0.72 | 0.85 | Tyr | 0.21 | 0.26 |
| Pro | 0.61 | 0.74 | Phe | 0.36 | 0.43 |

small contribution. With the exception of total amino acid nitrogen, the contributions of all other excreta nitrogen compounds to their respective nitrogen correction factor were higher in excreta collected without preservative than excreta collected with preservative. The energetic contributions are expected based on the proportion of excreta total nitrogen that each of the excretory nitrogen compounds represent (Table 8). The proportion of excreta total nitrogen as uric acid, urea, ammonia, and creatinine nitrogen were higher in excreta collected without preservative. The proportion as excreta total amino acid nitrogen was higher in excreta collected with preservative. The correction factors determined were 37.33 kJ/gN for the unfed birds without preservative and 37.67 kJ/gN for the birds with preservative.

III. EXPERIMENT 3. Effect of different feedstuffs, and of poultry excreta collected with and without preservative, on the proportion of excretory nitrogen compounds and the contribution to a nitrogen correction factor for the TME assay.

A. Effect of Preservative

Nitrogen levels from excretory nitrogen compounds in excreta collected with and without preservative from birds force fed Marshall wheat are presented in Table 10. The treatment means for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta. Excreta total nitrogen was significantly ($P<0.05$) higher in the excreta without preservative (166.74 mg) than with preservative (142.11 mg). Similarly, excreta without preservative (16.20 mg) contained significantly ($P<0.05$) higher levels of ammonia nitrogen than excreta with preservative (10.13 mg). Creatinine nitrogen levels were significantly ($P<0.05$) lower in excreta without preservative (1.22 mg) than with

**Table 10.** Effect of Marshall wheat – Excreta[1] collected with (+) and without (–) preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 3.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Marshall (–)Preservative | 166.74$^{A2}$ | 139.90$^{A}$ (83.90)[3] | 2.54$^{A}$ (1.52) | 16.20$^{A}$ (9.72) | 1.22$^{B}$ (0.73) | 9.40$^{A}$ (5.64) |
| Marshall (+)Preservative | 142.11$^{B}$ | 118.86$^{A}$ (83.64) | 1.60$^{A}$ (1.13) | 10.13$^{B}$ (7.13) | 1.53$^{A}$ (1.08) | 9.19$^{A}$ (6.47) |
| Root MSE | 4.71 | 7.74 | 0.86 | 1.49 | 0.17 | 0.50 |

* Individual amino acid component

| Amino Acid | (-) preservative | (+) preservative | Amino Acid | (-) preservative | (+) preservative |
|---|---|---|---|---|---|
| Lys | 1.20$^{A}$ (0.72) | 1.08$^{A}$ (0.76) | Ala | 0.72$^{A}$ (0.43) | 0.69$^{A}$ (0.49) |
| His | 0.69$^{A}$ (0.41) | 0.63$^{A}$ (0.44) | Cys | 0.40$^{A}$ (0.24) | 0.40$^{A}$ (0.28) |
| Arg | 1.35$^{A}$ (0.81) | 1.33$^{A}$ (0.94) | Val | 0.46$^{A}$ (0.28) | 0.45$^{A}$ (0.32) |
| Asp | 0.71$^{A}$ (0.43) | 0.68$^{A}$ (0.48) | Met | 0.21$^{A}$ (0.13) | 0.19$^{A}$ (0.13) |
| Thr | 0.43$^{A}$ (0.26) | 0.43$^{A}$ (0.30) | Ile | 0.31$^{A}$ (0.19) | 0.30$^{A}$ (0.21) |
| Ser | 0.45$^{A}$ (0.27) | 0.47$^{A}$ (0.33) | Leu | 0.50$^{A}$ (0.30) | 0.48$^{A}$ (0.34) |
| Glu | 0.91$^{A}$ (0.55) | 0.89$^{A}$ (0.63) | Tyr | 0.16$^{A}$ (0.01) | 0.16$^{A}$ (0.11) |
| Pro | 0.60$^{A}$ (0.36) | 0.57$^{A}$ (0.40) | Phe | 0.31$^{A}$ (0.19) | 0.30$^{A}$ (0.21) |

[1] excreta weight: (–) preservative – 10.11 g; (+) preservative – 12.65 g

[2] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[3] ( ) represents proportion (as a %) of total nitrogen.

preservative (1.53 mg). There were no significant ($P<0.05$) differences in levels of excreta uric acid, urea, individual amino acid, and total amino acid nitrogen between the two treatments.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in Table 7) and treatment values are presented in Table 19. The proportion (Table 10), and therefore contribution to the correction factor, of excreta total nitrogen as uric acid (83.90% vs. 83.64%), urea (1.52% vs. 1.13%), and ammonia (9.72% vs. 7.13%) nitrogen were higher in the excreta collected without preservative. The proportion as creatinine nitrogen (1.08% vs. 0.73%) and total amino acid nitrogen (6.47% vs. 5.64%) were higher in the excreta with preservative. The correction factors (Table 19) determined were 39.96 kJ/gN for excreta collected without preservative and 40.47 kJ/gN for excreta collected with preservative.

Nitrogen levels from excretory nitrogen compounds in excreta collected with and without preservative from birds force fed Glenlea wheat are presented in Table 11. The treatment means for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta. Excreta total, ammonia, and creatinine nitrogen were significantly ($P<0.05$) higher in excreta without preservative (165.93, 17.20, and 1.24 mg respectively) than with preservative (144.58, 10.06, and 0.45 gm respectively). There were no significant ($P>0.05$) differences in levels of excreta uric acid nitrogen and excreta urea nitrogen between treatments. Among excreta individual amino acid nitrogen, histidine was significantly ($P<0.05$) higher in excreta without preservative (0.78 mg) than with preservative (0.62 mg). There were no significant ($P>0.05$) treatment differences between any of the

**Table 11.** Effect of Glenlea wheat – Excreta[1] collected with (+) and without (–) preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 3.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Glenlea (–)Preservative | 165.93$^{A2}$ | 131.24$^{A}$ (79.09)[3] | 2.09$^{A}$ (1.26) | 17.20$^{A}$ (10.37) | 1.24$^{A}$ (0.75) | 10.48$^{A}$ (6.32) |
| Glenlea (+)Preservative | 144.58$^{B}$ | 118.88$^{A}$ (82.22) | 1.20$^{A}$ (0.83) | 10.06$^{B}$ (6.96) | 0.45$^{B}$ (0.31) | 9.20$^{A}$ (6.36) |
| Root MSE | 4.71 | 7.74 | 0.86 | 1.49 | 0.17 | 0.50 |

* Individual amino acid component

| Amino Acid | (-) preservative | (+) preservative | Amino Acid | (-) preservative | (+) preservative |
|---|---|---|---|---|---|
| Lys | 1.26$^{A}$ (0.76) | 1.07$^{A}$ (0.74) | Ala | 0.82$^{A}$ (0.49) | 0.58$^{A}$ (0.40) |
| His | 0.78$^{A}$ (0.47) | 0.62$^{B}$ (0.43) | Cys | 0.45$^{A}$ (0.27) | 0.40$^{A}$ (0.28) |
| Arg | 1.45$^{A}$ (0.87) | 1.32$^{A}$ (0.91) | Val | 0.48$^{A}$ (0.29) | 0.62$^{A}$ (0.43) |
| Asp | 0.77$^{A}$ (0.46) | 0.69$^{A}$ (0.48) | Met | 0.22$^{A}$ (0.13) | 0.16$^{A}$ (0.11) |
| Thr | 0.52$^{A}$ (0.31) | 0.46$^{A}$ (0.32) | Ile | 0.34$^{A}$ (0.21) | 0.29$^{A}$ (0.20) |
| Ser | 0.54$^{A}$ (0.33) | 0.48$^{A}$ (0.33) | Leu | 0.56$^{A}$ (0.34) | 0.48$^{A}$ (0.33) |
| Glu | 1.07$^{A}$ (0.65) | 0.89$^{A}$ (0.62) | Tyr | 0.19$^{A}$ (0.12) | 0.17$^{A}$ (0.12) |
| Pro | 0.69$^{A}$ (0.42) | 0.66$^{A}$ (0.46) | Phe | 0.34$^{A}$ (0.21) | 0.30$^{A}$ (0.21) |

[1] excreta weight: (–) preservative – 10.91 g; (+) preservative – 13.59 g

[2] means between treatment groups followed by the same superscript are not significantly different at $P>0.05$.

[3] ( ) represents proportion (as a %) of total nitrogen.

remaining excreta individual amino acid nitrogen levels. Excreta total amino acid nitrogen was not significantly (P>0.05) different between treatments.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in Table 7) and treatment values are presented in Table 19. The proportion (Table 11), and therefore contribution to the correction factor, of excreta total nitrogen as urea (1.26% vs. 0.83%), ammonia (10.37% vs. 6.96%), and creatinine (0.75% vs. 0.31%) nitrogen were higher in the excreta without preservative. The proportion as excreta uric acid nitrogen (82.22% vs. 79.09%) and total amino acid nitrogen (6.36% vs. 6.32%) were higher in the excreta with preservative. The correction factors (Table 19) determined were 39.47 kJ/gN for excreta collected without preservative and 39.60 kJ/gN for excreta collected with preservative.

Nitrogen levels from excretory nitrogen compounds in excreta collected with and without preservative from birds force fed HY320 wheat are presented in Table 12. The treatment means for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta. Excreta total nitrogen and excreta creatinine nitrogen were significantly (P<0.05) higher in excreta without preservative (158.57 mg and 1.44 mg, respectively) than with preservative (139.34 mg and 0.54 mg, respectively). There were no significant (P>0.05) differences in the levels of excreta uric acid, urea, and ammonia nitrogen between treatments. Among excreta individual amino acid nitrogen, the only differences were between histidine and threonine which were significantly (P<0.05) higher in excreta without preservative (0.76 mg and 0.55 mg, respectively) than with preservative (0.63 mg and 0.44 mg, respectively). Excreta total amino acid nitrogen levels were not significantly (P>0.05) different between treatments.

**Table 12.** Effect of HY320 wheat – Excreta[1] collected with (+) and without (–) preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 3.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| HY320 wheat (–)Preservative | 158.47$^{A2}$ | 124.10$^{A}$ (78.31)[3] | 1.23$^{A}$ (0.78) | 16.12$^{A}$ (10.17) | 1.44$^{A}$ (0.91) | 11.13$^{A}$ (7.02) |
| HY320 wheat (+)Preservative | 139.34$^{B}$ | 110.96$^{A}$ (79.63) | 1.75$^{A}$ (1.26) | 11.03$^{A}$ (7.92) | 0.54$^{B}$ (0.39) | 9.26$^{A}$ (6.65) |
| Root MSE | 4.71 | 7.74 | 0.86 | 1.49 | 0.17 | 0.50 |

* Individual amino acid component

| Amino Acid | (-) preservative | (+) preservative | Amino Acid | (-) preservative | (+) preservative |
|---|---|---|---|---|---|
| Lys | 1.29$^{A}$ (0.81) | 1.10$^{A}$ (0.79) | Ala | 0.85$^{A}$ (0.54) | 0.70$^{A}$ (0.50) |
| His | 0.76$^{A}$ (0.48) | 0.63$^{B}$ (0.45) | Cys | 0.42$^{A}$ (0.27) | 0.38$^{A}$ (0.27) |
| Arg | 1.62$^{A}$ (1.02) | 1.32$^{A}$ (0.95) | Val | 0.54$^{A}$ (0.34) | 0.49$^{A}$ (0.35) |
| Asp | 0.86$^{A}$ (0.54) | 0.71$^{A}$ (0.51) | Met | 0.23$^{A}$ (0.15) | 0.19$^{A}$ (0.14) |
| Thr | 0.55$^{A}$ (0.35) | 0.44$^{B}$ (0.32) | Ile | 0.39$^{A}$ (0.25) | 0.30$^{A}$ (0.22) |
| Ser | 0.55$^{A}$ (0.35) | 0.46$^{A}$ (0.33) | Leu | 0.63$^{A}$ (0.40) | 0.54$^{A}$ (0.39) |
| Glu | 1.14$^{A}$ (0.72) | 0.92$^{A}$ (0.66) | Tyr | 0.22$^{A}$ (0.14) | 0.18$^{A}$ (0.13) |
| Pro | 0.70$^{A}$ (0.44) | 0.58$^{A}$ (0.42) | Phe | 0.38$^{A}$ (0.24) | 0.31$^{A}$ (0.22) |

[1] excreta weight: (–) preservative – 11.24 g; (+) preservative – 14.13 g

[2] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[3] ( ) represents proportion (as a %) of total nitrogen.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay were determined (using the energetic values shown in Table 7) and treatment values are presented in Table 19. The proportion (Table 12), and therefore contribution to the correction factor, of excreta total nitrogen as ammonia (10.17% vs. 7.92), creatinine (0.91% vs. 0.39%), and total amino acid (7.02% vs. 6.65%) nitrogen were higher in the excreta without preservative. The proportion as uric acid nitrogen (79.63% vs. 78.31%) and urea nitrogen (1.26% vs. 0.78%) were higher in excreta with preservative. The correction factors (Table 19) determined were 40.27 kJ/gN for excreta collected without preservative and 39.45 kJ/gN for excreta collected with preservative.

B. Effect of feedstuff – Excreta collected with and without preservative

Nitrogen levels from excretory nitrogen compounds in excreta collected without and with preservative from birds force fed HY320 wheat or Marshall wheat are presented in Tables 13 and 14. The treatment means for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta.

In the treatment group without preservative (Table 13), there were no significant ($P>0.05$) differences in excreta total, uric acid, urea, ammonia, and creatinine nitrogen levels between birds force fed HY320 or Marshall wheat. Among excreta individual amino acid nitrogen, the levels for threonine, glutamic acid, leucine, tyrosine, and phenylalanine were significantly ($P<0.05$) higher from birds force fed HY320 wheat (0.55, 1.14, 0.63, 0.22, and 0.38 mg, respectively) than from birds force fed Marshall wheat (0.43, 0.91, 0.50, 0.16, and 0.31 mg, respectively). There were no significant ($P>0.05$) differences between the remaining individual amino acid nitrogen

**Table 13.** Effect of HY320 wheat and Marshall wheat – Excreta collected without preservative on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 3.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| HY320 | 158.47[A1] | 124.10[A] (78.31)[2] | 1.23[A] (0.78) | 16.12[A] (10.17) | 1.44[A] (0.91) | 11.13[A] (7.02) |
| Marshall | 166.74[A] | 139.90[A] (83.90) | 2.54[A] (1.62) | 16.20[A] (9.72) | 1.22[A] (0.73) | 9.40[A] (5.64) |
| Root MSE | 4.71 | 7.74 | 0.86 | 1.49 | 0.17 | 0.50 |

* Individual amino acid component

| Amino Acid | HY320 | Marshall | Amino Acid | HY320 | Marshall |
|---|---|---|---|---|---|
| Lys | 1.29[A] (0.81) | 1.20[A] (0.72) | Ala | 0.85[A] (0.54) | 0.72[A] (0.43) |
| His | 0.76[A] (0.48) | 0.69[A] (0.41) | Cys | 0.42[A] (0.27) | 0.40[A] (0.28) |
| Arg | 1.62[A] (1.02) | 1.35[A] (0.81) | Val | 0.54[A] (0.34) | 0.46[A] (0.32) |
| Asp | 0.86[A] (0.54) | 0.71[A] (0.43) | Met | 0.23[A] (0.15) | 0.21[A] (0.13) |
| Thr | 0.55[A] (0.35) | 0.43[B] (0.26) | Ile | 0.39[A] (0.25) | 0.31[A] (0.21) |
| Ser | 0.55[A] (0.35) | 0.45[A] (0.27) | Leu | 0.63[A] (0.40) | 0.50[B] (0.34) |
| Glu | 1.14[A] (0.72) | 0.91[B] (0.55) | Tyr | 0.22[A] (0.14) | 0.16[B] (0.11) |
| Pro | 0.70[A] (0.44) | 0.60[A] (0.36) | Phe | 0.38[A] (0.24) | 0.31[B] (0.21) |

[1] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[2] ( ) represents proportion (as a %) of total nitrogen.

**Table 14.** Effect of HY320 wheat and Marshall wheat – Excreta collected with preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 3.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| HY320 | 139.34[A1] | 110.96[A] (79.63)[2] | 1.75[A] (1.26) | 11.03[A] (7.92) | 0.54[A] (0.39) | 9.26[A] (6.65) |
| Marshall | 142.11[A] | 118.86[A] (83.64) | 1.60[A] (1.13) | 10.13[A] (7.13) | 0.53[A] (1.08) | 9.19[A] (6.47) |
| Root MSE | 4.71 | 7.74 | 0.86 | 1.49 | 0.17 | 0.50 |

* Individual amino acid component

| Amino Acid | HY320 | Marshall | Amino Acid | HY320 | Marshall |
|---|---|---|---|---|---|
| Lys | 1.10[A] (0.79) | 1.08[A] (0.76) | Ala | 0.70[A] (0.50) | 0.69[A] (0.49) |
| His | 0.63[A] (0.45) | 0.63[A] (0.44) | Cys | 0.38[A] (0.27) | 0.40[A] (0.28) |
| Arg | 1.32[A] (0.95) | 1.33[A] (0.94) | Val | 0.49[A] (0.35) | 0.45[A] (0.32) |
| Asp | 0.71[A] (0.51) | 0.68[A] (0.48) | Met | 0.19[A] (0.14) | 0.19[A] (0.13) |
| Thr | 0.44[A] (0.32) | 0.43[A] (0.30) | Ile | 0.30[A] (0.22) | 0.30[A] (0.21) |
| Ser | 0.46[A] (0.33) | 0.47[A] (0.33) | Leu | 0.54[A] (0.39) | 0.48[A] (0.34) |
| Glu | 0.92[A] (0.66) | 0.89[A] (0.63) | Tyr | 0.18[A] (0.13) | 0.16[A] (0.11) |
| Pro | 0.58[A] (0.42) | 0.57[A] (0.40) | Phe | 0.31[A] (0.22) | 0.30[A] (0.21) |

[1] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[2] ( ) represents proportion (as a %) of total nitrogen.

levels. Excreta total amino acid nitrogen levels were not significantly (P>0.05) different between HY320 and Marshall wheat.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic value shown in Table 7) and treatment values are presented in Table 19. The proportion (Table 13), and therefore contribution to the correction factor, of excreta total nitrogen as ammonia (10.17% vs. 9.72%), creatinine (0.91% vs. 0.73%), and total amino acid nitrogen (7.02% vs. 5.64%) were higher from birds force fed HY320 wheat. The proportion as uric acid nitrogen (83.90% vs. 78.31%) and urea nitrogen (1.62% vs. 0.78%) was higher in the birds force fed Marshall wheat. The correction factors (Table 19) determined were 40.27 kJ/gN for excreta collected from birds force fed HY320 wheat and 39.96 kJ/gN for excreta collected from birds force fed Marhsall wheat.

In the treatment group with preservative (Table 14) there were no significant (P>0.05) differences in excreta total nitrogen or in nitrogen levels from any of the excretory nitrogen compounds between birds force fed HY320 or Marshall wheat.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in Table 7) and treatment values are presented in Table 19. The proportion (Table 14), and therefore contribution to the nitrogen correction factor, of excreta total nitrogen as uric acid nitrogen (83.64% vs. 79.63%) and creatinine nitrogen (1.08% vs. 0.39%) were higher from birds force fed Marshall wheat. The proportion as urea (1.26% vs. 1.13%), ammonia (7.92% vs. 7.13%), and total amino acid (6.65% vs. 6.47%) nitrogen were higher for birds force fed HY320 wheat. The correction factors (Table 19) determined were 39.45 kJ/gN for excreta collected from birds force fed HY320 wheat and 40.47 kJ/gN for birds force fed Marshall wheat.

Nitrogen levels from excretory nitrogen compounds in excreta collected without and with preservative from birds force fed HY320 wheat or Glenlea wheat are presented in Tables 15 and 16. The treatment means for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta.

In the treatment group without preservative (Table 15), there were no significant ($P>0.05$) differences in excreta total nitrogen or in nitrogen levels from any of the excretory nitrogen compounds between birds force fed HY320 or Glenlea wheat.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay were determined (using the energetic value shown in Table 7) and treatment values are presented in Table 19. The proportion (Table 15), and therefore contribution to the nitrogen correction factor, of excreta total nitrogen as uric acid (79.09% vs. 78.31%), urea (1.26% vs. 0.78%), and ammonia nitrogen (10.37% vs. 10.17%) were higher in birds force fed Glenlea wheat. The proportion as creatinine nitrogen (0.91% vs. 0.75%) and total amino acid (7.02% vs. 6.32%) nitrogen were higher in the birds force fed HY320 wheat. The correction factors (Table 19) determined were 40.27 kJ/gN for excreta collected from birds force fed HY320 wheat and 39.47 kJ/gN for birds force fed Glenlea wheat.

In the treatment group with preservative (Table 16), there were no significant ($P>0.05$) differences in excreta total nitrogen or in nitrogen levels from any of the excretory nitrogen compounds between birds force fed HY320 or Glenlea wheat.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay were determined (using the energetic values shown in Table 7) and treatment values are presented in Table 19. The proportion (Table 16), and therefore contribution to the nitrogen correction factor, of excreta total nitrogen

**Table 15.** Effect of HY320 wheat and Glenlea wheat – Excreta collected without preservative on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 3.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| HY320 | 158.47[A1] | 124.10[A] (78.31)[2] | 1.23[A] (0.78) | 16.12[A] (10.17) | 1.44[A] (0.91) | 11.13[A] (7.02) |
| Glenlea | 165.93[A] | 131.24[A] (79.09) | 2.09[A] (1.26) | 17.20[A] (10.37) | 1.24[A] (0.75) | 10.48[A] (6.32) |
| Root MSE | 4.71 | 7.74 | 0.86 | 1.49 | 0.17 | 0.50 |

* Individual amino acid component

| Amino Acid | HY320 | Glenlea | Amino Acid | HY320 | Glenlea |
|---|---|---|---|---|---|
| Lys | 1.29[A] (0.81) | 1.26[A] (0.76) | Ala | 0.85[A] (0.54) | 0.82[A] (0.49) |
| His | 0.76[A] (0.48) | 0.78[A] (0.47) | Cys | 0.42[A] (0.27) | 0.45[A] (0.27) |
| Arg | 1.62[A] (1.02) | 1.45[A] (0.87) | Val | 0.54[A] (0.34) | 0.48[A] (0.29) |
| Asp | 0.86[A] (0.54) | 0.77[A] (0.46) | Met | 0.23[A] (0.15) | 0.22[A] (0.13) |
| Thr | 0.55[A] (0.35) | 0.52[A] (0.31) | Ile | 0.39[A] (0.25) | 0.34[A] (0.21) |
| Ser | 0.55[A] (0.35) | 0.54[A] (0.33) | Leu | 0.63[A] (0.40) | 0.56[A] (0.34) |
| Glu | 1.14[A] (0.72) | 1.07[A] (0.65) | Tyr | 0.22[A] (0.14) | 0.19[A] (0.12) |
| Pro | 0.70[A] (0.44) | 0.69[A] (0.42) | Phe | 0.38[A] (0.24) | 0.34[A] (0.21) |

[1] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[2] ( ) represents proportion (as a %) of total nitrogen.

**Table 16.** Effect of HY320 wheat and Glenlea wheat – Excreta collected with preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 3.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| HY320 | 139.34[A1] | 110.96[A] (79.63)[2] | 1.75[A] (1.26) | 11.03[A] (7.92) | 0.64[A] (0.39) | 9.26[A] (6.65) |
| Glenlea | 144.58[A] | 118.88[A] (82.22) | 1.20[A] (0.83) | 10.06[A] (6.96) | 0.45[A] (0.31) | 9.20[A] (6.36) |
| Root MSE | 4.71 | 7.74 | 0.86 | 1.49 | 0.17 | 0.50 |

* Individual amino acid component

| Amino Acid | HY320 | Glenlea | Amino Acid | HY320 | Glenlea |
|---|---|---|---|---|---|
| Lys | 1.10[A] (0.79) | 1.07[A] (0.74) | Ala | 0.70[A] (0.50) | 0.58[A] (0.40) |
| His | 0.63[A] (0.45) | 0.62[A] (0.43) | Cys | 0.38[A] (0.27) | 0.40[A] (0.28) |
| Arg | 1.32[A] (0.95) | 1.32[A] (0.91) | Val | 0.49[A] (0.35) | 0.62[A] (0.43) |
| Asp | 0.71[A] (0.51) | 0.69[A] (0.48) | Met | 0.19[A] (0.14) | 0.16[A] (0.11) |
| Thr | 0.44[A] (0.32) | 0.46[A] (0.32) | Ile | 0.30[A] (0.22) | 0.29[A] (0.20) |
| Ser | 0.46[A] (0.33) | 0.48[A] (0.33) | Leu | 0.54[A] (0.39) | 0.48[A] (0.33) |
| Glu | 0.92[A] (0.66) | 0.89[A] (0.62) | Tyr | 0.18[A] (0.13) | 0.17[A] (0.12) |
| Pro | 0.58[A] (0.42) | 0.66[A] (0.46) | Phe | 0.31[A] (0.22) | 0.30[A] (0.21) |

[1] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[2] ( ) represents proportion (as a %) of total nitrogen.

as uric acid nitrogen (82.22% vs. 79.63%) was higher for birds force fed Glenlea wheat. The proportion as urea (1.26% vs. 0.83%), ammonia (7.92% vs. 6.96%), creatinine (0.39% vs. 0.31%), and amino acid (6.65% vs. 6.36%) nitrogen were higher for birds force fed HY320 wheat. The correction factors (Table 19) determined were 39.45 kJ/gN for excreta collected from birds force fed HY320 wheat and 40.47 kJ/gN for birds force fed Marshall wheat.

Nitrogen levels from excretory nitrogen compounds in excreta collected without and with preservative from birds force fed Marshall wheat or Glenlea wheat are presented in Tables 17 and 18. The treatment means for total, uric acid, urea, ammonia, creatinine, and ammino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta.

In the treatment group without preservative (Table 17), there were no significant ($P>0.05$) differences in excreta total nitrogen or in nitrogen levels from any of the excretory nitrogen compounds between birds force fed Marshall or Glenlea wheat.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay were determined (using the energetic values shown in Table 7) and treatment values are presented in Table 19. The proportion (Table 17), and therefore contribution to the nitrogen correction factor, of excreta total nitrogen as uric acid nitrogen (83.90% vs. 79.09%) and urea nitrogen (1.62% vs. 1.26%) were higher in birds force fed Marshall wheat. The proportion as ammonia (10.37% vs. 9.72%), creatinine (0.75% vs. 0.73%), and total amino acid (6.32% vs. 5.64%) nitrogen were higher in birds force fed Glenlea wheat. The correction factors (Table 19) determined were 39.96 kJ/gN for excreta collected from birds force fed Marshall wheat and 40.27 kJ/gN for birds force fed Glenlea wheat.

**Table 17.** Effect of Marshall wheat and Glenlea wheat – Excreta collected without preservative on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 3.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Marshall | 166.74[A1] | 139.90[A] (83.90)[2] | 2.54[A] (1.62) | 16.20[A] (9.72) | 1.22[A] (0.73) | 9.40[A] (5.64) |
| Glenlea | 165.93[A] | 131.24[A] (79.09) | 2.09[A] (1.26) | 17.20[A] (10.37) | 1.24[A] (0.75) | 10.48[A] (6.32) |
| Root MSE | 4.71 | 7.74 | 0.86 | 1.49 | 0.17 | 0.50 |

* Individual amino acid component

| Amino Acid | Marshall | Glenlea | Amino Acid | Marshall | Glenlea |
|---|---|---|---|---|---|
| Lys | 1.20[A] (0.72) | 1.26[A] (0.76) | Ala | 0.72[A] (0.43) | 0.82[A] (0.49) |
| His | 0.69[A] (0.41) | 0.78[A] (0.47) | Cys | 0.40[A] (0.28) | 0.45[A] (0.27) |
| Arg | 1.35[A] (0.81) | 1.45[A] (0.87) | Val | 0.46[A] (0.32) | 0.48[A] (0.29) |
| Asp | 0.71[A] (0.43) | 0.77[A] (0.46) | Met | 0.21[A] (0.13) | 0.22[A] (0.13) |
| Thr | 0.43[A] (0.26) | 0.52[A] (0.31) | Ile | 0.31[A] (0.21) | 0.34[A] (0.21) |
| Ser | 0.45[A] (0.27) | 0.54[A] (0.33) | Leu | 0.50[A] (0.34) | 0.56[A] (0.34) |
| Glu | 0.91[A] (0.55) | 1.07[A] (0.65) | Tyr | 0.16[A] (0.11) | 0.19[A] (0.12) |
| Pro | 0.60[A] (0.36) | 0.69[A] (0.42) | Phe | 0.31[A] (0.21) | 0.34[A] (0.21) |

[1] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[2] ( ) represents proportion (as a %) of total nitrogen.

**Table 18.** Effect of Marshall wheat and Glenlea wheat – Excreta collected with preservatives (boric acid powder plus 0.3 percent w/w mercuric chloride) on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 3.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Marshall | 142.11[A1] | 118.86[A] (83.64)[2] | 1.60[A] (1.13) | 10.13[A] (7.13) | 0.53[A] (1.08) | 9.19[A] (6.47) |
| Glenlea | 144.58[A] | 118.88[A] (82.22) | 1.20[A] (0.83) | 10.06[A] (6.96) | 0.45[A] (0.31) | 9.20[A] (6.36) |
| Root MSE | 4.71 | 7.74 | 0.86 | 1.49 | 0.17 | 0.50 |

* Individual amino acid component

| Amino Acid | Marshall | Glenlea | Amino Acid | Marshall | Glenlea |
|---|---|---|---|---|---|
| Lys | 1.08[A] (0.76) | 1.07[A] (0.74) | Ala | 0.69[A] (0.49) | 0.58[A] (0.40) |
| His | 0.63[A] (0.44) | 0.62[A] (0.43) | Cys | 0.40[A] (0.28) | 0.40[A] (0.28) |
| Arg | 1.33[A] (0.94) | 1.32[A] (0.91) | Val | 0.45[A] (0.32) | 0.62[A] (0.43) |
| Asp | 0.68[A] (0.48) | 0.69[A] (0.48) | Met | 0.19[A] (0.13) | 0.16[A] (0.11) |
| Thr | 0.43[A] (0.30) | 0.46[A] (0.32) | Ile | 0.30[A] (0.21) | 0.29[A] (0.20) |
| Ser | 0.47[A] (0.33) | 0.48[A] (0.33) | Leu | 0.48[A] (0.34) | 0.48[A] (0.33) |
| Glu | 0.89[A] (0.63) | 0.89[A] (0.62) | Tyr | 0.16[A] (0.11) | 0.17[A] (0.12) |
| Pro | 0.57[A] (0.40) | 0.66[A] (0.46) | Phe | 0.30[A] (0.21) | 0.30[A] (0.21) |

[1] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[2] ( ) represents proportion (as a %) of total nitrogen.

In the treatment group with preservative (Table 18), there were no significant ($P>0.05$) differences in excreta total nitrogen or in nitrogen levels from any of excretory nitrogen compounds between birds force fed Marshall or Glenlea wheat.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay were determined (using the energetic values shown in Table 7) and treatment values are presented in Table 19. The proportion (Table 18), and therefore contribution to the nitrogen correction factor, of excreta total nitrogen as uric acid (83.64% vs. 82.22%), urea (1.13% vs. 0.83%), ammonia (7.13% vs. 6.96%), creatinine (1.08% vs. 0.31%), and total amino acid (6.47% vs. 6.36%) nitrogen were higher for birds force fed Marshall wheat. The correction factors (Table 19) determined were 40.47 kJ/gN for excreta collected from birds force fed Marshall wheat and 39.60 kJ/gN for birds force fed Glenlea wheat.

IV. EXPERIMENT 4. Effect of different feedstuffs, and of poultry excreta collected with and without preservative, on the proportion of excretory nitrogen compounds and the contribution to a nitrogen correction factor for the TME assay.

A. Effect of preservative

Nitrogen levels from excretory nitrogen compounds in excreta collected with and without preservative from birds force fed fish meal are presented in Table 20. The treatment means for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta. With the exception of excreta ammonia nitrogen, there were no significant ($P>0.05$) differences in excreta total nitrogen or in nitrogen levels of any of the excretory nitrogen compounds between the two treatments. Excreta ammonia nitrogen was significantly

**Table 19.** Energetic contribution of individual excretory nitrogen compounds to a nitrogen correction factor for the TME assay – Experiment 3.

| Treatment | Uric Acid | Urea | Ammonia | Creatinine | Amino Acids | Correction Factor |
|---|---|---|---|---|---|---|
| | | | kJ/gN | | | |
| Marshall wheat without preservative | 28.75 | 0.34 | 2.20 | 0.41 | 8.26 | 39.96 |
| Marshall wheat with preservative | 28.66 | 0.25 | 1.61 | 0.60 | 9.35 | 40.47 |
| Glenlea wheat without preservative | 27.11 | 0.28 | 2.35 | 0.42 | 9.31 | 39.47 |
| Glenlea wheat with preservative | 28.18 | 0.19 | 1.58 | 0.17 | 9.48 | 39.60 |
| HY320 wheat without preservative | 26.84 | 0.17 | 2.30 | 0.51 | 10.45 | 40.27 |
| HY320 wheat with preservative | 27.29 | 0.28 | 1.79 | 0.22 | 9.87 | 39.45 |

**Table 20.** Effect of fish meal - Excreta[1] collected with (+) and without (-) preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the levels of the excretory nitrogen compounds in poultry excreta - Experiment 4.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Fish meal (-)Preservative | 175.98[A2] | 140.29[A] (79.72)[3] | 1.05[A] (0.60) | 19.73[A] (11.21) | 1.18[A] (0.67) | 10.68[A] (6.07) |
| Fish meal (+)Preservative | 152.63[A] | 126.24[A] (82.71) | 2.27[A] (1.49) | 12.26[B] (8.03) | 1.22[A] (0.80) | 10.74[A] (7.04) |
| Root MSE | 6.63 | 5.59 | 0.47 | 1.83 | 0.15 | 0.77 |

* Individual amino acid component

| Amino Acid | (-) preservative | (+) preservative | Amino Acid | (-) preservative | (+) preservative |
|---|---|---|---|---|---|
| Lys | 2.16[A] (1.23) | 1.92[A] (1.26) | Ala | 0.74[A] (0.42) | 0.60[A] (0.39) |
| His | 0.94[A] (0.53) | 0.85[A] (0.56) | Cys | 0.35[A] (0.20) | 0.36[A] (0.24) |
| Arg | 1.35[A] (0.77) | 1.90[A] (1.25) | Val | 0.43[A] (0.24) | 0.38[A] (0.25) |
| Asp | 0.88[A] (0.50) | 0.95[A] (0.62) | Met | 0.18[A] (0.10) | 0.17[A] (0.11) |
| Thr | 0.36[A] (0.21) | 0.40[A] (0.26) | Ile | 0.33[A] (0.19) | 0.30[A] (0.20) |
| Ser | 0.43[A] (0.24) | 0.47[A] (0.31) | Leu | 0.52[A] (0.30) | 0.48[A] (0.32) |
| Glu | 0.96[A] (0.55) | 0.93[A] (0.61) | Tyr | 0.15[A] (0.09) | 0.15[A] (0.10) |
| Pro | 0.60[A] (0.34) | 0.59[A] (0.39) | Phe | 0.32[A] (0.18) | 0.31[A] (0.20) |

[1] excreta weight: (-) preservative - 17.21 g; (+) preservative - 19.36 g

[2] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[3] ( ) represents proportion (as a %) of total nitrogen.

($P<0.05$) higher in excreta collected without preservative (19.73 mg) than with preservative (12.26 mg).

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in Table 7) and treatment values are presented in Table 29. The proportion (Table 20), and therefore contribution to the correction factor, of excreta total nitrogen as uric acid (82.71% vs. 79.72%), urea (1.49% vs. 0.60%), creatinine (0.80% vs. 0.67%), and total amino acid (7.04% vs. 6.07%) nitrogen were higher in the excreta with preservative. The proportion as ammonia nitrogen (11.21% vs. 8.03%) was higher in the excreta without preservative. The correction factors (Table 29) determined were 39.01 kJ/gN for excreta collected without preservative and 40.68 kJ/gN for excreta collected with preservative.

Nitrogen levels from excretory nitrogen compounds in excreta collected with and without preservative from birds force fed alfalfa meal are presented in Table 21. The treatment means for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta. There were no significant ($P>0.05$) differences in excreta total, uric acid, urea, ammonia, and creatinine nitrogen between the two treatments. Among excreta individual amino acid nitrogen, aspartic acid and isoleucine were significantly ($P<0.05$) higher in excreta without preservative (0.77 mg and 0.38 mg respectively) than with preservative (0.57 mg and 0.25 mg respectively). There were no significant ($P>0.05$) treatment differences among the remaining individual amino acid nitrogen levels. Excreta total amino acid nitrogen was not significantly ($P<0.05$) different between treatments.

**Table 21.** Effect of alfalfa meal - Excreta[1] collected with (+) and without (-) preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the levels of the excretory nitrogen compounds in poultry excreta - Experiment 4.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Alfalfa meal (-)Preservative | 82.97[A2] | 60.80[A] (73.28)[3] | 1.42[A] (1.71) | 9.87[A] (11.90) | 0.67[A] (0.81) | 10.93[A] (13.17) |
| Alfalfa meal (+)Preservative | 75.72[A] | 63.50[A] (83.86) | 1.43[A] (1.89) | 3.63[A] (4.79) | 0.41[A] (0.54) | 9.30[A] (12.28) |
| Root MSE | 6.63 | 5.59 | 0.47 | 1.83 | 0.15 | 0.77 |

* Individual amino acid component

| Amino Acid | (-) preservative | (+) preservative | Amino Acid | (-) preservative | (+) preservative |
|---|---|---|---|---|---|
| Lys | 1.46[A] (1.76) | 1.43[A] (1.89) | Ala | 0.77[A] (0.93) | 0.56[A] (0.74) |
| His | 2.06[A] (2.48) | 2.02[A] (2.67) | Cys | 0.31[A] (0.37) | 0.31[A] (0.41) |
| Arg | 1.12[A] (1.35) | 0.94[A] (1.24) | Val | 0.46[A] (0.55) | 0.38[A] (0.50) |
| Asp | 0.77[A] (0.93) | 0.57[B] (0.75) | Met | 0.19[A] (0.23) | 0.16[A] (0.21) |
| Thr | 0.42[A] (0.51) | 0.35[A] (0.46) | Ile | 0.38[A] (0.46) | 0.25[B] (0.33) |
| Ser | 0.45[A] (0.54) | 0.38[A] (0.50) | Leu | 0.55[A] (0.66) | 0.35[A] (0.46) |
| Glu | 0.87[A] (1.05) | 0.69[A] (0.91) | Tyr | 0.17[A] (0.21) | 0.12[A] (0.16) |
| Pro | 0.59[A] (0.71) | 0.51[A] (0.67) | Phe | 0.35[A] (0.42) | 0.27[A] (0.36) |

[1] excreta weight: (-) preservative - 22.17 g; (+) preservative - 25.34 g

[2] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[3] ( ) represents proportion (as a %) of total nitrogen.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in Table 7) and treatment values are presented in Table 29. The proportion (Table 21), and therefore contribution to the correction factor, of excreta total nitrogen as uric acid nitrogen (83.86% vs. 73.28%) and urea nitrogen (1.89% vs. 1.71%) were higher in the excreta collected with preservative. The proportion as ammonia (11.90% vs. 4.79%), creatinine (0.81% vs. 0.54%), and total amino acid (13.17% vs. 12.28%) nitrogen were higher in the excreta collected without preservative. The correction factors (Table 29) determined were 47.12 kJ/gN for excreta collected without preservative and 47.15 kJ/gN for excreta collected with preservative.

Nitrogen levels from excretory nitrogen compounds in excreta collected with and without preservative from birds force fed Marshall wheat are presented in Table 22. The treatments for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta. There were no significant ($P>0.05$) differences in excreta total, uric acid, urea, and creatinine nitrogen. Excreta ammonia nitrogen was significantly ($P<0.05$) higher in excreta collected without preservative (20.18 mg) than with preservative (9.53 mg). Among excreta individual amino acid nitrogen, aspartic acid, glutamic acid, and isoleucine were significantly ($P<0.05$) higher in excreta collected without preservative (0.86, 1.09, and 0.40 mg respectively) than with preservative (0.66, 0.87, and 0.27 mg respectively). There were no significant ($P>0.05$) treatment differences among the remaining individual amino acid nitrogen levels. Excreta total amino acid nitrogen was not significantly ($P>0.05$) different between treatments.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in

**Table 22.** Effect of wheat – Excreta[1] collected with (+) and without (–) preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 4.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Wheat (–)Preservative | 155.34[A2] | 117.24[A] (75.47)[3] | 2.15[A] (1.38) | 20.18[A] (12.99) | 1.11[A] (0.72) | 11.03[A] (7.10) |
| Wheat (+)Preservative | 131.46[A] | 106.93[A] (81.34) | 2.13[A] (1.62) | 9.53[B] (7.25) | 0.58[A] (0.44) | 8.53[A] (6.49) |
| Root MSE | 6.63 | 5.59 | 0.47 | 1.83 | 0.15 | 0.77 |

* Individual amino acid component

| Amino Acid | (-) preservative | (+) preservative | Amino Acid | (-) preservative | (+) preservative |
|---|---|---|---|---|---|
| Lys | 1.29[A] (0.83) | 0.96[A] (0.73) | Ala | 0.89[A] (0.57) | 0.56[A] (0.43) |
| His | 0.79[A] (0.51) | 0.62[A] (0.47) | Cys | 0.41[A] (0.26) | 0.38[A] (0.29) |
| Arg | 1.67[A] (1.08) | 1.28[A] (0.97) | Val | 0.56[A] (0.36) | 0.40[A] (0.30) |
| Asp | 0.86[A] (0.55) | 0.66[B] (0.50) | Met | 0.21[A] (0.14) | 0.16[A] (0.12) |
| Thr | 0.52[A] (0.34) | 0.43[A] (0.33) | Ile | 0.40[A] (0.26) | 0.27[B] (0.21) |
| Ser | 0.57[A] (0.37) | 0.47[A] (0.36) | Leu | 0.56[A] (0.36) | 0.46[A] (0.35) |
| Glu | 1.09[A] (0.70) | 0.87[B] (0.66) | Tyr | 0.18[A] (0.12) | 0.15[A] (0.11) |
| Pro | 0.70[A] (0.45) | 0.57[A] (0.43) | Phe | 0.33[A] (0.21) | 0.29[A] (0.22) |

[1] excreta weight: (–) preservative – 8.87 g; (+) preservative – 11.28 g

[2] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[3] ( ) represents proportion (as a %) of total nitrogen.

Table 7) and treatment values are presented in Table 29. The proportion (Table 22), and therefore contribution to the correction factor, of excreta total nitrogen as uric foracid nitrogen (81.34% vs. 75.47%) and urea nitrogen (1.62% vs. 1.38%) were higher in excreta collected with preservative. The proportion as ammonia (12.99% vs. 7.25%), creatinine (0.72% vs. 0.44%), and total amino acid nitrogen (7.10% vs. 6.49%) were higher in the excreta collected without preservative. The correction factors (Table 29) determined were 39.86 kJ/gN for excreta collected without preservative and 39.72 kJ/gN for excreta collected with preservative.

B. Effect of feedstuff – Excreta collected with and without preservative

Nitrogen levels from excretory nitrogen compounds in excreta collected without and with preservative from birds force fed fish meal or alfalfa meal are presented in Tables 23 and 24. The treatment means for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta.

In the treatment group without preservative (Table 23), excreta total nitrogen was significantly ($P<0.05$) higher in the excreta from birds force fed fish meal (175.98 mg) than from birds force fed alfalfa meal (82.97 mg). Similarly, both excreta uric acid nitrogen and excreta ammonia nitrogen were significantly ($P<0.05$) higher from birds force fed fish meal (140.29 mg and 19.73 mg respectively) than from birds force fed alfalfa meal (60.80 mg and 9.87 mg respectively). There were no significant ($P>0.05$) differences in excreta urea nitrogen or excreta creatinine nitrogen between birds force fed fish or alfalfa meal. Among excreta individual amino acid nitrogen, histidine was significantly ($P<0.05$) higher in excreta from birds force fed alfalfa meal (2.06 mg) than from birds force fed fish meal (0.24 mg). There were no significant

**Table 23.** Effect of fish meal and alfalfa meal – Excreta collected without preservative on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 4.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Fish meal | 175.98[A1] | 140.29[A] (79.72)[2] | 1.05[A] (0.60) | 19.73[A] (11.21) | 1.18[A] (0.67) | 10.68[A] (6.07) |
| Alfalfa meal | 82.97[B] | 60.80[B] (73.28) | 1.42[A] (1.71) | 9.87[B] (11.90) | 0.67[A] (0.81) | 10.93[A] (13.17) |
| Root MSE | 6.63 | 5.59 | 0.47 | 1.83 | 0.15 | 0.77 |

* Individual amino acid component

| Amino Acid | Fish meal | Alfalfa meal | Amino Acid | Fish meal | Alfalfa meal |
|---|---|---|---|---|---|
| Lys | 2.16[A] (1.23) | 1.46[A] (1.76) | Ala | 0.74[A] (0.42) | 0.77[A] (0.93) |
| His | 0.24[B] (0.53) | 2.06[A] (2.48) | Cys | 0.35[A] (0.20) | 0.31[A] (0.37) |
| Arg | 1.35[A] (0.77) | 1.12[A] (1.35) | Val | 0.43[A] (0.24) | 0.46[A] (0.55) |
| Asp | 0.88[A] (0.50) | 0.77[A] (0.93) | Met | 0.18[A] (0.10) | 0.19[A] (0.23) |
| Thr | 0.36[A] (0.21) | 0.42[A] (0.51) | Ile | 0.33[A] (0.19) | 0.38[A] (0.46) |
| Ser | 0.43[A] (0.24) | 0.45[A] (0.54) | Leu | 0.52[A] (0.30) | 0.55[A] (0.55) |
| Glu | 0.96[A] (0.55) | 0.87[A] (1.05) | Tyr | 0.15[A] (0.09) | 0.17[A] (0.21) |
| Pro | 0.60[A] (0.34) | 0.59[A] (0.71) | Phe | 0.32[A] (0.18) | 0.35[A] (0.42) |

[1] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[2] ( ) represents proportion (as a %) of total nitrogen.

**Table 24.** Effect of fish meal and alfalfa meal - Excreta collected with preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the levels of the excretory nitrogen compounds in poultry excreta - Experiment 4.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Fish meal | 152.63[A1] | 126.24[A] (82.71)[2] | 2.27[A] (1.49) | 12.26[A] (8.03) | 1.22[A] (0.80) | 10.74[A] (7.04) |
| Alfalfa meal | 75.72[B] | 63.50[B] (83.86) | 1.43[A] (1.89) | 3.63[B] (4.79) | 0.41[B] (0.54) | 9.30[A] (12.28) |
| Root MSE | 6.63 | 5.59 | 0.47 | 1.83 | 0.15 | 0.77 |

* Individual amino acid component

| Amino Acid | Fish meal | Alfalfa meal | Amino Acid | Fish meal | Alfalfa meal |
|---|---|---|---|---|---|
| Lys | 1.92[A] (1.26) | 1.43[A] (1.89) | Ala | 0.60[A] (0.39) | 0.56[A] (0.74) |
| His | 0.85[B] (0.56) | 2.02[A] (2.67) | Cys | 0.36[A] (0.24) | 0.31[A] (0.41) |
| Arg | 1.90[A] (1.25) | 0.94[B] (1.24) | Val | 0.38[A] (0.25) | 0.38[A] (0.50) |
| Asp | 0.95[A] (0.62) | 0.57[B] (0.75) | Met | 0.17[A] (0.11) | 0.16[A] (0.21) |
| Thr | 0.40[A] (0.26) | 0.35[A] (0.46) | Ile | 0.30[A] (0.20) | 0.25[A] (0.33) |
| Ser | 0.47[A] (0.31) | 0.38[A] (0.50) | Leu | 0.48[A] (0.32) | 0.35[A] (0.46) |
| Glu | 0.93[A] (0.61) | 0.69[B] (0.91) | Tyr | 0.15[A] (0.10) | 0.12[A] (0.16) |
| Pro | 0.59[A] (0.39) | 0.51[A] (0.67) | Phe | 0.31[A] (0.20) | 0.27[A] (0.36) |

[1] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[2] ( ) represents proportion (as a %) of total nitrogen.

(P>0.05) differences between the remaining individual amino acid nitrogen levels. Excreta total amino acid nitrogen levels were not significantly (P>0.05) different between fish and alfalfa meal.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in Table 7) and treatment values are presented in Table 29. The proportion (Table 23), and therefore contribution to the correction factor, of excreta total nitrogen as uric acid nitrogen (79.72% vs. 73.28%) was higher in excreta from birds force fed fish meal. The proportion as urea (1.71% vs. 0.60%), ammonia (11.90% vs. 11.21%), creatinine (0.81% vs. 0.67%), and total amino acid (13.17% vs. 6.07%) nitrogen were higher from birds force fed alfalfa meal. The correction factors (Table 29) determined were 39.01 kJ/gN for excreta collected from birds force fed fish meal and 47.12 kJ/gN from birds force fed alfalfa meal.

In the treatment group with preservative (Table 24), excreta total nitrogen was significantly (P<0.05) higher from the birds force fed fish meal (152.63 mg) than from the birds force fed alfalfa meal (75.72 mg). Excreta uric acid, ammonia, and creatinine nitrogen were also significantly (P<0.05) higher from the birds force fed fish meal (126.24, 12.26, and 1.22 mg respectively) than from the birds force fed alfalfa meal (63.50, 3.63, and 0.41 mg respectively). Excreta urea nitrogen was not significantly (P>0.05) different between the birds force fed fish meal or alfalfa meal. Among excreta individual amino acid nitrogen, histidine was significantly (P<0.05) higher from the birds force fed alfalfa meal (2.02 mg) than from the birds force fed fish meal (0.85 mg). Conversely, arginine, aspartic acid, and glutamic acid levels were significantly (P<0.05) higher from the birds force fed fish meal (1.90, 0.95, and 0.93 mg respectively) than from the birds force fed alfalfa meal (0.94, 0.57, and 0.69 mg

respectively). There were no significant (P>0.05) differences between the remaining excreta individual amino acid nitrogen levels. Excreta total amino acid nitrogen was not significantly (P>0.05) different between fish and alfalfa meal.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in Table 7) and treatment values are presented in Table 29. The proportion (Table 24), and therefore contribution to the correction factor, of excreta total nitrogen as uric acid (83.86% vs. 82.71%), urea (1.89% vs. 1.49%), and total amino acid (12.28% vs. 7.04%) nitrogen were higher from birds force fed alfalfa meal. The proportion as ammonia nitrogen (8.03% vs. 4.79%) and creatinine nitrogen (0.80% vs. 0.54%) were higher from birds force fed fish meal. The correction factors (Table 29) determined were 40.68 kJ/gN for excreta collected from birds force fed fish meal and 47.15 kJ/gN from birds force fed alfalfa meal.

Nitrogen levels from excretory nitrogen compounds in excreta collected without and with preservative from birds force fed fish meal or Marshall wheat are presented in Tables 25 and 26. The treatment means for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta.

In the treatment group without preservative (Table 25), excreta total nitrogen was not significantly (P>0.05) different between the birds force fed fish meal and Marshall wheat. Excreta uric acid was significantly (P<0.05) higher from the birds force fed fish meal (140.29 mg) than the birds force fed Marshall wheat (117.24 mg). There were no significant (P>0.05) differences between the levels of excreta urea, ammonia, and creatinine nitrogen. Among excreta individual amino acid nitrogen levels, threonine and serine were significantly (P<0.05) higher from birds force fed

**Table 25.** Effect of fish meal and wheat – Excreta collected without preservative on the levels of excretory nitrogen compounds in poultry excreta – Experiment 4.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Fish meal | 175.98[A1] | 140.29[A] (79.72)[2] | 1.05[A] (0.60) | 19.73[A] (11.21) | 1.18[A] (0.67) | 10.68[A] (6.07) |
| Wheat | 155.34[A] | 117.24[B] (75.47) | 2.15[A] (1.38) | 20.18[A] (12.99) | 1.11[A] (0.72) | 11.03[A] (7.10) |
| Root MSE | 6.63 | 5.59 | 0.47 | 1.83 | 0.15 | 0.77 |

* Individual amino acid component

| Amino Acid | Fish meal | Marshall wheat | Amino Acid | Fish meal | Marshall wheat |
|---|---|---|---|---|---|
| Lys | 2.16[A] (1.23) | 1.29[A] (0.83) | Ala | 0.74[A] (0.42) | 0.89[A] (0.57) |
| His | 0.94[A] (0.53) | 0.79[A] (0.51) | Cys | 0.35[A] (0.20) | 0.41[A] (0.26) |
| Arg | 1.35[A] (0.77) | 1.67[A] (1.08) | Val | 0.43[A] (0.24) | 0.56[A] (0.36) |
| Asp | 0.88[A] (0.50) | 0.86[A] (0.55) | Met | 0.18[A] (0.10) | 0.21[A] (0.14) |
| Thr | 0.36[B] (0.21) | 0.52[A] (0.34) | Ile | 0.33[A] (0.19) | 0.40[A] (0.26) |
| Ser | 0.43[B] (0.24) | 0.57[A] (0.37) | Leu | 0.52[A] (0.30) | 0.56[A] (0.36) |
| Glu | 0.96[A] (0.55) | 1.09[A] (0.70) | Tyr | 0.15[A] (0.09) | 0.18[A] (0.12) |
| Pro | 0.60[A] (0.34) | 0.70[A] (0.45) | Phe | 0.32[A] (0.18) | 0.33[A] (0.21) |

[1] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[2] ( ) represents proportion (as a %) of total nitrogen.

**Table 26.** Effect of fish meal and wheat – Excreta collected with preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 4.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Fish meal | 152.63[A1] | 126.24[A] (82.71)[2] | 2.27[A] (1.49) | 12.26[A] (8.03) | 1.22[A] (0.80) | 10.74[A] (7.04) |
| Wheat | 131.46[A] | 106.93[A] (81.34) | 2.13[A] (1.62) | 9.53[A] (7.25) | 0.58[B] (0.44) | 8.53[A] (6.49) |
| Root MSE | 6.63 | 5.59 | 0.47 | 1.83 | 0.15 | 0.77 |

* Individual amino acid component

| Amino Acid | Fish meal | Marshall wheat | Amino Acid | Fish meal | Marshall wheat |
|---|---|---|---|---|---|
| Lys | 1.92[A] (1.26) | 0.96[A] (0.73) | Ala | 0.60[A] (0.39) | 0.56[A] (0.43) |
| His | 0.58[A] (0.56) | 0.62[A] (0.47) | Cys | 0.36[A] (0.24) | 0.38[A] (0.29) |
| Arg | 1.90[A] (1.25) | 1.28[A] (0.97) | Val | 0.38[A] (0.25) | 0.40[A] (0.30) |
| Asp | 0.95[A] (0.62) | 0.66[B] (0.50) | Met | 0.17[A] (0.11) | 0.16[A] (0.12) |
| Thr | 0.40[A] (0.26) | 0.43[A] (0.33) | Ile | 0.30[A] (0.20) | 0.27[A] (0.21) |
| Ser | 0.47[A] (0.31) | 0.47[A] (0.36) | Leu | 0.48[A] (0.32) | 0.46[A] (0.35) |
| Glu | 0.93[A] (0.61) | 0.87[A] (0.66) | Tyr | 0.15[A] (0.10) | 0.15[A] (0.11) |
| Pro | 0.59[A] (0.39) | 0.57[A] (0.43) | Phe | 0.31[A] (0.20) | 0.29[A] (0.22) |

[1] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[2] ( ) represents proportion (as a %) of total nitrogen.

Marshall wheat (0.52 mg and 0.57 mg respectively) than the birds force fed fish meal (0.36 mg and 0.43 mg respectively). There were no significant (P>0.05) differences between the remaining excreta individual amino acid nitrogen levels. Excreta total amino acid nitrogen was not significantly (P>0.05) different between fish meal and Marshall wheat.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in Table 7) and treatment values are presented in Table 29. The proportion (Table 25), and therefore contribution to the correction factor, of excreta total nitrogen as uric acid nitrogen (79.72% vs. 75.47%) was higher from the birds force fed fish meal. The proportion as urea (1.38% vs. 0.60%), ammonia (12.99% vs. 11.21%), creatinine (0.72% vs. 0.67%), and total amino acid (7.10% vs. 6.07%) nitrogen were higher from the birds force fed Marshall wheat. The correction factors (Table 29) determined were 39.01 kJ/gN for excreta collected from birds force fed fish meal and 39.86 kJ/gN from birds force fed Marshall wheat.

In the treatment group with preservative (Table 26), there were no significant (P>0.05) differences in excreta total, uric acid, urea, and ammonia nitrogen. Excreta creatinine nitrogen was significantly (P<0.05) higher from the birds force fed fish meal (1.22 mg) than the birds force fed Marshall wheat (0.58 mg). Among excreta individual amino acid nitrogen, the only significant (P<0.05) difference which occurred was with aspartic acid, which was higher in birds force fed fish meal (0.95 mg) than in birds force fed Marshall wheat (0.66 mg). Excreta total amino acid nitrogen was not significantly (P>0.05) different between fish meal and Marshall wheat.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in

Table 7) and treatment values are presented in Table 29. The proportion (Table 26), and therefore contribution to the correction factor, of excreta total nitrogen as uric acid (82.71% vs. 81.34%), ammonia (8.03% vs. 7.25%), creatinine (0.80% vs. 0.44%), and total amino acid (7.04% vs. 6.49%) nitrogen were higher from the birds force fed fish meal. The proportion as urea nitrogen (1.62% vs. 1.49%) was higher from the birds force fed Marshall wheat. The correction factors (Table 29) determined were 40.68 kJ/gN for excreta collected from birds force fed fish meal and 39.72 kJ/gN from birds force fed Marshall wheat.

Nitrogen levels from excretory nitrogen compounds in excreta collected without and with preservative from birds force fed alfalfa meal or Marshall wheat are presented in Tables 27 and 28. The treatment means for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta.

In the treatment group without preservative (Table 27), excreta total, uric acid, and ammonia nitrogen were significantly higher from the birds force fed Marshall wheat (155.34, 117.24, and 20.18 mg respectively) than from the birds force fed alfalfa meal (82.97, 60.80, and 9.87 mg respectively). There were no significant ($P>0.05$) differences between the levels of excreta urea nitrogen and excreta creatinine nitrogen. Among excreta individual amino acid nitrogen, histidine was significantly ($P<0.05$) higher from the birds force fed alfalfa meal (2.06 mg) than from the birds force fed Marshall wheat (0.79 mg). Conversely, glutamic acid was significantly ($P<0.05$) higher from the birds force fed Marshall wheat (1.09 mg) than from the birds force fed alfalfa meal (0.87 mg). There were no significant ($P>0.05$) differences betwen the remaining excreta individual amino nitrogen levels. Excreta total amino acid

**Table 27.** Effect of alfalfa meal and wheat – Excreta collected without preservative on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 4.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Alfalfa meal | 82.97$^{B1}$ | 60.80$^{B}$ (73.28)[2] | 1.42$^{A}$ (1.71) | 9.87$^{B}$ (11.90) | 0.67$^{A}$ (0.81) | 10.93$^{A}$ (13.17) |
| Wheat | 155.34$^{A}$ | 117.24$^{A}$ (75.47) | 2.15$^{A}$ (1.38) | 20.18$^{A}$ (12.99) | 1.11$^{A}$ (0.72) | 11.03$^{A}$ (7.10) |
| Root MSE | 6.63 | 5.59 | 0.47 | 1.83 | 0.15 | 0.77 |

* Individual amino acid component

| Amino Acid | Alfalfa meal | Marshall wheat | Amino Acid | Alfalfa meal | Marshall wheat |
|---|---|---|---|---|---|
| Lys | 1.46$^{A}$ (1.76) | 1.29$^{A}$ (0.83) | Ala | 0.77$^{A}$ (0.93) | 0.89$^{A}$ (0.57) |
| His | 2.06$^{A}$ (2.48) | 0.79$^{B}$ (0.51) | Cys | 0.31$^{A}$ (0.37) | 0.41$^{A}$ (0.26) |
| Arg | 1.12$^{A}$ (1.35) | 1.67$^{A}$ (1.08) | Val | 0.46$^{A}$ (0.55) | 0.56$^{A}$ (0.36) |
| Asp | 0.77$^{A}$ (0.93) | 0.86$^{A}$ (0.55) | Met | 0.19$^{A}$ (0.23) | 0.21$^{A}$ (0.14) |
| Thr | 0.42$^{A}$ (0.51) | 0.52$^{A}$ (0.34) | Ile | 0.38$^{A}$ (0.46) | 0.40$^{A}$ (0.26) |
| Ser | 0.45$^{A}$ (0.54) | 0.57$^{A}$ (0.37) | Leu | 0.55$^{A}$ (0.66) | 0.56$^{A}$ (0.36) |
| Glu | 0.87$^{B}$ (1.05) | 1.09$^{A}$ (0.70) | Tyr | 0.17$^{A}$ (0.21) | 0.18$^{A}$ (0.12) |
| Pro | 0.59$^{A}$ (0.71) | 0.70$^{A}$ (0.45) | Phe | 0.35$^{A}$ (0.42) | 0.33$^{A}$ (0.21) |

[1] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[2] ( ) represents proportion (as a %) of total nitrogen.

**Table 28.** Effect of alfalfa meal and wheat – Excreta collected with preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the levels of the excretory nitrogen compounds in poultry excreta – Experiment 4.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Alfalfa meal | 75.72[B1] | 63.50[B] (83.86)[2] | 1.43[A] (1.89) | 3.63[A] (4.79) | 0.41[A] (0.54) | 9.30[A] (12.28) |
| Wheat | 131.46[A] | 106.93[A] (81.34) | 2.13[A] (1.62) | 9.53[A] (7.25) | 0.58[A] (0.44) | 8.53[A] (6.49) |
| Root MSE | 6.63 | 5.59 | 0.47 | 1.83 | 0.15 | 0.77 |

* Individual amino acid component

| Amino Acid | Alfalfa meal | Marshall wheat | Amino Acid | Alfalfa meal | Marshall wheat |
|---|---|---|---|---|---|
| Lys | 1.43[A] (1.89) | 0.96[A] (0.73) | Ala | 0.56[A] (0.74) | 0.56[A] (0.43) |
| His | 2.02[A] (2.67) | 0.62[B] (0.47) | Cys | 0.31[A] (0.41) | 0.38[A] (0.29) |
| Arg | 0.94[A] (1.24) | 1.28[A] (0.97) | Val | 0.38[A] (0.50) | 0.40[A] (0.30) |
| Asp | 0.57[A] (0.75) | 0.66[A] (0.50) | Met | 0.16[A] (0.21) | 0.16[A] (0.12) |
| Thr | 0.35 (0.46) | 0.43[A] (0.33) | Ile | 0.25[A] (0.33) | 0.27[A] (0.21) |
| Ser | 0.38[A] (0.50) | 0.47[A] (0.36) | Leu | 0.35[A] (0.46) | 0.46[A] (0.35) |
| Glu | 0.69[A] (0.91) | 0.87[A] (0.66) | Tyr | 0.12[A] (0.16) | 0.15[A] (0.11) |
| Pro | 0.51[A] (0.67) | 0.57[A] (0.43) | Phe | 0.27[A] (0.36) | 0.29[A] (0.22) |

[1] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[2] ( ) represents proportion (as a %) of total nitrogen.

nitrogen was not significantly (P>0.05) different between alfalfa meal and Marshall wheat.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in Table 7) and treatment values are presented in Table 29. The proportion (Table 27), and therefore contribution to the correction factor, of excreta total nitrogen as uric acid nitrogen (75.47% vs. 73.28%) and ammonia nitrogen (12.99% vs. 11.90%) were higher from birds force fed Marshall wheat. The proportion as urea (1.71% vs. 1.38%), creatinine (0.81% vs. 0.72%), and total amino acid (13.17% vs. 7.10%) nitrogen were higher from the birds force fed alfalfa meal. The correction factors (Table 29) determined were 47.12 kJ/gN for excreta collected from birds force fed alfalfa meal and 39.86 kJ/gN from birds force fed Marshall wheat.

In the treatment group with preservative (Table 28), excreta total nitrogen and excreta uric acid were significantly (P<0.05) higher from birds force fed Marshall wheat (131.46 mg and 106.93 mg respectively) than from birds force fed alfalfa meal (75.72 mg and 63.50 mg respectively). There were no significant (P>0.05) differences between the levels of excreta urea, ammonia, and creatinine nitrogen. Among excreta individual amino acid nitrogen, the only significant (P<0.05) difference which occurred was with histidine, which was higher from the birds force fed alfalfa meal (2.02 mg) than the birds force fed Marshall wheat (0.62 mg). Excreta total amino acid nitrogen was not significantly (P>0.05) different between alfalfa meal and Marshall wheat.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay was determined (using the energetic values shown in Table 7) and treatment values are presented in Table 29. The proportion (Table 28), and therefore contribution to the correction factor, of excreta total nitrogen as uric

**Table 29.** Energetic contribution of individual excretory nitrogen compounds to a nitrogen correction factor for the TME assay – Experiment 4.

| Treatment | Uric Acid | Urea | Ammonia | Creatinine | Amino Acids | Correction Factor |
|---|---|---|---|---|---|---|
| | | | kJ/gN | | | |
| Fish meal without preservative | 27.32 | 0.13 | 2.54 | 0.37 | 8.65 | 39.01 |
| Fish meal with preservative | 28.35 | 0.33 | 1.82 | 0.45 | 9.73 | 40.68 |
| Alfalfa meal without preservative | 25.11 | 0.38 | 2.69 | 0.45 | 18.49 | 47.12 |
| Alfalfa meal with preservative | 28.74 | 0.42 | 1.09 | 0.30 | 16.60 | 47.15 |
| Wheat without preservative | 25.87 | 0.31 | 2.94 | 0.40 | 10.34 | 39.86 |
| Wheat with preservative | 27.88 | 0.36 | 1.64 | 0.25 | 9.59 | 39.72 |

acid (83.86% vs. 81.34%), urea (1.89% vs. 1.62%), creatinine (0.54% vs. 0.44%), and total amino acid (12.28% vs. 6.49%) nitrogen were higher from the birds force fed alfalfa meal. The proportion as ammonia nitrogen (7.25% vs. 4.79%) was higher from the birds force fed Marshall wheat. The correction factors (Table 29) determined were 47.15 kJ/gN for excreta collected from birds force fed alfalfa meal and 39.72 kJ/gN from birds force fed Marshall wheat.

V. EXPERIMENT 5. Effect of supplemental energy on the proportion of excretory nitrogen compounds and the contribution to a nitrogen correction factor for the TME assay.

Excreta total nitrogen and nitrogen levels from the excretory nitrogen compounds are presented in Table 30. The treatment means for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta. Excreta total nitrogen levels between the unfed birds (232.88 mg) and the birds force fed supplemental energy (232.59 mg) were almost identical. There was no significant ($P>0.05$) difference between the two treatments.

Excreta uric acid nitrogen was higher for the unfed birds (199.97 mg). This value, however, was not significantly ($P>0.05$) different than the excreta uric acid nitrogen level of the force fed birds (189.87 mg).

Excreta urea nitrogen was significantly ($P<0.05$) different between the two treatments with a higher level excreted by the unfed birds. The unfed birds excreted 3.77 mg while the force fed birds excreted 1.67 mg. Excreta ammonia nitrogen was also affected by the provision of supplemental energy. The force fed birds excreted 28.10 mg which was significantly ($P<0.05$) higher than that excreted by the unfed birds (22.81 mg).

**Table 30.** Effect of supplemental energy (α D (+) glucose) on the excretory nitrogen compounds in poultry excreta[1] – Experiment 5.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Unfed | 232.88[A2] | 199.97[A] (85.87)[3] | 3.77[A] (1.62) | 22.81[B] (9.79) | 0.52[A] (0.22) | 11.47[A] (4.93) |
| Force Fed (30g α D (+) glucose) | 232.59[A] | 189.98[A] (81.68) | 1.67[B] (0.72) | 28.10[A] (12.08) | 0.47[A] (0.20) | 12.67[A] (5.45) |
| Root MSE | 12.44 | 15.65 | 1.35 | 3.68 | 0.06 | 1.32 |

* Individual amino acid component

| Amino Acid | Unfed | Force fed (30g α D (+) glucose) | Amino Acid | Unfed | Force fed (30g α D (+) glucose) |
|---|---|---|---|---|---|
| Lys | 1.91[A] (0.82) | 1.80[A] (0.77) | Ala | 0.81[B] (0.35) | 1.02[A] (0.44) |
| His | 1.16[A] (0.50) | 1.06[A] (0.46) | Cys | 0.40[A] (0.17) | 0.45[A] (0.19) |
| Arg | 1.49[A] (0.64) | 1.60[A] (0.69) | Val | 0.55[B] (0.24) | 0.68[A] (0.29) |
| Asp | 0.74[A] (0.32) | 0.86[A] (0.37) | Met | 0.15[A] (0.06) | 0.21[A] (0.10) |
| Thr | 0.52[A] (0.22) | 0.53[A] (0.23) | Ile | 0.35[B] (0.15) | 0.45[A] (0.19) |
| Ser | 0.49[A] (0.21) | 0.59[A] (0.25) | Leu | 0.55[A] (0.24) | 0.67[A] (0.29) |
| Glu | 1.01[A] (0.43) | 1.18[A] (0.51) | Tyr | 0.22[B] (0.10) | 0.29[A] (0.12) |
| Pro | 0.83[A] (0.36) | 0.90[A] (0.39) | Phe | 0.31[A] (0.13) | 0.39[A] (0.17) |

[1] excreta weight: unfed – 6.55 g; force fed – 6.16 g

[2] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[3] ( ) represents proportion (as a %) of total nitrogen.

As with excreta total nitrogen and excreta uric acid nitrogen there was no significant ($P>0.05$) difference in the level of excreta creatinine nitrogen between the two treatments. The unfed birds excreted 0.52 mg and the force fed birds excreted 0.47 mg.

With respect to excreta individual amino acid nitrogen, alanine (1.02 mg), valine (0.68 mg), isoleucine (0.45 mg), and tyrosine (0.29 mg) levels of the force fed birds were significantly ($P<0.05$) higher than those of the unfed birds (0.81, 0.55, 0.35, and 0.22 mg respectively). There were no significant ($P>0.05$) treatment differences between any of the other individual amino acids. However, with the exception of lysine and histidine, levels tended to be higher in the force fed birds than the unfed birds. There was no significant ($P>0.05$) difference in the level of excreta total amino acid nitrogen between the unfed (11.47 mg) and force fed (12.67 mg) birds.

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay were determined (using the energetic values shown in Table 7) and treatment values are presented in Table 31. In both treatments uric acid nitrogen represented the largest contribution to the nitrogen correction factor. Total amino acid nitrogen was also shown to make a considerable contribution to the correction factor. Ammonia nitrogen was the third largest contributor while both urea nitrogen and creatinine nitrogen provided only a small contribution. The proportion of excreta total nitrogen that each of the excretory nitrogen compounds represent are shown in Table 30. The proportion, and therefore contribution to the correction factor, of excreta total nitrogen as uric acid (85.87% vs. 81.86%), urea (1.62% vs. 0.72%), and creatinine (0.22% vs. 0.20%) nitrogen was higher in the unfed birds. The proportion as ammonia nitrogen (12.08% vs. 9.79%) and total amino acid nitrogen (5.45% vs.

**Table 31.** Energetic contribution of individual excretory nitrogen compounds to a nitrogen correction factor for the TME assay – Experiment 5.

| Treatment | Uric Acid | Urea | Ammonia | Creatinine | Amino Acids | Correction Factor |
|---|---|---|---|---|---|---|
| | | | kJ/gN | | | |
| Unfed | 29.43 | 0.36 | 2.22 | 0.13 | 7.06 | 39.20 |
| Force fed (30g α D (+) glucose) | 27.99 | 0.16 | 2.74 | 0.11 | 8.04 | 39.04 |

4.93%) was higher in force fed birds. The correction factors determined were 39.20 kJ/gN for the unfed birds and 39.04 kJ/gN for the force fed birds.

VI. EXPERIMENT 6. Effect of cecectomy on the proportion of excretory nitrogen compounds and the contribution to a nitrogen correction factor for the TME assay.

Excreta total nitrogen levels from the excretory nitrogen compounds are presented in Table 32. The treatment means for total, uric acid, urea, ammonia, creatinine, and amino acid nitrogen are expressed as milligrams of nitrogen per gram of excreta. There was no significant ($P>0.05$) difference in excreta total nitrogen between the intact birds (229.00 mg) and the cecectomized birds (220.52 mg).

Excreta uric acid nitrogen was higher in the intact birds (179.80 mg) than in the cecectomized birds (171.09 mg). However, the values were not significantly ($P>0.05$) different.

Excreta urea nitrogen as lower in the intact birds (3.71 mg). This value was significantly less than the excreta urea nitrogen level in the cecectomized birds (6.49 mg).

Excreta ammonia nitrogen was also affected by cecectomy. Levels were significantly ($P<0.05$) higher in the intact birds (23.30 mg) than the cecectomized birds (18.62 mg).

There was no significant ($P>0.05$) effect of cecectomy on excreta creatinine nitrogen. The intact birds excreted 0.41 mg and the cecectomized birds excreted 0.46 mg.

Excreta individual amino acid nitrogen was unaffected between the two treatments. There were no significant ($P>0.05$) differences in excreta individual amino

**Table 32.** Effect of cecectomy on the excretory nitrogen compounds in poultry excreta[1] – Experiment 6.

Milligrams of nitrogen per gram of excreta

| Treatment | Total Nitrogen | Uric Acid | Urea | Ammonia | Creatinine | Total Amino Acids* |
|---|---|---|---|---|---|---|
| Unfed intact | 229.00[A2] | 179.80[A] (78.52)[3] | 3.71[B] (1.62) | 23.30[A] (10.18) | 0.41[A] (0.18) | 11.48[A] (5.01) |
| Unfed cecectomy | 220.52[A] | 171.09[A] (77.59) | 6.49[A] (2.94) | 18.62[B] (8.44) | 0.46[A] (0.21) | 10.41[A] (4.72) |
| Root MSE | 11.41 | 14.20 | 1.53 | 3.41 | 0.07 | 1.67 |

* Individual amino acid component

| Amino Acid | Unfed Intact | Unfed Cecectomy | Amino Acid | Unfed Intact | Unfed Cecectomy |
|---|---|---|---|---|---|
| Lys | 1.70[A] (0.74) | 1.41[A] (0.64) | Ala | 0.87[A] (0.38) | 0.79[A] (0.36) |
| His | 0.89[A] (0.39) | 0.77[A] (0.35) | Cys | 0.40[A] (0.18) | 0.43[A] (0.20) |
| Arg | 1.47[A] (0.64) | 1.39[A] (0.63) | Val | 0.60[A] (0.26) | 0.55[A] (0.25) |
| Asp | 0.77[A] (0.34) | 0.74[A] (0.34) | Met | 0.14[A] (0.06) | 0.15[A] (0.07) |
| Thr | 0.54 (0.24) | 0.51[A] (0.23) | Ile | 0.39[A] (0.17) | 0.35[A] (0.16) |
| Ser | 0.56[A] (0.25) | 0.53[A] (0.24) | Leu | 0.58[A] (0.25) | 0.53[A] (0.24) |
| Glu | 1.02[A] (0.45) | 0.94[A] (0.43) | Tyr | 0.28[A] (0.12) | 0.23[A] (0.10) |
| Pro | 0.95[A] (0.42) | 0.79[A] (0.36) | Phe | 0.34[A] (0.14) | 0.30[A] (0.14) |

[1] excreta weight: intact – 7.65 g; cecectomy – 7.14 g

[2] means between treatment groups followed by the same superscript are not significantly different at P>0.05.

[3] ( ) represents proportion (as a %) of total nitrogen.

acid nitrogen levels between the intact and cecectomized birds. However, with the exception of cystine and methionine, levels were higher in the intact birds. Excreta total amino acid nitrogen from the intact birds (11.48 mg) was not significantly ($P>0.05$) different from that of the cecectomized birds (10.41 mg).

The contribution of each excreta nitrogen compound to the nitrogen correction factor for the TME assay were determined (using the energetic value shown in Table 7) and treatment values are presented in Table 33. In both treatments uric acid nitrogen represented the largest contribution to the nitrogen correction factor. Amino acid nitrogen was also shown to make a considerable contribution to the correction factor. Ammonia nitrogen was the third largest contributor while both urea nitrogen and creatinine nitrogen provided only a small contribution. The proportion of total nitrogen of the excretory nitrogen compounds are shown in Table 32. The proportion, and therefore contribution to the correction factor, of excreta total nitrogen as uric acid (78.52% vs. 77.59%), ammonia (10.18% vs. 8.44%), and total amino acid (5.01% vs. 4.72%) nitrogen were higher in the intact birds. The proportion as urea nitrogen (2.94% vs. 1.62%) and creatinine nitrogen (0.21% vs. 0.18%) were higher in the cecectomized birds. The correction factors determined were 37.12 kJ/gN for the intact birds and 36.30 kJ/gN for the cecectomized birds.

**Table 33.** Energetic contribution of individual excretory nitrogen compounds to a nitrogen correction factor for the TME assay - Experiment 6.

| Treatment | Uric Acid | Urea | Ammonia | Creatinine | Amino Acids | Correction Factor |
|---|---|---|---|---|---|---|
| | | | kJ/gN | | | |
| Unfed intact | 26.91 | 0.36 | 2.31 | 0.10 | 7.44 | 37.12 |
| Unfed cecectomy | 26.59 | 0.66 | 1.91 | 0.12 | 7.02 | 36.30 |

# DISCUSSION

## I. EXPERIMENT 1. Examination and quantification of nitrogen compounds in poultry excreta collected with preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) and the contribution to a nitrogen correction factor for the TME assay.

Little or no work on the preservation of poultry excreta to prevent nitrogen loss (via ammonia lost to the atmosphere) or changes due to microbial activity relative to the TME assay have been conducted. Data presented in Table 5 shows the levels of excretory nitrogen compounds which occur under such conditions.

Clearly, uric acid nitrogen is the major nitrogenous component (80.6%) of the total nitrogen excreted (Table 5). This is expected since uric acid is the major metabolic end product of protein utilization, regardless of whether the protein catabolized is of endogenous (body tissue) or exogenous (dietary) origin, as demonstrated in Table 34.

Ammonia nitrogen excretion presented in Table 5 was found to be the second largest contributor (7.55%) to excreta total nitrogen. This is expected since ammonia and uric acid nitrogen excretion have been shown to parallel each other (Tasaki and Okumura, 1964; Teekell et al., 1968). As with uric acid nitrogen, ammonia nitrogen excretion is also an end product of protein utilization. In fowl, urinary ammonia is formed by the deamination of protein in the kidney tubule cells (Sturkie, 1976). The ammonia then diffuses into the acidic luminal fluid to form $NH_4^+$ which is then excreted with anions to maintain proper acid-base balance (Sykes, 1971).

Amino acid nitrogen was found to be third largest contributor (5.54%) to excreta total nitrogen (Table 5). There have not been any previous studies to

Table 34. 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 | | | | |
| Mayrs (1924)[2] | 65.7 | | 6.0 | | | | |
| Davis (1927) | 62.9 | 10.4 | 17.3 | 8.0 | | | 1.4 |
| Coulson and Hughes (1930) | 65.8 | 6.5 | 7.6 | 4.6 | | 13.4 | 2.8 |
| Edwards and Wilson (1954)[2] | 60.0 | | | | 10.0 | 20.0 | |
| O'Dell et al. (1960) | 80.7 | 4.5 | 10.5 | 0.9 | 2.2 | 1.2 | |
| Waring and Brown (1965) | 50-60 | | | | | | |
| Teekell et al. (1968) | 60.0 | 6.0 | 23.0 | 4.0 | 2.0 | | |
| Sykes (1971): Fed | 84.1 | 5.2 | 6.8 | 0.5 | 1.7 | | 1.7 |
| Unfed | 57.8 | 2.9 | 23.0 | 4.3 | 2.8 | | 9.2 |
| 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 |
| Humphreys (1985): Unfed | | | | | | | |
| - preservative | 93.4 | 1.5 | 9.3 | 0.2 | | | |
| + preservative | 95 | 0.4 | 5.7 | 0.3 | 1.4 | | |
| Humphreys (1985): Force fed | | | | | | | |
| - preservative | 86.3 | 0.3 | 12.9 | 0.2 | | | |
| + preservative | 92.4 | 0.3 | 3.7 | 0.3 | 2.4 | | |

[1] cited in Coulson and Hughes (1930)
[2] cited in O'Dell et al. (1960)

determine the levels of amino acid nitrogen in excreta collected with a preservative. Under normal conditions (Table 34), amino acid nitrogen has been shown to contribute levels varying from 1.7% to 10% of the total nitrogen excreted.

Urea nitrogen excretion by the chicken was small (Table 5) and therefore accounted for only a small (0.73%) proportion of excreta total nitrogen. Under normal dietary conditions the source of urea is dietary arginine which is hydrolyzed in the liver and kidney by arginase to produce, as one of its end products, urea (Sykes, 1971). Urea is then excreted, but not before over 99% of the excreted urea is reabsorbed by the renal tubules. Urea therefore is excreted in only small quantities (Sykes, 1971) as shown in Table 34. Under conditions where the bird remains unfed over a short period of time, the level of urea should remain similar, if not lower, to that of urea excreted under normal dietary conditions. The level reported in Table 5 demonstrates this and is consistent with the level obtained from excreta collected with a preservative (Humphreys, 1985).

Creatinine nitrogen excretion was also small (0.21% of total nitrogen) (Table 5) and was similar to that reported by Humphreys (1985). Creatinine excretion is the result of utilization of phosphocreatine as an energy form (Lehninger, 1977). Glycine, arginine, and methionine are hydrolyzed in the kidney to form as one of the end products, guanido-acetate. Guanido-acetate in turn is transmethylated in the liver to form creatine. Creatine then combines with ATP to form phosphocreatine, a high energy phosphate storage compound in muscle tissue. Upon a demand for energy, dephosphorylation occurs producing creatinine which is excreted in the urine (Lehninger, 1977). Sturkie (1976) states that creatinine exists only in minute amounts in chicken urine.

The trend in the proportion of excreta total nitrogen that each of the excretory nitrogen compounds represents (Table 5) from excreta collected with a preservative, followed the same pattern as that reported by Humphreys (1985) (Table 34). That is, uric acid nitrogen was the largest excretory nitrogen compound followed in turn by ammonia, amino acid, urea, and creatinine nitrogen.

The contribution the individual excreta nitrogen compounds to a nitrogen correction factor for the TME assay were determined and are presented in Table 6. Uric acid nitrogen provided the largest energetic contribution to the correction factor. Amino acid nitrogen also made a considerable contribution to the correction factor, followed in turn by ammonia nitrogen. Urea nitrogen and creatinine nitrogen provided only a small contribution. These results were expected considering the results in Table 5 and the energetic values of the individual nitrogen compounds shown in Table 7. The correction factor determined was 37.57 kJ/gRN which is similar to the value of 37.70 kJ/gRN obtained by Humphreys (1985). However, it should be noted that Humphreys (1985) used an inflated value of 83.43 kJ/gN for the energetic value of ammonia nitrogen. Additionally, the energetic contribution of amino acid nitrogen was not considered. Using the correct value of 22.64 kJ/gN for ammonia nitrogen, the correction factor would have been 34.27 kJ/gRN which was lower than that reported in this experiment. The correction factor reported in this experiment (37.57 kJ/gRN) was also higher than the more commonly reported values of Hill and Anderson (1958) (34.39 kJ/gRN) and Titus (1956) (36.53 kJ/gRN) using full fed birds and excreta collected under normal conditions. The Hill and Anderson (1958) value was calculated only on the energetic value of uric acid nitrogen, on the assumption that uric acid is the sole nitrogen excretory compound. The value of Titus (1956), obtained by determining the gross energy of a sample of nondescript chicken

urine, was based on the fact that catabolism of body tissue protein produces urea, creatinine, ammonia, and other nitrogenous compounds in addition to uric acid.

II. EXPERIMENT 2. Examination and quantification of nitrogen compounds in poultry excreta collected with and without preservative and the contribution to a nitrogen correction factor for the TME assay.

The data presented in experiment 1 provided analysis of the levels of excretory nitrogen compounds present in excreta collected in a preservative. Experiment 2 was designed to determine the direct effect of a preservative on the nitrogen compounds in poultry excreta and the effect on a nitrogen correction factor for the TME assay.

Total excreta nitrogen was significantly lower in excreta collected with preservative than without preservative (Table 8). These differences are similar to those reported by Humphreys (1985). It is possible that the use of a preservative prevents the proliferation of microbial populations thereby preventing loss or change in excreta nitrogen due to microbial activity. Normally excreta is collected in bags which remain moist and at room temperature for 48 hours, conditions ideal for increased microbial activity. Early studies in excreta preservation by Flatt (1957), Jacobsen et al. (1959), and Rocks (1977) support this.

Although the level of uric acid nitrogen excreted was significantly higher from excreta collected without preservative (Table 8), the proportion of total nitrogen as uric acid nitrogen was similar between treatments. It has been suggested that excreta uric acid nitrogen may be decreased through the action of uric acid degrading bacteria present in poultry excreta (Barnes and Impey, 1974). The data from this experiment suggest that bacterial degradation of uric acid nitrogen may not be a factor. The higher occurrence of uric acid nitrogen from excreta collected without preservative is

likely a reflection of a higher level of total nitrogen. A similar effect was also shown by Humphreys (1985). The proportion as uric acid nitrogen in excreta collected without preservative from unfed birds is between that value reported by Waring and Brown (1965), Sykes (1971), and Humphreys (1985) (Table 34).

Excreta urea nitrogen was higher, although not significantly ($P>0.05$), in excreta collected without preservative (Table 8). The proportion of total nitrogen as urea nitrogen was slightly higher from excreta collected without preservative. In general, for both treatments the proportion of urea was low, which is consistent with the data of Sykes (1971) and Humphreys (1985). As a result, urea provides only a small energetic contribution and therefore changes which occur will have little effect on the nitrogen correction factor.

The excretion of ammonia nitrogen (Table 8) was significantly higher from excreta collected without preservative. Similarly, the proportion of total nitrogen as ammonia nitrogen was also higher from excreta collected without preservative. This is consistent with the data of Humphreys (1985). In part, the reduction in ammonia level may be due to the prevention, by the preservative, of microbial degradation of urea which results in the production of ammonia. The proportion of total nitrogen as ammonia nitrogen in excreta collected without preservative from the unfed birds was considerably lower than the value reported by Sykes (1971).

The excretion of creatinine nitrogen (Table 8) was also significantly higher from excreta collected without preservative. Similarly, the proportion of total nitrogen as creatinine nitrogen was also higher. These findings are contrary to those reported by Humphreys (1985). The proportion of total nitrogen as creatinine nitrogen in excreta collected without preservative from the unfed birds was also lower than the value reported by Sykes (1971). However, as reported by Teekell et al. (1968) and Sturkie

(1976), creatinine is excreted in negligible amounts. Therefore, changes in creatinine nitrogen will have little effect on the nitrogen correction factor.

Excreta total amino acid nitrogen was higher (although not significantly) in excreta collected without preservative (Table 8). However, the proportion of total nitrogen as total amino acid nitrogen was higher from excreta collected with preservative, suggesting that a preservative may influence amino acid nitrogen levels in poultry excreta. As mentioned previously, there have been no studies to determine excreta amino acid levels from poultry excreta collected in a preservative. The proportionate level of amino acid nitrogen collected without preservative from the unfed birds is considerably higher than that reported by both Sykes (1971) and Humphreys (1985). The effect of a preservative may be important since amino acid nitrogen has the highest energetic value of all of the excretory nitrogen compounds. Any changes which occur would therefore have a significant effect on the correction factor.

The contribution of the individual nitrogen containing compounds to a nitrogen correction factor for the TME assay was determined and are presented in Table 9. In both treatments uric acid nitrogen provided the largest energetic contribution to the correction factor, followed by total amino acid nitrogen. Ammonia nitrogen was the third largest energetic contributor, while both urea nitrogen and creatinine nitrogen provided only a small contribution. From the data presented in Tables 7 and 8, the correction factors determined were 37.33 kJ/gRN from excreta collected without preservative and 37.67 kJ/gRN from excreta collected with preservative. The correction factors are similar to the one determined in experiment 1. Between the two treatments, the nitrogen correction factors were almost identical. Although there is an effect of preservative on the proportions of the individual excretory nitrogen

compounds, the differences are negated by the resulting energetic contributions. As a result, the use of a preservative appears to have no effect on the nitrogen correction factor. The correction factors determined are higher than the values of 34.39 kJ/gRN proposed by Hill and Anderson (1958) and 36.53 kJ/gRN proposed by Titus (1956). The correction factors determined by Humphreys (1985), after adjusting for the incorrect energetic value for ammonia nitrogen (i.e. 22.64 kJ/gN not 83.43 kJ/gN), were 34.65 kJ/gRN from excreta collected without preservative and 34.27 kJ/gRN with preservative. These results also appear to substantiate a lack of effect of preservative on the nitrogen correction factor. These values were lower than those reported in this experiment. This is due to several factors. In addition to using an incorrect energetic value for ammonia nitrogen, Humphreys (1985) failed to include the energetic contribution of the amino acid nitrogen. The value of Hill and Anderson (1958) was calculated only on the energetic value of uric acid nitrogen, on the assumption that uric acid is the sole nitrogen excretory compound. The value of Titus (1956), was obtained by determining the gross energy of a sample of nondescript chicken urine, as opposed to investigating the specific energetic contributions of each individual excretory nitrogen compound. The correction factors determined in this experiment suggest that the use of the nitrogen correction factors determined by Hill and Anderson (1958), Titus (1956), and Humphreys (1985) underestimate the metabolic and endogenous nitrogen (and therefore energy) losses, and may be inappropriate for use in the TME assay.

III. EXPERIMENT 3. Effect of different feedstuffs, and of poultry excreta collected with and without preservative, on the proportion of excretory nitrogen compounds and the contribution to a nitrogen correction factor for the TME assay.

A. Effect of Preservative

The purpose of this experiment was to examine the effect of excreta collected in a preservative from birds force fed different samples of wheat. The results of excreta collected with and without preservative are shown in Tables 10 to 12. Essentially, the addition of a preservative had a similar effect among all of the treatment comparisons. In all comparisons, excreta total nitrogen was significantly higher from the excreta collected without preservative. As explained in experiment 1, the use of a preservative may be important in preventing changes in excreta nitrogen due to microbial activity by eliminating an environment suitable for microbial activity (Flatt, 1957; Jacobsen et al., 1959; Rocks, 1977).

There were no significant differences among any of the treatment comparisons in excreta uric acid nitrogen. The percentage of total excreta nitrogen as uric acid nitrogen was also similar, which supports the results of experiment 2 and those of Humphreys (1985) that bacterial degradation of uric acid nitrogen (Barnes and Impey 1974) is not a factor concerning the levels of uric acid nitrogen in excreta. The proportion of excreta total nitrogen as uric acid nitrogen falls within the ranges of those previously reported (Table 34).

As with excreta uric acid nitrogen, there was no significant effect of the use of a preservative on the levels of excreta urea nitrogen among any of the treatment comparisons. There was inconsistency in the proportion of total nitrogen as urea

nitrogen. In the treatments involving Marshall wheat and Glenlea wheat, a higher percentage as urea nitrogen occurred from excreta collected without preservative. In the treatment involving HY320 wheat a higher percentage as urea nitrogen occurred from excreta collected with preservative. Humphreys (1985), upon force feeding cracked wheat, observed no differences in the proportion of total excreta nitrogen as urea nitrogen. However, since urea provides only a small energetic contribution to the nitrogen correction factor, any changes resulting from the addition of a preservative to excreta will have little effect. Overall, the proportion of excreta total nitrogen as urea nitrogen is slightly lower than previously reported (Table 34).

The effect of a preservative on the levels of excreta ammonia nitrogen varied among the treatment comparisons. In the treatments involving Marshall wheat and Glenlea wheat, excreta ammonia nitrogen was significantly higher from excreta collected without preservative. In the treatment involving HY320 wheat, ammonia nitrogen levels were not significantly different, although the levels were still higher from the excreta collected without preservative. Among all of the treatment comparisons, the proportion of total nitrogen as ammonia nitrogen was higher from the excreta collected without preservative. These results are supported by those reported by Humphreys (1985). In part, the reduction in ammonia levels may be due to the prevention, by the preservative, of microbial degradation of urea which results in the production of ammonia. The proportion of excreta total nitrogen as ammonia nitrogen from the excreta collected without preservative falls within the ranges previously reported (Table 34).

As with excreta ammonia nitrogen, there were varied responses in excreta creatinine levels resulting from the addition of a preservative to excreta. In the treatment involving Marshall wheat, creatinine nitrogen was significantly higher from

the excreta collected with preservative. In the treatments involving Glenlea wheat and HY320 wheat, creatinine nitrogen was significantly higher from excreta collected without preservative. The differences are likely a result of biological variation. Humphreys (1985) reports that the excreta creatinine nitrogen level was higher from excreta collected with preservative. Teekell et al. (1968) and Sturkie (1976) report that creatinine is excreted in negligible amounts. As a result, changes in excreta creatinine nitrogen will have little effect on the nitrogen correction factor. The proportion of excreta total nitrogen as creatinine is generally lower than previously reported (Table 34).

The proportion of excreta total nitrogen as total amino acid nitrogen varied among treatment comparisons. In the treatments involving Marshall wheat and Glenlea wheat, a higher percentage as total amino acid nitrogen occurred from excreta collected with preservative. In the treatment involving HY320 wheat, a higher percentage as total amino acid nitrogen occurred from excreta collected without preservative. As with creatinine nitrogen the differences are likely a result of biological variation. The percentage of excreta total nitrogen as total amino acid nitrogen falls within the ranges, but is generally higher, than previously reported (Table 34).

The contribution of the individual nitrogen compounds to a nitrogen correction factor for the TME assay was determined and are presented in Table 19. In all treatments uric acid nitrogen provided the largest energetic contribution to the correction factor, followed by total amino acid nitrogen. Ammonia nitrogen was the third largest energetic contributor, while both urea nitrogen and creatinine nitrogen provided only a small contribution. Similar trends were observed in experiments 1 (Table 6) and experiment 2 (Table 9). These results were expected considering the

data presented in Tables 10 to 12 and the energetic values of the individual nitrogen compounds shown in Table 7. The correction factors between the excreta collected without preservative and with preservative were similar in all treatments. They were as follows: Marshall wheat 39.96 kJ/gRN and 40.47 kJ/gRN respectively; Glenlea wheat 39.47 kJ/gRN and 39.60 kJ/gRN respectively; HY320 wheat 40.27 kJ/gRN and 39.45 kJ/gRN respectively. Although there were variations in the proportions of the individual excretory nitrogen compounds, the differences were negated by the resulting energetic contributions. As a result, the use of a preservative appears to have no effect on the nitrogen correction factor. Similar results are reported in experiment 2 (Table 9) and by Humphreys (1985).

The nitrogen correction factors in this experiment (Table 19) are higher than those reported in experiment 2 (Table 9). The primary difference is due to an increase in the proportion of excreta total nitrogen (and thus contribution to the correction factor) as amino acid nitrogen from the birds force fed 25 g of test material. The values for excreta uric acid, urea, and ammonia nitrogen remained constant whether the birds were unfed (as in experiment 2) or force fed 25 g of test material. Variations may be dependent upon a number of factors. The differences may be due, in part, to the fact that the use of fasted roosters may not accurately estimate the endogenous losses of the fed birds due to the physiological stress derived from the starvation period. This is supported by both Dale and Fuller (1982) and Parsons et al. (1982a). Dale and Fuller (1982) stated that in fed birds, tissue catabolism is minimized to the extent that the feedstuff being tested is able to supply the required energy. This is supported by Sibbald and Wolynetz (1984b) who suggested that the energy/gram of nitrogen may not be constant under different conditions, implying that the proportion of energy containing nitrogenous compounds voided as a result of tissue catabolism,

varies. The increase in creatinine nitrogen and amino acid nitrogen supports these suggestions, while the consistency in the proportions of excreta uric acid, urea, and ammonia nitrogen do not. The contradictions may be explained as follows. Generally, in situations of low to zero feed intake, stored body fat and tissue protein are catabolized to meet the birds maintenance energy requirements. However, Siregar and Farrell (1980) state that there is no preferential utilization of body fat versus tissue protein. It is possible therefore, that the birds may be using stored body fat rather than tissue protein over the short period of starvation, causing excretions of the various endogenous nitrogen compounds to remain unaffected and constant. Additionally, it is also possible that the increase in excreta amino acid nitrogen may come from sloughed intestinal cells due to erosion of the intestinal mucosa resulting from feed passage or from an undigested portion of the feed. Regardless, the differences in the nitrogen correction factors determined in this experiment and experiment 2 suggest that individual correction factors for unfed and force fed birds should be applied.

The nitrogen correction factors determined (Table 19) were higher than those reported by Hill and Anderson (34.39 kJ/gRN) (1958) and Titus (36.53 kJ/gRN) (1956). The correction factors determined by Humphreys (1985) from birds force fed cracked wheat, after adjusting for the incorrect energetic value for ammonia nitrogen (i.e. 22.64 kJ/gN not 83.43 kJ/gN), were 32.76 kJ/gRN from excreta collected without preservative and 32.87 kJ/gRN from excreta collected with preservative. Although the values from Humphreys (1985) also substantiate a lack of effect of preservative on the correction factor, they are lower than reported in this experiment (Table 19). This suggests that the use of the correction factors determined by Hill and Anderson

(1958), Titus (1956) and Humphreys (1985) underestimate fecal and urinary nitrogen (and therefore energy) losses, and may be inappropriate for use in the TME assay.

B. Effect of feedstuff – Excreta collected with and without preservative.

The purpose of these comparisons were to determine the effect of different samples of wheat on the proportion of excretory nitrogen compounds and the resulting effect on a nitrogen correction factor for the TME assay. The samples of wheat were compared as follows: 1) HY320 wheat versus Marshall wheat; 2) HY320 wheat versus Glenlea wheat; 3) Marshall wheat versus Glenlea wheat. Each comparison was done from excreta collected with and without preservative. The results are shown in Tables 13 to 18. There were no significant differences within any of the treatment groups in the levels of, excreta total, uric acid, urea, ammonia, creatinine, and total amino acid nitrogen. However, consistent with the trend noted in experiment 2, the levels of excreta total nitrogen and the excretory nitrogen compounds were higher from the excreta collected without preservative. A lack of variation in excretory nitrogen (and thus energy) losses is supported by several authors (Sibbald and Price 1980; Sibbald 1981a; Ranaweera and Nano 1981; Sibbald 1982). The lack of significance may be due to the similarity in nutrient composition (Table 3a,b,c) between the samples of wheat. As a result the levels of excretory nitrogen compounds would be expected to be similar. Dale and Fuller (1982) state that in fed birds, tissue catabolism is minimized to the extent that the feedstuff being tested is able to supply the required energy. It is therefore likely that each of the samples were able to provide a similar nutritional contribution, affecting a similar physiological response between the birds. The proportion of excreta total nitrogen that each of the excretory nitrogen compounds represents were similar among all of the treatments. However, with the exception of

excreta ammonia nitrogen which was higher from excreta collected without preservative, there were no consistent trends between excreta collected with and without preservative.

The contribution of the individual nitrogen containing compounds to a nitrogen correction factor for the TME assay was determined and are presented in Table 19. among all of the treatments uric acid nitrogen provided the largest energetic contribution to the correction factor, followed by total amino acid nitrogen. Ammonia nitrogen was the third largest energetic contributor, while both urea nitrogen and creatinine nitrogen provided only a small contribution. These results were expected considering the data presented in Tables 13 to 18 and the energetic values of the individual nitrogen compounds shown in Table 7. The correction factors determined from excreta collected without preservative for Marshall, Glenlea, and HY320 wheat were 39.96, 39.47, and 40.27 kJ/gRN respectively. The correction factors determined from excreta collected with preservative for Marshall, Glenlea, and HY320 wheat were 40.47, 39.60, and 39.45 kJ/gRN respectively. Although there was variation in the proportion of the individual excretory nitrogen compounds, the differences were negated by the resulting energetic contributions. As a result, the variety of wheats tested appear to have no effect on the nitrogen correction factor. The similarities, as discussed previously, are likely a reflection of the similarities in nutrient composition of the samples of wheat.

IV. EXPERIMENT 4. Effect of different feedstuffs, and of poultry excreta collected with and without preservative, on the proportion of excretory nitrogen compounds and the contribution to a nitrogen correction factor for the TME assay.

A. Effect of Preservative

The purpose of these comparisons was to examine the effect of excreta collected in a preservative from birds force fed a feedstuff containing low (alfalfa meal), medium (wheat), and high (fish meal) levels of available amino acids. The results of excreta collected with and without preservative are shown in Tables 20 to 22. The only significant effect of a preservative was in the level of excreta ammonia nitrogen from the treatments involving fish meal (Table 20) and wheat (Table 22). In both cases excreta ammonia nitrogen was significantly higher from excreta collected without preservative than with preservative. In the treatment involving alfalfa meal (Table 21), ammonia nitrogen levels were not significantly different, however the levels were still higher from the excreta collected without preservative. There were no significant differences within any of the treatment groups in the levels of excreta total, uric acid, urea, creatinine, and total amino acid nitrogen. The lack of effect on excreta total nitrogen is inconsistent with that reported in experiments 2 and 3. This may be due to the nature of the feedstuff. Fish meal (Table 4a) has a high level of available protein which may be digested and excreted in varying levels from bird to bird. alfalfa meal (Table 4b), on the other hand, is a more fibrous feedstuff with a lower level of protein which is also more unavailable. Also, its physical nature may act to increase intestinal mucosal erosion and thus increase the loss of endogenous nitrogen. The lack of effect with wheat is inconsistent with the results of experiment 3, which

showed a significant effect of the preservative on excreta total nitrogen. It may therefore be possible that providing an exogenous (dietary) source of nitrogen may result in variable levels of total excreta nitrogen. A possible result may be to overshadow the effect of a preservative to prevent changes in excreta nitrogen due to microbial growth and activity. The lack of effect on excreta uric acid nitrogen again supports the observation that bacterial degradation of uric acid nitrogen (Barnes and Impey 1974) is not a factor concerning levels of excreta uric acid nitrogen.

The proportion of excreta total nitrogen as uric acid, urea, creatinine, and amino acid nitrogen were similar between excreta collected with and without preservative. The proportion as ammonia nitrogen was generally higher from excreta collected without preservative. The results again indicate that the preservative may play a role in the prevention of microbial degradation of urea which results in increased levels of ammonia. This is consistent with that reported in experiments 2 and 3 and by Humphreys (1985).

The proportions of excretory nitrogen compounds generally fall within the ranges previously reported (Table 34). The proportion as urea nitrogen and creatinine nitrogen are in the lower end of the ranges while the proportion as amino acid nitrogen from alfalfa meal is higher than reported.

The contribution of the individual nitrogen compounds to a nitrogen correction factor for the TME assay was determined and are presented in Table 29. In all treatments uric acid nitrogen provided the largest energetic contribution to the correction, followed by total amino acid nitrogen. Ammonia was the third highest energetic contributor, while both urea nitrogen and creatinine nitrogen provided only a small contribution. Similar trends were observed in experiments 1 (Table 6), 2 (Table 9) and 3 (Table 19). These results were expected considering the data

presented in Tables 20 to 22 and the energetic values of the individual nitrogen compounds shown in Table 7. The correction factors between the excreta collected without preservative and with preservative were similar in all treatments. They were as follows: fish meal, 39.01 kJ/gRN and 40.68 kJ/gRN respectively; alfalfa meal, 47.12 kJ/gRN and 47.15 kJ/gRN respectively; wheat, 39.86 kJ/gRN and 39.72 kJ/gRN respectively. Although there were slight variations in the proportions of the individual excretory nitrogen compounds, the differences were negated by the resulting energetic contributions. As a result, the use of a preservative appears to have no effect on the nitrogen correction factor. A lack of effect of preservative was also shown in experiments 2 (Table 9) and 3 (Table 19), and by Humphreys (1985).

With the exception of alfalfa meal (which will be discussed later), the nitrogen correction factors were similar to those reported in experiment 3 (Table 19). In both cases, the correction factors were higher than those for the unfed birds in experiment 2 (Table 9). The primary differences from experiment 2 were an increase in the proportion of excreta total nitrogen as creatinine nitrogen and amino acid nitrogen. The possible explanations for the differences are as explained previously in experiment 3. The differences in the nitrogen correction factors lend further support that individual correction factors for unfed and fed birds should be applied.

The nitrogen correction factors determined were again higher than the values of Hill and Anderson (34.39 kJ/gRN) (1958), Titus (36.53 kJ/gRN) (1956) and Humphreys (32.76 kJ/gRN and 32.87 kJ/gRN – see experiment 3) (1985). The values reported again suggest that the use of the correction factors determined by Hill and Anderson (1958), Titus (1956) and Humphreys (1985) underestimate fecal and urinary nitrogen (and thus energy) losses, and may be inappropriate for use in the TME assay.

B. Effect of feedstuff – Excreta collected with and without preservative.

The purpose of these comparisons were to examine the effect of different feedstuffs on the proportion of the excretory nitrogen compounds and the resulting effect on a nitrogen correction factor for the TME assay. The feedstuffs were compared as follows: 1) fish meal versus alfalfa meal; 2) fish meal versus wheat; 3) alfalfa meal versus wheat. Each comparison was done from excreta collected with and without preservative. The results are shown in Tables 23 to 28.

The largest number of significant differences occurred in the comparison of fish meal versus alfalfa meal. From excreta collected without preservative (Table 23), total, uric acid, and ammonia nitrogen were significantly higher from the fish meal. From excreta collected with preservative (Table 24), total, uric acid, ammonia, and creatinine nitrogen were also significantly higher from the fish meal.

The lowest number of significant differences occurred in the comparison of fish meal versus wheat. From excreta collected without preservative (Table 25), the only significant difference occurred with uric acid nitrogen which was higher in fish meal. From excreta collected with preservative (Table 26), the only significant difference occurred with creatinine nitrogen which was also higher in fish meal.

In the comparison of alfalfa meal versus wheat, total, uric acid, and ammonia nitrogen were significantly higher for wheat from excreta collected without preservative (Table 27). From excreta collected with preservative (Table 28), total nitrogen and uric acid nitrogen were also significantly higher for wheat.

The levels of excretory nitrogen compounds were generally variable showing no consistent trends between fish meal and wheat, whether the excreta was collected with or without preservative. The levels for alfalfa meal were generally lower. The

proportions of total nitrogen that each of the excretory nitrogen compounds represent did show consistency. The proportions as uric acid, urea, ammonia, and creatinine nitrogen were similar among feedstuffs whether excreta was collected with or without preservative. The only exception occurred with amino acid nitrogen. For fish meal and wheat the proportions were similar, whereas the proportion for alfalfa meal was considerably higher.

The contribution of the individual nitrogen containing compounds to a nitrogen correction factor for the TME assay was determined and are presented in Table 29. Among all of the treatments uric acid nitrogen provided the largest energetic contribution to the correction factor, followed by total amino acid nitrogen. Ammonia nitrogen was the third largest energetic contributor, while both urea nitrogen and creatinine nitrogen provided only a small contribution. These results were as expected considering the data presented in Tables 23 to 28 and the energetic values of individual nitrogen compounds shown in Table 7. The correction factors determined from excreta collected without preservative for fish meal, alfalfa meal, and wheat were 39.01, 47.12, and 39.86 kJ/gRN respectively. The correction factors determined from excreta collected with preservative for fish meal, alfalfa meal, and wheat were 40.68, 47.15, and 39.72 kJ/gRN respectively. With the exception of alfalfa meal, there was no effect of fish meal or wheat on the nitrogen correction factor. The correction factors for fish meal and wheat were also similar to the correction factors of the different samples of wheat in experiment 2.

The difference in the correction factor from alfalfa meal is likely due to several reasons. Relative to fish meal and wheat, alfalfa meal is a more fibrous feedstuff and lower in available amino acid content. Hallsworth and Coates (1962) originally suggested that the severity of erosion of the intestinal mucosa increased with the

intake of dietary fibre, thereby increasing fecal and urinary nitrogen (and thus energy) excretion. This effect has been reported by several authors (Mutzer and Slinger, 1980d, 1980e; Parsons et al., 1983; Parsons, 1984). These authors also reported an increase in endogenous amino acid excretion with increased dietary fibre. This is consistent with the results obtained from this experiment. The increase in excreta amino acid nitrogen may also be due to the unavailability of the amino acids. The result would be an increase in the undigested amino acid nitrogen content of the excreta. Since the energetic value of amino acid nitrogen is high, the result of a large increase in excreta amino acid nitrogen would be to greatly increase the energetic contribution of amino acid nitrogen, and thus inflate the value of the correction factor. This effect was observed in the comparisons involving alfalfa meal.

The effect of fish meal also requires discussion. Several authors (Teekell et al., 1968; McNabb and McNabb, 1975; Ward et al., 1975; Parsons et al., 1982b) have reported an increase in the various excretory nitrogen compounds following an increase in dietary protein. However, among comparisons with fish meal there were no significant effects on the excretion patterns of the excretory nitrogen compounds. In comparison with the unfed birds from experiment 2 its therefore possible (as discussed previously in experiment 3) that the birds are using stored body fat rather than tissue protein over the short period of starvation. Initially, the lack of differences observed among the samples of wheat in experiment 3 was attributed to the similarity in the nutrient composition. However, in light of the results of this experiment, it appears that a further explanation is required. Over short term periods of starvation, whether the birds remain unfed or are force fed a digestible test material, it is possible that the birds are able to maintain a physiological equilibrium with respect to excretory nitrogen compounds. The energetic contributions of the

excretory nitrogen compounds and the resulting correction factors through experiments 2, 3, and 4 support these suggestions.

V. EXPERIMENT 5. Effect of supplemental energy on the proportion of excretory nitrogen compounds and the contribution to a nitrogen correction factor for the TME assay.

There was no significant difference in total nitrogen excreted between unfed birds and birds force fed supplemental glucose (Table 30). In fact, the levels excreted between the two treatments were almost identical. This contradicts findings by both Dale and Fuller (1982) and Sibbald and Morse (1983b) that nitrogen excretion decreased with the provision of supplemental energy. Richardson et al. (1968) and Okumura et al. (1981) reported a decrease in nitrogen excretion as dietary energy levels were increased. These findings suggest that excreta nitrogen losses due to degradation of body protein to provide energy to meet the basal energetic needs can be reduced, if not eliminated, by the provision of dietary energy. The results were similar however to those of Sibbald (1975b; 1976a) who found that excretion levels were independent of energy intake. Similarly, Campbell et al. (1983) observed a lack of a trend towards either an increase or decrease in nitrogen and thus energy excretion. The lack of an influence of supplemental energy on nitrogen excretion draw support from Sibbald and Morse (1983b) who state that "there is an inevitable metabolic fecal plus endogenous urinary nitrogen loss that cannot be reduced by the provision of supplementary energy".

Excreta uric acid nitrogen levels did not change significantly with the provision of supplemental glucose (Table 30). However, the proportion of total nitrogen as uric acid nitrogen was higher in the unfed birds. The reduction in uric acid nitrogen

excretion through the provision of supplemental energy is in agreement with that reported by Richardson et al. (1968). Teekell et al. (1968) also observed comparatively lower uric acid nitrogen excretion from chickens fed a non-protein basal diet (Table 34).

The urea nitrogen excretion was significantly lower with the administration of supplemental glucose (Table 30). Thus, the proportion of total nitrogen as urea nitrogen was also lower. Richardson et al. (1968) observed similar trends on the effect of increased dietary energy. The proportion observed by Teekell et al. (1968) was higher than that identified in this experiment.

The provision of supplemental glucose resulted in significantly higher levels of excreta ammonia nitrogen and thus, a corresponding increase in the proportion of excreta total nitrogen as ammonia nitrogen (Table 30). Richardson et al. (1968) observed similar trends. The proportion of total nitrogen as ammonia nitrogen was considerably lower than that obtained by Teekell et al. (1968).

Excreta amino acid nitrogen (Table 30) was slightly higher when expressed as either the actual amount (although not significantly) or as a percentage of excreta total nitrogen upon administration of supplemental glucose. Richardson et al. (1968) observed a similar effect due to increased energy intake while Sibbald (1979a) observed no effect on amino acid nitrogen excretion. The percentage of total nitrogen as amino acid nitrogen was higher than that reported by Teekell et al. (1968).

Administration of supplemental glucose had no effect on the excretion of creatinine nitrogen. Also, the proportion of total nitrogen as creatinine nitrogen was similar between the two treatments. As reported by Richardson et al. (1968), creatinine nitrogen excretion appears to be relatively constant regardless of energy

intake. The percentage of total nitrogen as creatinine nitrogen from birds force fed supplemental glucose was considerably lower than reported by Teekell et al. (1968).

It is possible that the changes noted in the excretory nitrogen products may have resulted from a change in the nature of nitrogen catabolism due to the provision of supplemental glucose which is a non-protein, non-nitrogen containing feedstuff. This is supported by Dale and Fuller (1982) who state that tissue catabolism is minimized to the extent that the feedstuff being tested is able to supply the required energy. Sibbald and Morse (1983b) suggest that excreta nitrogen, and thus excreta energy losses, are dependent upon the nature of the material being catabolized to meet the energy requirements. However, the changes (i.e. variation) are consistent with the variation shown in previous experiments. Therefore, as explained previously, it is possible that the birds (whether unfed or force fed a digestible test material) may be able to maintain a physiological equilibrium with respect to the excretory nitrogen compounds over the short starvation period. In the case of unfed birds, it may be maintained through utilization of body fat. The supplemental glucose (1 gram of glucose provides 3.64 kcal metabolizable energy) provided approximately 20 percent of the energy required per day for maintenance. Therefore, in this experiment body fat utilization may, in part, have been spared by the supplemental glucose.

The contribution of the individual nitrogen containing compounds to a nitrogen correction factor was determined and are presented in Table 31. In both treatments uric acid nitrogen provided the largest energetic contribution to the correction factor, followed by total amino acid nitrogen. Ammonia nitrogen was the third largest energetic contributor, while both urea nitrogen and creatinine nitrogen provided only a small contribution. These results were as expected considering the results in Table 30 and the energetic values of the individual nitrogen compounds shown in Table 7.

The correction factors determine were 39.20 kJ/gRN for the unfed birds and 39.04 kJ/gRN for the birds force fed supplemental glucose. Between the two treatments, the nitrogen correction factors are almost identical. Although there is an effect of supplemental glucose on the proportions of the individual excretory nitrogen compounds, possibly due to biological variation, the differences are negated by the resulting energetic contributions. Therefore, it appears that the provision of supplemental glucose, at the level administered, has no effect on the nitrogen correction factor. The correction factors determined for both the unfed birds and the birds force fed supplemental glucose were higher than the correction factors determined by both Hill and Anderson (1958) (34.39 kJ/gRN) and Titus (1956) (36.53 kJ/gRN). This suggests that the correction factors proposed by Hill and Anderson (1958) and Titus (1956) underestimate the metabolic and endogenous nitrogen (and therefore energy) losses, and may be inappropriate for use in the TME assay.

VI. EXPERIMENT 6. Effect of cecectomy on the proportion of excretory nitrogen compounds and the contribution to a nitrogen correction factor for the TME assay.

The function of the microflora in the gastrointestinal tract is considered both important and controversial. One of the major sites of microbial activity is in the ceca. The ceca is involved in protein digestion and non-protein absorption and therefore may be important in the excretion of nitrogen compounds in poultry excreta. The degradative ability of microbes in the ceca is well documented (McNab, 1973; March, 1979). Experiment 6 was designed to evaluate the effect of the ceca on the excretory nitrogen compounds, and the resulting effect on the nitrogen correction factor.

Total nitrogen excretion was unaffected by cecectomy (Table 32). There were no significant differences in excreta total nitrogen between the intact and cecectomized birds, although the level excreted was slightly lower from the cecectomized birds. The lack of a significant effect on nitrogen (and thus energy) excretion was similarly reported by Kessler and Thomas (1981) and Parsons (1985).

There was no significant difference in the level of excreta uric acid nitrogen between the intact and cecectomized birds (Table 32). Although the level obtained was lower from the cecectomized birds, the percentage of total nitrogen as uric acid nitrogen was similar to the intact birds. It has been suggested that a decrease in excreta uric acid nitrogen may occur as a result of uric acid degrading bacteria of cecal origin being present in excreta samples (Barnes and Impey, 1974). However, the results from this experiment agree with those of Kese and March (1975) who reported a lack of effect of cecectomy on uric acid nitrogen excretion.

Excreta urea nitrogen was significantly lower from the intact birds (Table 32). The decrease in urea which occurred may have been the result of urea degrading bacteria of cecal origin in the excreta. Okumura et al. (1976) have reported the effect of urea degrading bacteria in the gastrointestinal tract of the chicken. In the ceca bacterial urease acts freely on urea. The end product of this bacterial urealysis is ammonia. In the intestine the ammonia would normally be absorbed and excreted as uric acid (Okumura et al., 1976). However, in the excreta, bacterial release of ammonia would result in an increase in the ammonia nitrogen content in the excreta. The data presented in Table 32 support this. Excreta urea nitrogen is significantly lower while excreta ammonia nitrogen is significantly higher in the intact birds. A similar effect is noted on the proportion of total nitrogen as urea nitrogen and ammonia nitrogen. Additionally, although there were considerable shifts in excreta

content of urea nitrogen and ammonia nitrogen, excreta total nitrogen content remained relatively similar.

Excreta creatinine nitrogen was similar between the intact and cecectomized birds (Table 32). Since creatinine nitrogen is a relatively high energy nitrogen compound, it would be expected that microbial degradation of creatinine would occur. However, the data in this experiment does not suggest this. Bacterial degradation of creatinine in the ceca has not been reported in the literature.

Amino acid nitrogen excretion was not significantly affected by cecectomy (Table 34). This contradicts reports by several authors (Rérat, 1978; Thomas, 1980; Kessler et al., 1981; Parsons, 1984; Parson, 1985) who reported that fasted, cecectomized birds excreted significantly higher levels of individual amino acid nitrogen. Rerat (1978) explains that in the fasted bird, proteolysis and deamination of amino acids are likely the primary mode of cecal microbial action as a result of a low level of fermentable carbohydrate present in the lower gut. Therefore, microbial action would explain the reduction of individual amino acid nitrogen by fasted, intact hens. The data presented in this experiment (Table 32) suggest otherwise. Although not significant, amino acid nitrogen excretion (both individual and total), and the proportion of excreta total nitrogen as amino acid nitrogen, were slightly higher from the intact birds.

The contribution of the individual nitrogen containing compounds to a nitrogen correction factor was determined and are presented in Table 33. In both treatments uric acid nitrogen provided the largest energetic contribution to the correction factor, followed by total amino acid nitrogen. Ammonia nitrogen was the third largest energetic contributor, while both urea nitrogen and creatinine nitrogen provided only a small contribution. These results were as expected considering the results in Table

33 and the energetic values of the individual nitrogen compounds shown in Table 7. The correction factors determined were 37.12 kJ/gRN for the intact birds and 36.30 kJ/gRN for the cecectomized birds. As shown the nitrogen correction factors between the two treatments were similar, with the correction factor for the intact birds being slightly higher. This was primarily due to a slightly higher proportion of excreta total nitrogen as ammonia nitrogen and amino acid nitrogen. Although there is an effect of cecectomy on the proportions of the individual excretory nitrogen compounds, the differences were essentially negated by the resulting energetic contributions. Therefore, cecectomy appears to have no effect on the nitrogen correction factor. The correction factors for both the intact and cecectomized birds were higher than the correction factor determined by Hill and Anderson (1958) (34.39 kJ/gRN). The correction factor of Titus (1956) (36.53 kJ/gRN) falls between the factors determined for the intact birds (37.12 kJ/gRN) and the cecectomized birds (36.30 kJ/gRN). In light of the previous experiments, this again suggests that the correction factors proposed by Hill and Anderson (1958) and Titus (1956) do not accurately estimate the metabolic and endogenous nitrogen (and therefore energy) losses, and may be inappropriate for use in the TME assay.

# SUMMARY AND CONCLUSIONS

Six experiments were designed to study some of the factors which may affect the levels of the excretory nitrogen containing compounds and thus the nitrogen correction factor used in the true metabolizable energy assay. Four of the six experiments were conducted to evaluate the effect of excreta collected in a preservative. Three of the six experiments evaluated the effect of different feedstuffs while one examined the effect of cecectomy. Examination of the data from these experiments has led to the following conclusions.

I. Proportion of nitrogen containing compounds in poultry excreta and their contribution to the nitrogen correction factor.

1. Uric acid nitrogen represents the greatest proportion (77.59–85.87%) of total excreta nitrogen but not the sole excretory nitrogen compound. The relative proportions of the additional nitrogen containing compounds were 6.96–12.99% for ammonia, 4.56–13.17% for amino acids, 0.60–2.94% for urea, and 0.18–1.08% for creatinine.

2. Uric acid nitrogen provides the largest energetic contribution to the correction factor. Amino acid nitrogen is the second largest energetic contributor, followed in turn by ammonia nitrogen. Urea nitrogen and creatinine nitrogen provide only a small contribution.

II. Effect of preservative (boric acid powder plus 0.3 percent w/w mercuric chloride) on the nitrogen correction factor.

1. There was a lack of effect of preservative on the nitrogen correction factor.

2. For unfed birds, the correction factor from excreta collected without preservative was 37.33 kJ/gRN and 37.67 kJ/gRN from excreta collected with preservative.

3. For birds force fed a sample of Marshall wheat, the correction factors from excreta collected with and without preservative were 40.47 and 39.96 kJ/gRN (experiment 3) respectively. For birds force fed a sample of Glenlea wheat, the correction factors were 39.60 and 39.47 kJ/gRN respectively. For birds force fed a sample of HY320 wheat, the correction factors were 39.45 and 40.27 kJ/gRN respectively.

4. For birds force fed a sample of fish meal, the correction factors from excreta collected with and without preservative were 40.68 and 39.01 kJ/gRN.

5. For birds force fed a sample of alfalfa meal, the correction factors from excreta collected with and without preservative were 47.15 and 47.12 kJ/gRN.

6. For birds force fed a sample of wheat (experiment 4), the correction factors from excreta collected with and without preservative were 39.72 and 39.86 kJ/gRN.

III. Effect of feedstuff on the nitrogen correction factor

1. With the exception of alfalfa meal, there was a lack of effect of feedstuff on the correction factor, regardless of whether the excreta was collected with or without preservative.

2. The correction factors for feedstuffs from excreta collected without preservative were as follows: Marshall wheat – 39.96 kJ/gRN; Glenlea wheat – 39.47 kJ/gRN; HY320 wheat – 40.27 kJ/gRN; fish meal – 39.01 kJ/gRN; wheat (experiment 4) – 39.86 kJ/gRN.

3. The correction factors for feedstuffs from excreta collected with preservative were as follows: Marshall wheat - 40.47 kJ/gRN; Glenlea wheat - 39.60 kJ/gRN; HY320 wheat - 39.45 kJ/gRN; fish meal - 40.68 kJ/gRN; wheat (experiment 4) - 39.72 kJ/gRN.

4. The increased value for the correction factor for alfalfa meal (47.12 kJ/gRN from excreta collected without preservative and 47.15 kJ/gRN from excreta collected with preservative) suggests that a separate correction factor be used for fibrous feedstuffs that are lower in available amino acid content.

IV. Effect of supplemental energy on the nitrogen correction factor

There was a lack of effect of supplemental energy (α D (+) glucose) on the correction factor. The correction factors for both the unfed birds and the birds force fed α D (+) glucose were similar. The values were 39.20 and 39.04 kJ/gRN respectively.

V. Effect of cecectomy on the nitrogen correction factor

There was a lack of effect of cecectomy on the correction factor. The correction factors for both the intact and cecectomized birds were similar. The values were 37.12 and 36.30 kJ/gRN respectively.

# GENERAL CONCLUSION

Generally, one of two nitrogen correction factors have been used when applying the nitrogen correction. Hill and Anderson (1958) proposed a value of 34.39 kJ/gRN, while Titus (1956) proposed a value of 36.53 kJ/gRN. In light of the results of this study, it is suggested that the values of Hill and Anderson (1958) and Titus (1956) underestimate the metabolic and endogenous nitrogen (and thus energy) losses.

Additionally, the nitrogen correction factors for the unfed birds and the birds force fed supplemental energy were lower than the nitrogen correction factors for the birds force fed the various feedstuffs. This suggests that specific nitrogen correction factors for unfed and force fed birds should be applied.

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