THE NATURE AND DETERMINATION OF METABOLIZABLE ENERGY

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ABSTRACT

Part 1 of this thesis involves a review along quantitative lines of investigations concerning metabolizable energy (ME) in poultry nutrition. A model for the mechanism of nitrogen (N) excretion is presented and examined in an experimental section comprising Part 2.

In Part 1, Chapter 1 is an exposition of the distribution and utilization of feed energy and raises the subject of additivity and questions of standardization of ME assay procedure. Chapter 2 consists of 2 sections. Section 1 provides a literature review of the bioassay determination of apparent metabolizable energy (AME). It covers an analysis of the nature of AME and explains basic concepts, provides mathematical definitions and perspectives and N corrected AME values (AME_n) are discussed. Further it describes methods of determination and provides an examination of the factors involved in AME variation. Section 2 deals with the nature of true metabolizable energy (TME) in which definitions and derivations of TME are provided, the relationship between TME and AME given, deviations from linearity of the energy excreted (EO) on energy input (EI) regression investigated and N corrected TME values (TME_n) discussed. Additionally, methods and evidence bearing on the central premise to TME are presented and other areas that have gained attention reviewed.

In Part 2, the subject of Chapter 3 is a linear experimental model developed by King (1984) to explain deviations in linearity of the relationship between N excreted (NO) and N intake (NI) as it may apply to adult cockerels and the nature of the correction of TME values to zero N balance (ZNB). Chapter 4 deals with 2 experiments, LN 202 and LN 204. The primary objective of Experiment LN 202 was to examine and investigate the regression relationship, EO on EI obtained for adult cockerels, and to assess the effect on it of correcting EO to ZNB. Experiment LN 204 was set up to study the impact of diet and assay procedure on TME of meat and bone meal (M & B) and to examine the effect on these values of correcting to ZNB. The effect of assay procedure on TME and TME_n of a whole diet was also explored.

In Experiment LN 202 2 slopes were obtained for the regression relationship, EO on EI, and on correcting EO to ZNB 1 slope satisfactorily represented the relationship. This was consistent with expectations arising from the model. The model suggests that this slope contains a bias element that causes TME values to deviate from unbiassed TME.

In Experiment LN 204 the TME values of M & B as determined by dietary inclusion and by direct supply were compared and assessed in terms of the model. Correction of the TMEs to ZNB resulted in a single value. TME assessment of a whole diet by 2 different assays resulted in similar values when the values, according to the model, estimated the same quantity and different values when the quantities measured were, as predicted by the model, different. Correction to ZNB caused like values to deviate and unlike values to come closer together.

Chapter 5 provides an overview of the model and the experimental findings and outlines the conclusions that have been drawn from this work.

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LIST OF ABBREVIATIONS

a_N	The intercept on Y of the slope for N excretion on N input.
ADE	Apparent digestible energy.
AME	Apparent metabolizable energy.
a_{Nm}	Nitrogen excretion constant arising from day to day wasting of N from the body. It is the result of normal maintenance activity. It is body weight related and independent of the food fed.
a_{Np}	Nitrogen excreted resulting from tissue protein catabolism that takes place for the purpose of supplying energy needs and takes place during starvation.
AME_b	AME obtained by bioassay.
AME_c	The conceptual measure of AME.
AME_n	Nitrogen corrected AME (commonly to zero N balance).
$AME_{(n)}$	Refers to AME or AME_n.
b	The slope for the subscript by which it is defined.
b_{Nr}	The N excreted per unit of N input over subsequent values of N input.
b_{Nna}	The proportion of N input that is not metabolized and is eliminated directly.
BE	Bioavailable energy.
BM	Basal metabolism.
BW	Body weight.
BMR	The standard or basal metabolic rate.
C.L.	Confidence limits.
Cr_2O_3	Chromic oxide.
DE	Digestible energy.
E	Energy.
EE	Refer to text for specific meaning: (i) Excreta energy. (ii) Endogenous energy.
EE_n	Energy excreted (by control birds) corrected for energy equivalent zero N balance.
EI	Energy input.
EO	Energy excreted.
EO_n	Energy excreted (by fed birds) corrected for energy equivalent zero N balance.

E_{NB}	Energy equivalent of N balance.
EO_s	Energy excreted over subsequent values of food intake.
Ea_{Nm}	The intercept of the energy of N excreted arising from normal maintenance activity.
Ea_{Np}	The intercept of the energy of N excreted arising from tissue protein breakdown during starvation.
FE	Faecal energy.
Fi	Weight of food residues.
FeE	The energy of microflora and microbial debris voided as faeces.
FeN	The N content of microflora and microbial debris voided as faeces.
FfE	The energy of food residues passaged through the gut.
FfN	The N content of food residues passaged through the gut.
FiE	The energy of food residues.
F_{ir}	Weight of excreta of food residues remaining which are eliminated via the gut or the urine.
FmE	Energy of products of the gut specified as cells of the gut wall, bile mucous and unabsorbed digestible juices and gas.
FmN	The N content of the gut specified as cells of the gut wall, bile mucous and unabsorbed digestible juices and gas.
FMR	Fasting metabolic rate.
FuE	The energy of materials absorbed across the intestine and excreted directly in the urine without undergoing metabolic change.
FuN	The N content of materials absorbed across the intestine and excreted directly in the urine without undergoing metabolic change.
F_{ign}	Weight of N containing products of the food not absorbed and passaged via the gut.
F_{iun}	Weight of N products that are absorbed across the gut wall and excreted directly in the urine without undergoing metabolic change.
$F_{ir}E$	Energy of excreta of food residues remaining which are eliminated via the gut or the urine.
$F_{ign}E$	Energy of N containing products of the food not absorbed and passaged via the gut.

$F_{iun}E$	Energy of N products that are absorbed across the gut wall and excreted directly in the urine without undergoing metabolic change.
Fm+eE	FmE + FeE.
GE	Gross energy.
GEa	The sum of energy not lost as energy of N excretion products in the urine resulting from metabolism of food absorbed, GEr, and that eliminated by the process UiE.
GEr	Energy not lost as energy of N excretion products in the urine resulting from metabolism of food absorbed.
HI	Heat increment.
HP	The total heat production of an animal consuming food in a thermally neutral environment.
HBC	Heat to keep body cold.
HBW	Heat to keep body warm.
LCT	Lower critical temperature.
ME	Metabolizable energy.
M & B	Meat and bone meal.
N	Nitrogen.
NB	Nitrogen balance.
NI	Nitrogen input.
NO	Nitrogen excreted.
NE_m	Maintenance energy.
NE_p	Production energy.
NE_w	Energy of work.
NO_i	Nitrogen excreted over initial values of N fed or input.
NO_s	Nitrogen excreted over subsequent values of N fed or input.
NI_{ge}	Nitrogen input per unit of food over gross energy input per unit of food.
NE_{m+p}	Net energy for body maintenance and for production purposes.
PRC	Poultry Research Centre.
r^2	Correlation.
S	Standard deviation.
S.E.	Standard error of the mean.
TME	True metabolizable energy.
TME_b	Biassed TME values.

TME_h	TME obtained over high food intake levels (equivalent to TME_s).
TME_L	TME at level of energy intake stated, e.g. TME_{L3} = TME at level 3.
TME_l	TME obtained over low food intake levels (equivalent to TME_{in}).
TME_n	TME corrected to zero N balance.
TME_o	Biassed TME values.
TME_s	TME for subsequent values of food intake or as obtained from the subsequent slope (equivalent to TME_h).
TME_u	Unbiassed TME values.
TME_{in}	TME for initial values of food intake or as obtained from the initial slope (equivalent to TME_l).
TME_{Ni}	TME corrected for N over initial values of food intake.
TME_{Ns}	TME corrected for N over subsequent values of food intake.
TME_{ZNB}	TME corrected to zero N balance.
$TME_{N_{UmE}}$	TME corrected for energy of N arising from UmE.
$TME_{N_{UmE+UeE}}$	TME corrected for energy of N arising from both UmE and UeE.
UE	Urinary energy.
UCT	Upper critical temperature.
UeE	Energy equivalent of N by-products in the urine resulting from the day to day wasting of N due to maintenance activity.
UeN	The N content of by-products in the urine resulting from the day to day wasting of N due to maintenance activity.
UiE	The energy of N excretion products in the urine resulting from metabolic breakdown of absorbed food.
UmE	Energy equivalent of N by-products in the urine resulting from tissue catabolism and evident under starvation conditions.
UmN	The N content of by-products in the urine resulting from tissue catabolism and evident under starvation conditions.
VAE	Energy of voluntary activity or activity increment.
ZNB	Zero N balance.
ZNR	Zero N retention.
Δ	Change in.

PART 1

CHAPTER 1

INTRODUCTION

The term "energy" derives from a combination of 2 Greek words: en, meaning "in" and ergon, meaning "work" (Scott et al., 1982). In the physical sciences, energy is the capacity of a body or system to do work or it is the measure of this capacity.

The nutritive value of a feed is normally expressed in terms of nutrients such as carbohydrates, fats, proteins, minerals, vitamins and water. Energy is not a nutrient but an important function of some nutrients. It is the substance that life processes use to support functions of maintenance, growth and reproduction.

Energy is stored in the carbohydrates, fats and proteins of foods. Carbohydrates and fats are main sources of energy. Proteins supply energy as well, but they are usually provided in diets to suppply amino acids (Schaible, 1970).

The gross energy (GE) of a feed indicates the total amount of energy present in a substance. It is obtained by measuring the heat produced when a substance is burned completely in the presence of oxygen. In animal nutrition it is commonly measured in an adiabatic oxygen-bomb calorimeter. The unit of measure of energy is joule (J) or calories (C). One kilocalorie (kcal) is equal to 4.185 kilojoule (kJ) or 1 kJ is equal to 0.239 kcal (Scott et al., 1982). However, as reported by Blaxter (1970), the International Union of Nutritional Sciences at a symposium on energy metabolism in Switzerland accepted the joule as the single unit of energy in nutritional work. Nevertheless, the joule has not yet met with universal acceptance (Kleiber, 1975).

The total energy value of a feedstuff is not used entirely by the animal. Energy losses occur in the digestion process and heat is generated in the assimilation of absorbed material. The distribution and utilization of feed energy has been partitioned by Vohra (1972), Farrell (1974) and Sibbald (1982a). The partition of Vohra (1972) is given in Fig. 1.1.

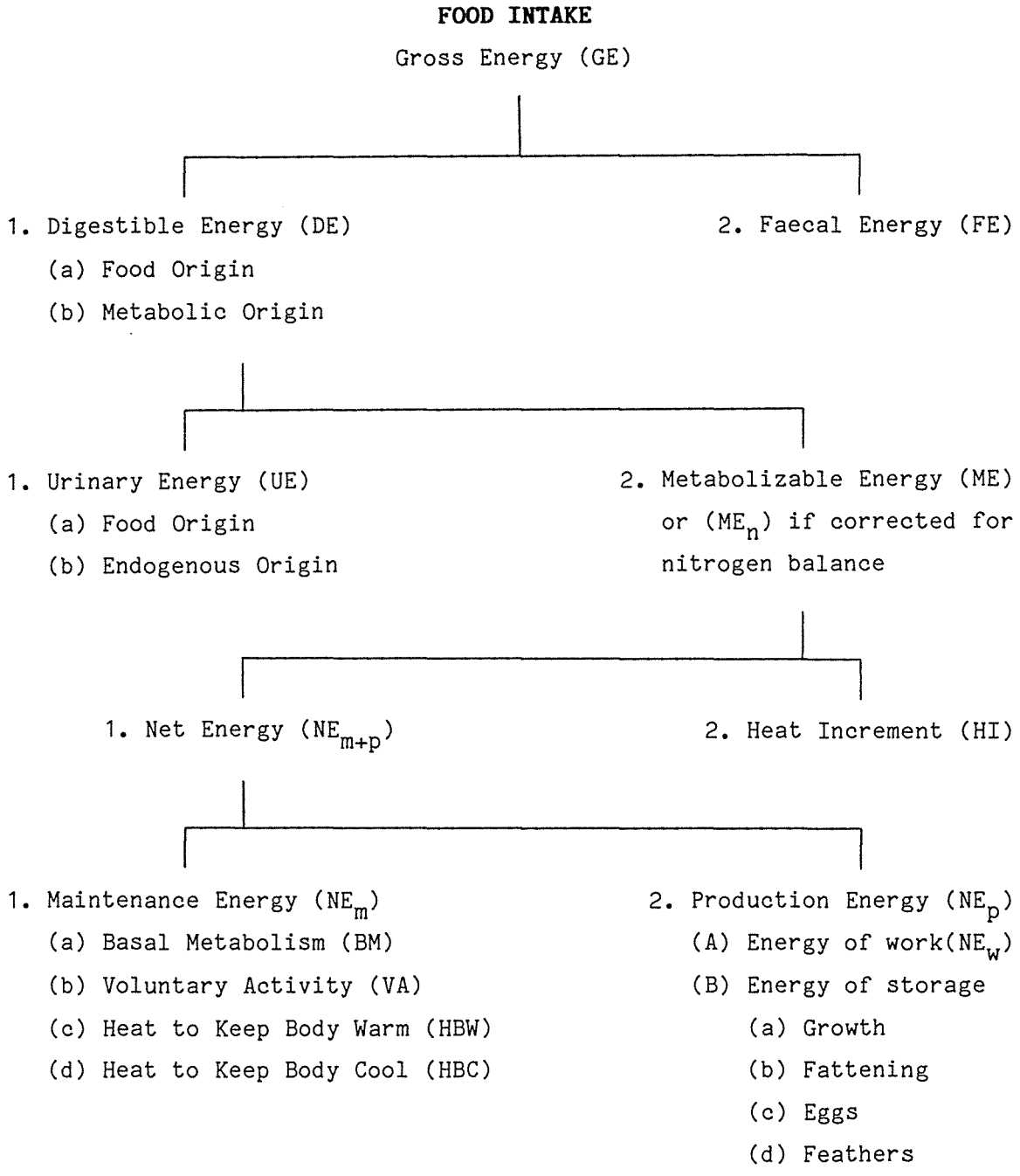


Fig. 1.1 The partition of gross energy of food (Vohra, 1972).

This partition illustrates measures of available energy, viz. digestible, metabolizable, net and productive energy. This thesis is concerned with measures of metabolizable energy (ME) in the context of poultry nutrition. It is a subject of considerable nutritional interest that in recent years has led to the development of tables of feed values. Metabolizable energy is one of a number of potential systems that yield estimates of the amount of energy in foodstuffs, that when eaten, becomes available to support in this case, growth, maintenance and production functions.

A basis for the use of ME is the assumption that the ME of a diet is the sum of MEs contributed by the dietary constituents. The accuracy of MEs quoted for diets, the formulation of ME levels of diets and the daily intake of ME of birds tied to feed out schedules all rely on the degree to which this assumption is realized. When whole diets are assayed for ME and the value obtained compared with the value predicted from summing constituent values, ME measurements can result in large discrepancies. For example, for a recent set of broiler breeder rations true metabolizable energy (TME) values obtained at the Poultry Research Centre (PRC) in kcal/kg were:

Diet	Calculated	Assayed	S.E. of mean
Starter	3081	3195	28.8
Grower	2980	3002	18.5
Layer	2980	3144	35.7

and only in the layer diet was tallow, a product known to cause deviations in additivity, included, and then at 0.5%. A consequence was that the birds received more TME than anticipated which it was conjectured was probably responsible for their greater than planned weight gain in lay and higher than necessary feed costs.

On the other hand, a large body of evidence has accrued at the PRC to suggest that for many diets a close relationship exists between calculated and assayed values. Such deviations that do occur may well

reflect a situation ever present in the feed industry of TME values used for calculation originating from different sources or batches of material to those materials making up diets in this case assayed diets. A list of calculated and assayed TME values are presented in Table 1.1 for complete diets as obtained from the PRC's "Annual Summary of Final Reports and Current Research" for the years 1978, 1979 and 1981 under Edmundson et al. (1978), Edmundson (1979) and Edmundson (1981). In this case the correlation between assayed whole diet values (X) and that calculated from summing ingredient components of whole diets (Y) was 79.0%. The regression equation:

$$Y = 2.47 + 0.812X$$

indicated that calculated values understated assayed values by a factor of 0.812.

An underlying objective of ME investigations has been the identification of measurements that result in improved additivity. This alone however is very much a simplification of the issue for additivity also depends on the updating of values as new season or batch of materials come to hand and the standardization of assay procedures so that sets of values can be established in which the MEs are related in as many of the variables known to affect additivity as is possible. Such variables include stock, age, size, temperature conditions and diets to name the more obvious and controllable elements. Additivity depends then also on standardization and routine examination and in circumstances in which funds and staff are limited, procedural simplicity becomes a necessary requirement. Without it the work does not get done.

The search for improved assay techniques or measures involves complex and drawn out procedures. The issues are basically these. What does the measure define? With what elements does it vary? Why does it vary and what is the mechanism? How can it be controlled? What does the new measure define? With what element does it vary? etc. This cycling is evident in the ME system of measurement which today comprises 2 measures, apparent metabolizable energy (AME), and TME, the latter a

Table 1.1

Calculated and Assayed TME Values of Whole Diets

Report	Diet	TME (Calculated) kJ/g	TME (Calculated) kcal/kg	TME (Assayed) kJ/g	TME (Assayed) kcal/kg	S.E. of mean kJ/g	95% C.L. ± kcal/kg
1978	Silo layer	12.18	2910	12.56	3000	-	-
"	Grower B	12.73	3041	12.34	2948	-	-
"	P/7/B	12.98	3101	13.05	3117	-	-
"	L/100	12.82	3061	13.16	3144	-	-
"	104/K	12.10	2891	11.90	2842	-	-
"	106/A	12.76	3047	12.56	3000	-	-
"	L/108/A	11.38	2717	11.31	2702	-	-
"	L/108/B	11.29	2696	11.36	2713	-	-
"	CN/162/Grower	12.94	3090	12.91	3084	-	-
"	CN/162/Layer	12.56	3000	12.64	3020	-	-
1979	L/110/A	11.93	2850	12.38	2957	-	64
"	L/112/A	13.57	3242	12.99	3103	-	39
"	L/112/B	12.98	3101	12.61	3013	-	33
"	L/112/C	12.44	2972	11.80	2818	-	45
"	L/112/D	11.95	2853	11.36	2713	-	69
"	L/112/E	11.48	2743	10.92	2608	-	73
"	CN/174/P/St	14.12	3373	13.95	3332	-	46
"	CN/174/Q/St	14.53	3470	15.07	3599	-	156
"	CN/174/R/St	14.57	3481	14.58	3482	-	43
"	CN/174/S/St	13.99	3341	14.16	3382	-	36
"	CN/174/U/St	13.94	3330	13.97	3336	-	62
"	CN/174/U/F	13.94	3330	13.82	3302	-	59
"	CN/174/PQ/F	14.69	3509	13.88	3314	-	39
"	CN/174/R/F	14.06	3358	14.37	3432	-	38
"	CN/174/ST/F	14.58	3482	13.74	3282	-	48
1981	L/133/A	12.98	3100	13.30	3176	0.12	-
"	L/133/B	12.98	3100	13.34	3186	0.18	-
"	L/133/C	12.98	3100	13.14	3138	0.16	-
"	L/133/D	12.98	3100	13.60	3247	0.17	-
"	L/135/A	12.98	3100	13.09	3126	0.08	-
"	L/135/B	12.98	3100	13.17	3146	0.27	-
"	L/135/C	12.98	3100	13.62	3253	0.06	-
"	L/135/D	12.98	3100	13.97	3336	0.11	-
"	L/148/A	12.98	3100	13.21	3155	0.12	-
"	Silo Layer	12.98	3100	12.08	2885	0.09	-

Source: Edmundson *et al.* (1978), Edmundson (1979) and Edmundson (1981).

development of the former. Developments in each have led to a variety of assay procedures some of which measure different quantities. An object of this thesis is to quantify the issues involved, to clarify what is being measured and to explore how additivity may be being influenced.

The question of additivity remains however a vexed one for always present is the prospect that the composition of diets affects the way constituents are metabolized and hence their ME values. This however is just part of a larger perspective, that additivity may vary with circumstances. That is, the proportion of change in ME between ingredients in a set may vary with each variable such as environmental temperature or age. This is likely and in the ME system no adjustment has been demonstrated or advocated that will control this feature. This means that that which is additive in one situation may not be in another. A part solution is to create one situation, one set of circumstances and work towards methods or measures that yield high levels of additivity within that. Hence standardization of assay procedure and environment is an essential feature of ME determination. The influence of dietary composition however remains uncontrolled. This influence, on the other hand, in many circumstances does not appear to be large and often is not evident.

Outside of these qualifications investigations of the ME system has led to considerable progress particularly in respect to understanding mechanisms and developing simpler assay techniques and has resulted in what is expected to be improved sets of values and increased accuracy in dietary ME prediction.

So far we have only briefly alluded to the function of ME as an element in the partition of feed energy. In this perspective absolute or unbiassed values are required if the value of elements in the partition when summed are to conform to the partition total. This requirement is for instance pertinent to the determination of the energy requirements of poultry.

Thus the situation exists where on the one hand we need ME measures that conform to the requirement for additivity but which do not necessarily have to conform strictly to ME as defined and for other purposes values whose usefulness depends entirely on the degree to which they define an absolute value.

As a prelude to discussions on the ME system of measurement, the remainder of this chapter is devoted to a description of the distribution and utilization of feed energy. It is intended in this way to bring into clear focus the nature of ME relative to other partition components some of which have been advocated as alternatives for feed energy evaluation in poultry nutrition.

1.1 Faecal Energy (FE)

Faecal energy is the gross energy of the faeces. It contains the energy of the undigested portion of feed together with materials of the digestive tract termed by Harris (1966) materials of metabolic origin.

1.2 Digestible Energy (DE)

Animals do not absorb all the feedstuffs they consume. The unabsorbed portion is voided as faeces. The difference GE - FE is the apparent digestible energy (ADE). The term "apparent" is used because faeces contain, apart from the energy of feed residues, a metabolic fraction consisting of bile, mucosal cells, unabsorbed intestinal secretions, gut flora and microbial debris (Sibbald, 1982a; Wolynetz and Sibbald, 1984). Because birds pass faeces and urine simultaneously it is difficult to separate these 2 components satisfactorily and energy is usually determined on excreta. Therefore, ADE is not a practical energy evaluating system using the avian species (Farrell, 1974).

1.3 Urinary Energy (UE)

The energy of urine according to the partition of Sibbald (1982a) comprises 2 parts. The energy content of the nutrients absorbed across the gut wall and excreted without having been catabolized -- they are of food origin, and the energy contained in the endogenous fraction which is the product of tissue catabolism. According to King (1984), there is a third component, the energy of by-products arising from assimilation of absorbed food.

1.4 Metabolizable Energy (ME)

Metabolizable energy is the total energy lost in urine and faeces subtracted from the gross energy of the feed ingested, the balance left being at the disposal of the animal. This is more aptly referred to as AME as the excreta contains endogenous material together with that derived from the feed. If these values are corrected for endogenous and metabolic energy losses they are referred to as TME.

1.5 Heat Increment (HI)

After food is consumed there is an increase in heat production above that observed during starvation. This increase is referred to as the heat increment (HI) of the diet (Crampton and Harris, 1969). It is measured in a thermally neutral environment. The ambient temperature, or range of temperatures, at which the metabolic rate is at a minimum is defined as the environment of thermal neutrality and is bounded by the upper and lower critical temperature (Freeman, 1971b).

The HI consists of the heat produced by microbial fermentation, the heat of digestion and absorption, the heat of product formation and the heat of waste formation and excretion (Sibbald, 1982a). The HI which is directly related to the amount of food ingested and characteristic of the type of food consumed (Ricklefs, 1974) is usually considered to be waste but it may be used to warm the animal as part of the net energy required for maintenance when the environmental temperature falls below the critical temperature (Harris, 1966). The critical temperature is the environmental temperature below which heat production of a fasting, quiescent animal will increase to prevent lowering of body temperature (Sibbald, 1982a).

However, the energy lost as HI is usually neglected in avian balance experiments but in ruminants the loss can be significant and is treated as a metabolic loss (Sibbald, 1982a).

Similar terms for HI are calorigenic effect, thermogenic action and specific dynamic action (Harris, 1966).

The HI of a diet can be determined by subtracting the heat production of the animal when fasting [the standard or basal metabolic rate (BMR)] from the heat production of an inactive animal when fed (the resting metabolic rate) or by feeding the animal at 2 or more levels of feed intake and calculating the difference in heat production (McDonald et al., 1981).

1.6 Net Energy (NE_{m+p})

When the ME of a food is corrected for HI, what remains is the net energy (NE_{m+p}). This is available to the bird for body maintenance (NE_m) and for production purposes (NE_p).

1.7 Maintenance Energy (NE_m)

Maintenance energy is the portion of the net energy needed to keep the animal in energy equilibrium -- the net energy required to replace the energy expended while carrying on "maintenance" life processes. In this state, there is no net gain or loss of energy in the body tissues (Harris, 1966). The major NE_m expense is that of basal energy metabolism. According to Brody (1945) it accounts for about 85% of the NE_m metabolism.

1.7.1 Basal Metabolism (BM)

Basal metabolism is the chemical change that occurs in the cells of an animal in the fasting and resting state when it uses just enough energy to maintain vital cellular activity, respiration, secretion and circulation as measured by the standard metabolic rate (Crampton and Harris, 1969).

The standard or basal metabolic rate (BMR) refers to the heat production per unit time determined when the animal is in a post-absorptive state, at rest, and maintained within the thermoneutral zone of environmental temperature (King and Farner, 1961). In adult fowl post-absorptive state is reached after about 48 h starvation (Mitchell and Haines, 1927 and others as cited by Freeman, 1971b).

However, Aschoff and Pohl (1970b) as cited by King (1974) observed that there were significant differences in BMR of resting birds measured between day and night. Measurement of the standard metabolic rate made during the day or normal active period (the experimental birds need to be in quiescent and wakeful) is sometimes referred to as the fasting metabolic rate (FMR) while when determined at night or during the normal sleeping period as BMR.

On the other hand, determination of the metabolic rate of the fully-fed but inactive bird in a thermally neutral environment is known as the resting metabolic rate (Freeman, 1971b).

Both BMR and the resting metabolic rate are determined using respiratory exchange (R.Q) techniques or direct calorimetry methods.

The BMR has been measured for animals of many different sizes. The BMR (kcal/h) of large animals is greater than that of small ones while BMR per unit body weight (kcal/g/h) is less in large animals (Whittow, 1976).

The relationship between BMR and body weight is thus exponential rather than linear (King and Farner, 1961):

$$M \propto W^n = kW^n$$

where M is the BMR in kcal/24h, W is the body weight in kg, and n is a constant ranging from 0.67 -- 1.00 (Zeuthen, 1953) standardized at 0.75 (Kleiber, 1965).

The equation, $M = 70W^{0.75}$, derived by Brody and Kleiber, has been generally found most appropriate by nutritionists to estimate BMR for all adult homeotherms (Crampton and Harris, 1969). However, Aschoff and Pohl (1970a,b) as cited by Calder and King (1974) reported that the BMR of birds can be expressed by the equation $M = 114.8W^{0.726}$ for passerine birds, and $M = 73.5W^{0.734}$ for nonpasserines whereas for FMR the equations $M = 140.9W^{0.704}$ and $M = 91.0W^{0.729}$ were described for passerines and nonpasserines respectively.

1.7.2 Energy of Voluntary Activity (VAE)

Energy of voluntary activity or activity increment (VAE) is the quantity of energy needed to sustain a certain unavoidable amount of activity by animals that are at "maintenance" living. Such activities may include getting up, lying down, standing, moving about to obtain food and drinking (Crampton and Harris, 1969).

The VAE is commonly expressed as a percentage of BMR. It may also be expressed in terms of body weight (Mitchell, 1962). However VAE was reported to be directly and linearly proportional to body weight in contrast to the case for BMR (Brody, 1945). Under normal conditions in chickens VAE amount to about 50% of the energy needed for basal metabolism (Scott et al., 1982).

1.7.3 Heat to Keep Body Warm (HBW)

Heat to keep body warm is the additional heat required to keep the animals body warm when the environmental temperature falls below the lower critical temperature (LCT). Below LCT, all other things being equal, heat loss exceeds heat production. Consequently catabolic rate is increased to prevent a fall in core temperature (Freeman, 1971a).

The heat increment, in total or in part, can be used for this purpose.

1.7.4 Heat to Keep Body Cold (HBC)

Heat to the keep body cold is the extra energy expended by the animal when the environmental temperature rises above its upper critical temperature (UCT). Above UCT sensible heat loss (the heat loss to the environment by a combination of the process of radiation, convection and conduction) is reduced. The bird relies increasingly on the insensible heat dissipating mechanism, a process of evaporation which is largely respiratory evaporation (cutaneous evaporation being negligible because the bird does not possess sweat glands), in order to maintain its body temperature within its normal range. As a result respiratory rate increases (Freeman, 1971a).

The total heat production (HP) of an animal consuming food in a thermally neutral environment = HI + BMR + VAE. (Crampton and Harris, 1969). It is measured by either direct or indirect calorimetry.

1.8 Production Energy (NE_p)

Production energy is the fraction of NE available after maintenance energy (NE_m) requirements have been satisfied. It consists of 2 portions. One portion represents the energy expended in work or in pleasurable activities that exceed the involuntary activity of maintenance. This energy expenditure is irreversible and if the animal persists in activity requiring energy in excess of maintenance without consuming adequate energy components in the diet, it will lose body weight.

The other portion is the energy stored in tissues, such as in growth or fattening, or for the synthesis of products like eggs, feathers, etc. The energy of these latter products is permanently lost to the body, but the energy stored in tissues can be reused by catabolizing the tissues, as happens when intake is below the maintenance requirements (Crampton and Harris, 1969).

CHAPTER 2

METABOLIZABLE ENERGY

In the early literature the terms metabolizable energy (ME) and apparent metabolizable energy (AME) were interchangeable and used synonymously, the prefix "apparent" giving recognition to the fact that AME was a measure of energy lost in faeces and urine of an endogenous origin additional to that directly attributable to food. The development of the entity, true metabolizable energy (TME), has increasingly resulted in ME being described as AME in part perhaps to remove ambiguity but also in part in recognition of its descriptive nature. As both AME and TME are closely related in the sense that both are a measure of the dietary energy remaining following digestion, the term "ME" in the context of this thesis will be used as the root name of the energy system of measurement embracing both AME and TME. Thus where an author has used ME the term AME will be substituted where the intended meaning is unaltered.

SECTION 1 APPARENT METABOLIZABLE ENERGY

The AME of a foodstuff is defined as the gross energy of the food fed less the energy lost as faeces, urine and combustible gases (Harris, 1966, as cited by McNab and Fisher, 1981). Conversely it is a measure of the energy of food remaining following digestion and partitionable into heat increment and maintenance and production functions. Mathematically it has the following form:

$$AME = GE - (E_{faeces} + E_{urine} + E_{gas})$$

The AME values commonly are presented adjusted for zero nitrogen (N) retention and termed AME_n. The purpose of this correction is to reduce the variation in AME attributed to dietary, bird, food intake and environmental effects.

The AME is evaluated directly by feed assay but estimates are also obtainable from equations relating chemical composition to coefficients of combustion and digestibility. Direct assays require the heat of combustion of representative samples of the food and excreta and methods for determining the weight of excreta eliminated per unit of food intake. The "conventional" approach involves use of both reference and test diets, an acclimatization period and a period over which excreta is collected. The acclimatization and collection periods extend over a number of days. More recently "rapid" assay methods have been developed. These involve a food withholding period, a brief interval during which test material is fed and an excreta collection period of 24 h to 32 h.

The quantitative determination of excreta voided is obtained either by recovering the total excreta voided or by estimating the total recovery by testing representative samples of the excreta for the presence of an inert indicator fed at a known concentration in the test diet.

The AME values not corrected for N and determined by the conventional approach have frequently been referred to as "classical" or non corrected ME.

Since the development of techniques for determination of AME (Hill and Anderson, 1958; Hill et al., 1960) a system of food values based on AME has been developed and forms the basis around which the energy requirements of poultry are framed. For feed formulation purposes AME values are assumed to be additive and independent of dietary composition. This assumption is subject to qualification. Considerable variation in AME ingredient values exist. The cause of this variation is attributed in part to differences in chemical composition but differences in methods of determination, laboratory techniques, methods of excreta collection together with such factors as age, strains and species of bird have been implicated. As Miller (1974) notes, the value of a food is not constant but a variable factor, affected by all variables influencing digestion and assimilation of nutrients. Accordingly AME is situation specific and the most reliable estimate of its value is by direct assay.

The AME has been subject to considerable scrutiny. Methods and procedures have been critically examined by Rao and Clandinin (1970), Vohra (1972) and others as cited by Miller (1974) and most recently by Sibbald (1975,1982a), Farrell (1981), McNab and Fisher (1981) and Wolynetz and Sibbald (1984). In addition to factors mentioned above AME has been shown to vary according to length of the acclimatization period, nature of the reference diet, test food intake levels and levels of test material substitution and some investigations have cast doubt on the reproducibility of values.

This section provides a literature review of the bioassay determination of AME. It is broadly divided into 3 parts, an analysis of the nature of AME, a descriptive section on methods of determination and an examination of the factors involved in AME variation.

2.1 THE NATURE OF APPARENT METABOLIZABLE ENERGY

2.1.1 Point Perspective

The conceptual measure of AME (AME_c) is described by the partition of energy illustration Fig. 1.1 and defined in words by Harris (1966) as cited by McNab and Fisher (1981) as the gross energy of the food less the energy lost as faeces, urine and combustible gases. Conversely it is the energy value of food remaining after allowance for excretion products that is partitionable into heat increment and maintenance and production functions. Quantitatively this is represented as:

$$AME_c = GE_{food} - (E_{faeces} + E_{urine} + E_{gas})$$

$$GE - (E_{faeces}+E_{urine}+E_{gas}) = E_{heat\ increment}+E_{maint.}+E_{production}$$

Sibbald (1979f) defines AME in terms of the quantity measured by energy balance assays viz. AME is the gross energy of the feed minus the energy voided as faeces and urine. In these assays the gas component is not measured:

$$AME_b = GE - (E_{faeces} + E_{urine})$$

where AME_b = AME value obtained by bioassay.

This definition more accurately describes the values obtained by direct methods but remains an estimate of the conceptual value (AME_c).

Wolynetz and Sibbald (1984) contend that the contribution of combustible gas loss to excreta energy, for most products, is small and can be ignored. Sibbald (1983) has recognized that for some feedstuffs containing non digestible fermentable carbohydrates, appreciable gas loss may result. Given these arguments, for most products AME_b is a near estimate of AME_c.

In a theoretical study of the relationship between AME and TME Wolynetz and Sibbald (1984) partitioned excreta energy into component parts. Their model assumes excreta energy is the sum of 6 parts:

FfE = The energy of feed residues passaged through the gut.

FmE = Metabolic faecal energy comprising: cells sloughed from the gut wall, bile, mucous and unabsorbed digestive juices.

FeE = Endogenous faecal energy that is provided by microbial flora and debris that is voided in the faeces.

FuE = The energy of materials absorbed across the intestine and excreted directly in the urine without undergoing metabolic change.

UmE = Energy of by-products of N metabolism appearing in the urine and resulting from a special form of tissue catabolism caused by fasting and which can be relieved by feeding a N free sources of energy such as glucose (McNab and Fisher, 1981; Wolynetz and Sibbald, 1984).

UeE = Endogenous urinary energy arising as by-products of tissue turnover (an ongoing process), that is affected by body size (Mitchell, 1955), but which appears to be independent of food intake.

According to this model AME_b is represented as:

$$AME_b = GE - (FfE + FmE + FeE + FuE + UmE + UeE)$$

in which $FfE + FmE + FeE = E_{faeces} = FE$

and $FuE + UmE + UeE = E_{urine} = UE$

AME has also been described in terms of bioavailable energy by Wolynetz and Sibbald (1984), a quantity defined algebraically as:

$$BE = GE - (FfE + FuE)$$

which corresponds to unbiassed TME and which equates to the energy of the food less that of food residues (FfE) and energy of material absorbed across the intestinal wall but excreted in the urine without metabolic alteration (FuE). Under this concept AME has the form:

$$\begin{aligned} AME &= GE - (FfE + FuE) - (FmE + FeE + UmE + UeE) \\ &= BE - (FmE + FeE + UmE + UeE) \end{aligned}$$

and deviates from BE by:

$$- (FmE + FeE + UmE + UeE)$$

The measurements required for determination of AME_b by conventional assay procedures are derived below. The units of measure of AME are kilocalories (kcal), common in United States literature, or joules (System Internationals):

$$AME_b = GE - (E_{faeces} + E_{urine})$$

Dividing throughout by grams fed (g_f):

$$AME_b(kcal/g_f) = GE(kcal/g_f) - \frac{(E_{faeces}+E_{urine})(kcal/g_e) \times Excreta(g_e)}{\text{Weight Fed } (g_f)}$$

where g_e = grams of excreta.

Four measurements are required, energy per unit weight of diets involved, energy per unit weight of air dry or dry matter excreta, the weight of food fed and the air dry or dry matter weight of excreta.

2.1.2 Linear Perspective

A second perspective of the relationship of AME to energy of food fed and excreta energy has been developed from investigations concerning TME. For a wide variety of foodstuffs a linear relationship has been demonstrated between the energy input as food and the energy output as excreta (Sibbald, 1975; Shires et al., 1980; Jonsson, 1980 as cited by McNab and Fisher, 1981; Farrell, 1981; Sibbald, 1982a; Sibbald and Morse, 1983a). The regression line has the form:

$$Y = a + bX$$

in which the intercept "a" is positive and is postulated by Sibbald (1975,1982a) to correspond to energy losses of an endogenous nature and in which "b" is a constant describing the energy excreted per unit of energy consumed. Sibbald (1982a) refers to the losses associated with "a" as "FmE + UeE" (faecal metabolic and urinary endogenous energy) by which, in terms of the symbols used in section 2.1.1, is meant the sum of FmE, FeE, UmE and UeE. In this thesis "endogenous" will be the term used to describe the energy arising from this source. Under this perspective AME_b can be described by the following linear form (Sibbald, 1975; McNab and Fisher, 1981):

$$\begin{aligned} AME_b &= GE - (bX + a) \\ &= GE - (bGE + a) \\ &= (1 - b)GE - a \end{aligned}$$

where

AME_b = kcal
GE = kcal/g food x g food
b = kcal excreted/kcal fed
a = kcal of excreta at zero kcal input.

For operational purposes AME_b can be described in terms of weight (grams) fed:

$$AME_b = (1 - b)GE - a$$

Dividing throughout by the weight of food fed:

$$AME_b(kcal/g) = \frac{[1 - b(kcal_{exc.}/kcal_{food})]GE(kcal_{food}/g_{food} \times g\ food)}{g\ food} - \frac{a(kcal/g_{exc.} \times g\ exc.)}{g\ food}$$

$$AME_b(kcal/g) = GE(kcal/g\ food) - bGE(kcal_{exc.}/g\ food) - \frac{a}{g\ food}(kcal_{exc.}/g\ food)$$

The linear perspective shows AME_b to be a function of the energy retained (1 - b)GE less a constant energy loss "a". Since (1 - b) is also a constant the value of AME_b varies with the amount of food fed. In this connection Guillaume and Summers (1970), Sibbald (1975) and McNab and Fisher (1981) have demonstrated that AME_b varies asymptotically with the level of test food consumed.

In experiments of McNab and Fisher (1981) and Sibbald and Morse (1983a,b), there is evidence of an elevation of energy excreted at zero food intake to that extrapolated by regression lines based on higher food fed values. This suggests that (1 - b) and "a" may not be constant but indeed be subject to variation in accordance with food intake levels. The remarks of Sibbald and Morse (1983b) suggest that 2 regression lines might better fit the data of their experiment 2, one

for low and one for higher values of food input. Accordingly AME_b may be defined by:

$$AME_b = (1 - b_{low})GE - a_{low} \qquad \text{for low feed inputs}$$

and $$AME_b = (1 - b_{high})GE - a_{high} \qquad \text{for high feed inputs.}$$

In each (1 - b) and "a" are constants and AME_b subject to variation related to the amount of food fed.

The basis for the proposed partition of components of excreta in the linear perspective is the linearity in the relationship between energy input as feed (X) and energy eliminated as excreta (Y) given as Y=a+bX. As noted earlier this relationship is supported by a number of investigations though deviations have been identified at zero feed input by others. The central premise is that "a" which shows as positive and corresponds to energy losses of an endogenous nature is constant at all levels of food intake and consequently is independent of the type or level of food fed. In consequence all other excreta energy losses are described by bX and are direct by-products of the food eg. food residues. In quantitative terms this reasoning is described by Wolynetz and Sibbald (1984) as:

$$a = FmE + FeE + UmE + UeE$$

and $$bX = FfE + FuE$$

Thus, given $AME_b = (1 - b)GE - a$

then $$AME_b = GE - (FfE + FuE) - (FmE + FeE + UmE + UeE)$$

and $$AME_b/g \text{ food} = [GE - (FfE + FuE) - (FmE + FeE + UmE + UeE)]/g \text{ food}$$

In connection with these assumptions 3 lines of investigation have been pursued. One approach has been to feed incremental levels of a material of a type that enables determination of the existence or otherwise of a slope term of endogenous origin i.e. one which demonstrates the

existence of excreta energy other than that arising directly from food fed. Thus for materials considered completely digested, corn oil and dextrose (Sibbald, 1975,1976a), glucose and cornstarch (Dale and Fuller, 1981) the demonstration of an energy excreted per feed input slope would indicate variation in endogenous excreta with level of food fed. Similarly for non digestible materials eg. cellulose, sawdust (Sibbald, 1981a) departure of the slope from unity would suggest the presence of a slope term due to change in endogenous excreta energy. Of the work along these lines only Tenesaca and Sell (1981) have demonstrated an increase in endogenous energy excreted with levels of food fed. They fed incremental levels of silica gel, a material considered to be non combustible and non digestible. In a repeat of their first experiment they were unable to show the same effect.

A second approach revolves around the issue of whether the nature of the food influences endogenous energy loss. Shires et al. (1979) has reported that the protein content of the diet fed prior to assay affects endogenous energy loss. Farrell (1981) cites results which show an association between endogenous energy excreted of adult cockerels and levels of neutral detergent fibre in the diet. He reports a linear response in endogenous energy excreted with increasing levels of neutral detergent fibre below 120 g/kg of food. Taverner et al. (1981) notes that in pigs endogenous amino acids measured in the terminal ileum increased as the dietary fibre content of the diet increased. McNab and Fisher (1981) obtained different estimates of endogenous energy using intercepts of regression equations relating energy excreted to weight of food fed for wheat (102.86 kJ/48 h) and soybean meal (91.98 kJ/48 h) from values obtained from fasted adult cockerel controls of 132.80 kJ/48h. Farrell (1980b) on the basis of regression intercepts noted that endogenous energy appeared to vary among feedstuffs. For the 9 foodstuffs tested the intercepts ranged between 9.9 kJ for wheat and 28.2 kJ for barley.

A third approach is concerned with the linearity of the relationship between energy excreted and energy or food intake. References have previously been given to investigations in this area. Sibbald and Morse (1983b) have linked deviations from linearity occuring at low levels of food input with variation in urinary N excretion. They argue that

endogenous excreta energy may vary between fat and lean birds because it is a function, at least in part, of tissue protein degradation and lean birds are likely to catabolize more tissue protein than fat birds. They also raise the issue, one investigated by McNab and Fisher (1981), that severity of fasting may also contribute to the magnitude of N excretion and hence influence endogenous excreta energy values.

It should be noted that itemization of the various energy components of the partition of excreta energy does not in itself affect AME_b values. That is whether AME_b is determined using:

$$AME_b = GE - (FfE + FmE + FeE + FuE + UmE + UeE)$$

or the linear approach:

$$AME_b = GE - (FfE + FuE) - (FmE + FeE + UmE + UeE)$$

both estimate the quantity:

$$AME_b = GE - (E_{faeces} + E_{urine})$$

Values may differ but these differences are functions of methodology and calculation. Rather the partition has a conceptual value, one important in analyzing the attributes and meaning of measures of AME.

2.1.3 Nitrogen Corrected Apparent Metabolizable Energy (AME_n)

Nitrogen correction denotes reference back to a baseline. The baseline commonly used in ME studies is zero N balance (ZNB) also referred to as zero N retention (ZNR), although correction to other levels of N balance have been advocated. Hartfiel et al. (1970) and Reyntens (1972) as cited by Miller (1974), suggest it would be more appropriate to correct for a N retention of 25 or 33.3%, as this approximates closely N retention on practical diets. Morgan (1972) as cited by Leeson et al. (1977) has also advocated a correction related to the typical N retention of the animal.

The form of this correction is based on N balance (NB) or N retention as defined by:

$$NB = NI - NO$$

where NI = Nitrogen input
NO = Nitrogen excreted

and where ZNB occurs where NI = NO.

Nitrogen balance has been referred to as N retention (Miller, 1974; Scott et al., 1982) and net N retention (Leeson et al., 1977). In this connection the reference is to the net balance of N, a value which may be positive or negative and one defined by NI - NO as distinct from its other connotation that of the sum of dietary N retained plus dietary NO as the total of dietary N fed. By way of illustration of the dual meanings, at zero food intake the amount of N fed is zero and hence that retained is zero, but as N balance at zero food intake is negative (Sibbald and Morse, 1983a) the N retention, perhaps more aptly described as net N retention, is negative.

Nitrogen excretion has been partitioned by Wolynetz and Sibbald (1984) into N of an endogenous origin and that arising from the food fed:

$$NO = N_{food} + N_{endo.}$$
$$NO = (FfN + FuN) + (FmN + FeN + UmN + UeN)$$

where the symbols correspond to those used previously except they describe weight of N per component instead of component energy yield. Further Sibbald and Morse (1983a) have shown that for several foodstuffs NO appears to bear a linear relationship with dietary N fed. In consequence Wolynetz and Sibbald (1984) have described the partition of N in excreta in linear form as follows:

$$NO = (FmN + FeN + UmN + UeN) + b_nNI$$

in which b_n is the unit of excreta N per unit of N fed. From the linear relationship a different perspective can be obtained for N balance, one based on N retention as follows:

$$\begin{aligned} NB &= NI - NO \\ &= NI - [b_nNI + (FmN + FeN + UmN + UeN)] \\ &= (1 - b_n)NI - (FmN + FeN + UmN + UeN) \end{aligned}$$

NB = Nitrogen retained - Endogenous nitrogen

That is, N balance is the dietary N that is retained from which is subtracted N of an endogenous character. Zero N balance is achieved when N retained equals endogenous N.

The correction term applied, is N balance multiplied by an energy characteristic representative of NO end products appearing in the urine and resulting from in the main catabolism of tissue protein. The correction term has the form:

$$E_{NB}\ (kJ) = + [36.51\ (NI - NO)]$$

where 36.51 is the energy characteristic and E_{NB} is the energy equivalent of N balance to be added to energy of excreta output to obtain a zero balance N baseline.

The energy characteristic employed varies. That used by Hill and Anderson (1958), 34.39 kJ/gN is the energy content of uric acid (Leeson et al., 1977). Its use is based on the assumption that uric acid is the sole excretory product of catabolized protein. Hill and Anderson (1958) recognized that this was likely to be an overstatement of the actual situation. However this value has been used most frequently in N correction of AME values (Leeson et al., 1977). Leeson et al. (1977) cites Coulson and Hughes (1930) as obtaining an energy value of 36.40 kJ/gN for chicken urine. Titus et al. (1959) proposed a factor of 36.51 kJ/g of N because they found this more accurately described the energy value of the mixture of nitrogenous constituents of chicken urine. Zelenka (1970) as cited by Sibbald (1982a) concluded that AME values corrected according to Titus et al. (1959) were demonstrably more accurate than those corrected according to Hill and Anderson (1958). The value of 36.51 kJ/gN has been used by Sibbald and Slinger (1963a) and Sibbald and Morse (1983a,b).

The contribution of uric acid to urinary N has varied widely between investigations. Waring and Brown (1965) found uric acid N to account for from 51 to 94% of the urinary N. McNabb and McNabb (1975) analyzed avian urine and found the proportions of nitrogenous compounds in it were: uric acid 55 -- 72%, ammonia 11 -- 21% and urea 2 -- 11%. However, the value they obtained (55 -- 72% uric acid) were of a lower percentage than those reported by O'Dell et al. (1960) and Sykes (1971) who obtained values of 81% and 84% respectively.

The purpose of correcting AME values to a common baseline is to eliminate variations arising from variable N retention (Miller, 1974), or stated more explicitly (Wolynetz and Sibbald, 1984) variations in excreta energy arising from differences in the way in which N is utilized between birds, between levels of intake and between diets employed. In addition Farrell and Swain (1977) using starved adult cockerels, showed that environmental temperature and acclimation to it affected the magnitude of endogenous excreta energy. Dale and Fuller

(1981) have also related differences in endogenous excreta energy loss from starved birds to seasonal variations in environmental temperature. Energy loss via endogenous excreta was 133.9 kJ per 48 h for a winter mean temperature of 5°C versus 75.3 kJ/48 h for a summer temperature mean of 30°C.

Between bird endogenous energy loss has been shown to vary considerably. Farrell (1978b) reports values ranging from 33 to 82 kJ/24 h for adult cockerels and Sibbald and Price (1978), values for adult cockerels between 25 and 69 kJ/24 h per bird. McNab and Fisher (1981) report for 48 h periods, values between 47 to 238 kJ/bird over 14 experiments. The overall mean was 117 kJ/48h/bird with an average coefficient of variation within experiments (each of 6 replicates) of 36.8%. Shires et al. (1979) recorded a body weight effect on endogenous excreta energy loss.

Specifically and in terms of N components of excreta output Wolynetz and Sibbald (1984) argue that correcting FE + UE to ZNB should reduce or eliminate variation in the between bird differences due to FmN and FeN. The UeE is maintenance related and its contribution to between bird variation should likewise be minimized by N correction. For UmE they argue that for a specific feedstuff at a specific input the UmE varies in accordance with tissue catabolism, tissue synthesis and the utilization of absorbed nitrogenous compounds as sources of energy for the production of heat, fat and carbohydrate. The quantity should vary among feeds, and according to intake, and in so doing affect N balance. Correcting for this feed and quantity imposed variation should reduce or eliminate dietary differences in this component.

In connection with the latter Swift and French (1954), and Baldini (1961), as cited by Sibbald (1982a), question the correction on the grounds that protein storage is characteristic of growth and egg production and therefore N correction exacts a penalty on the diet i.e. smooths out real differences between diets. In this respect Mitchell (1964) as noted by Miller (1974) claims N correction may be appropriate when the object is to assess the value of a particular feedstuff for varying circumstances of feeding but not when the object is to assess AME intake in a particular animal feeding test. Sibbald (1982a)

acknowledges these arguments when complete diets are being compared, but claims they lack substance when the test feedstuff is assayed in imbalanced diets.

The use of N corrected AME values has been advocated on a number of grounds. Leeson et al. (1977) working with turkey poults found that high and low protein content of basal diets influenced AME of rapeseed meal and wheat. Correction of AME values for N retention decreased differences for each material. Sibbald (1982a) suggests that correction may help to reduce variation in AME values between bird types and would increase the applicability of AME values. He further notes that most AME data in use is AME_n and if data is to be added or compared it needs to be of the same type. On the other hand, Sibbald and Slinger (1963a) submit that the increase in precision claimed for AME_n is often a function of the method of calculation and produced a regression equation based on 1375 pairs of AME_n and AME values ($r = 0.995$) that indicated that the N corrected value was proportional to AME. Though Sibbald (1982a) acknowledges differences in dietary N retention must alter AME values, he argues that in general such differences have negligible effects provided that a single type of assay bird is used.

The partition equation for AME corrected to ZNB is shown as:

$$AME_n = GE/g - [(FfE+FuE) + (FmE+FeE+UmE+UeE) + k(NI-NO)]/g$$

where k = Energy characteristic
and AME = Energy units per g weight of material.

The operational formula is:

$$AME_n = GE/g - [(FE + UE) + k(NI - NO)]/g$$

2.2 CHEMICAL DETERMINATION OF AME

Equations to estimate AME values may be based on the crude nutrients or the digestible crude nutrients of feedstuffs.

In the former, prediction equations were published by Carpenter and Clegg (1956), Sibbald et al. (1963), Hartel et al. (1977) and others from work examining the interrelationship between the AME value of a feed and the corresponding crude nutrient contents using regression analysis. Carpenter and Clegg (1956) determined that AME can be predicted by the equation:

$$\text{AME} = 53 + 38(\%\ \text{crude protein} + 2.25 \times \%\ \text{ether extract} + 1.1 \times \%\ \text{starch} + \%\ \text{sugar})$$

In the latter, a number of regression equations based on digestible nutrients were developed by authors such as Fraps et al. (1940), Janssen et al. (1976) and Hartel et al. (1977). Fraps et al. (1940) as cited by Sibbald (1982a) stated that the AME values of chicken feeds can be estimated using the coefficients 18.4, 39.6 and 17.6 kJ/g for digestible protein, ether extract and N-free extract plus crude fibre respectively.

There are numerous regression equations which predict the AME values of specific feedstuffs such as wheat (Sibbald and Price, 1976,1977b; Coates et al., 1977a), oats (Sibbald and Price, 1976,1977b), barley (Sibbald and Price, 1976; Coates et al., 1977a), sorghum (Moir and Connor, 1977a,b) and rapeseed meal (Nwokolo and Bragg, 1978).

However, equations which predict AME values have limitations. The assumption that digestible value of a nutrient such as carbohydrate or protein is constant is questionable. For example, Bolton (1957) as cited by Sibbald (1982a) found the digestibility of pentosan to vary from 5.6% in maize germ meal to 36.5% in wheat fine middlings.

A single equation cannot be expected to be applicable for different kinds of feedstuffs as well as for mixed diets.

The presence of trypsin inhibitors and tannins in certain feedstuffs and other chemical entities not taken into account in the prediction may affect the AME values.

However, the development of rapid bioassays by Sibbald (1976a) and Farrell (1978b) for determining the ME have reduced the need for chemical determination of ME.

2.3 METHODS OF DETERMINATION OF APPARENT METABOLIZABLE ENERGY

The basic equation for the estimation of AME is:

$$\text{AME/g food} = \text{GE/g food} - \frac{\text{GE/g excreta x Total excretion (g)}}{\text{Total consumption (g)}}$$

where GE is the gross energy. Its derivation is shown here:

$$\text{AME} = \text{GE} - \text{EE (Excreta Energy)}$$

Dividing throughout by the amount of test food:

$$\text{AME/g} = \text{GE/g} - \text{EE/g}$$

In practice EE is determined as:

$$\text{EE} = \text{GE/g exc. x g exc.}$$

Thus:

$$\text{AME/g} = \text{GE/g} - \frac{\text{GE/g exc. x g exc.}}{\text{g}} \qquad [1]$$

From the equation it is apparent that the total quantities of excreta needs to be measured. There are 2 ways of measuring this, the total collection method or the indicator method.

Total excreta measured by the indicator method (chromic oxide) is as follows:

$$\text{g exc.} = \frac{\text{g chromic oxide/g fed x g fed}}{\text{g chromic oxide/g exc.}}$$

Substituting in [1]:

$$\text{AME/g} = \text{GE/g} - \frac{\text{GE/g exc. x g chromic oxide/g fed x g fed}}{\text{g fed x g chromic oxide/g exc.}}$$

$$\text{AME/g} = \text{GE/g} - \frac{\text{GE/g exc. x chromic oxide/g fed}}{\text{chromic oxide/g exc.}}$$

The choice between total collection and indicator method has been a controversial subject. The total collection method assumes that the excreta collected during a period of time corresponds to feed ingested during the same time. Miller (1974) pointed out that complete excreta recovery is not possible at the beginning and end of each collection but consequent errors are likely to be negligible if collection extends over several days after a preliminary acclimatization period. Major objections to the method are that feed intake and excreta output are not easy to measure especially in chick experiments. Feed spillage makes accurate consumption measurements difficult and may at the same time, result in contamination of excreta. Apart from spilled feed, contamination of excreta with chick feathers, down and scale not only makes accurate measurements difficult, but also changes the composition of the excreta. However, Terpstra and Janssen (1975) as cited by Sibbald (1982a) noted that the problems are less prevalent in studies with mature birds.

The use of indicators eliminates the need for measurement of feed intake and total excreta output. It reduces the amount of excreta to be stored for analysis and also allows contaminated excreta to be discarded. This latter must be done with care however, for samples for laboratory analysis need to be representative of the total excreta if errors associated with sampling are to be avoided. The method assumes that indicators are distributed evenly throughout the feed and excreta, totally inert and pass through the digestive tract at the same rate as other feed residues. A common indicator used in AME assays is chromic oxide (Cr_2O_3). Other indicators that have been used include crude fibre (Almquist and Halloran, 1971), polyethlene (Roudybush et al., 1974) and acid insoluble ash (Vogtmann et al., 1975). These materials require further investigation.

2.3.1 CONVENTIONAL METHODS

The conventional method of determining AME is a substitution approach. It is illustrated by the methods of Hill *et al.* (1960), Potter *et al.* (1960) and Sibbald and Slinger (1963a).

2.3.1 (a) The Assay of Hill et al. (1960) and Potter et al. (1960)

Hill *et al.* (1960) described a procedure which involves feeding of 2 semipurified diets. One, the reference diet, consisted of a basal mix together with glucose at 43.1 or 52.8% of the diet. The other, the test diet, matched the reference diet except that a 40% level of the glucose fraction was replaced with the test material. Glucose was used as a standard. Its determined AME value was considered fixed at 3.64 kcal/g. Chromic oxide was incorporated at approximately 0.3% in each diet.

Cockerel chicks used in the experiments were housed in electrically heated batteries. They were reared to 2 weeks of age and 10 chicks were then alloted to each experimental group on the basis of body weight. The experiments were conducted for the period from 2 to 4 weeks of age during which each experimental diet was fed to 2 duplicate groups. Feed and water were supplied *ad libitum*.

Excreta were collected at 24 h intervals on 4 successive days during the last week of the experimental period. The excreta collected could be dried using a draft drying oven or freeze dryer. The 4 daily collections were pooled after drying, ground and then, together with samples of the feeds, assayed for gross energy using a Parr Adiabatic Oxygen Bomb Calorimeter. Nitrogen was assayed by macro-Kjeldahl procedures, chromic oxide by the method of Hill and Anderson (1958), and moisture by air oven (16 -- 18 h at 105°C) or by vacuum oven (6 h or more at 50°C) techniques.

The $AME_{(n)}$ value of the test material where $AME_{(n)}$ refers to AME or AME_n of the test material can be calculated from the $AME_{(n)}$ values of the reference and test diets and that of glucose using the following formula:

$$AME_{(n)}/g \text{ test material} = 3.64 - \frac{AME_{(n)}/g \text{ reference diet} - AME_{(n)}/g \text{ test diet}}{\text{Proportion of test material in test diet}}$$

The above equation may be derived as shown below:

$$AME_{(n)}/g \text{ ref.} = \frac{X}{100} \times AME_{(n)} \text{ basal} + \frac{100 - X}{100} \times 3.64$$

$$AME_{(n)}/g \text{ test} = \frac{X}{100} \times AME_{(n)} \text{ basal} + \frac{100 - X}{100} \times Y$$

$$AME_{(n)}/g \text{ ref.} - AME_{(n)}/g \text{ test} = \left(\frac{X}{100} \times AME_{(n)} \text{ basal}\right) + \left(\frac{100 - X}{100} \times 3.64\right)$$

$$- \left(\frac{X}{100} \times AME_{(n)} \text{ basal}\right) - \left(\frac{100 - X}{100} \times Y\right)$$

$$AME_{(n)}/g \text{ ref.} - AME_{(n)}/g \text{ test} = \frac{100 - X}{100} (3.64 - Y)$$

$$-Y = \frac{AME_{(n)}/g \text{ ref.} - AME_{(n)}/g \text{ test}}{(100 - X)/100} - 3.64$$

$$Y = 3.64 - \frac{AME_{(n)}/g \text{ ref.} - AME_{(n)}/g \text{ test}}{(100 - X)/100 \} *}$$

$$= AME_{(n)}/g \text{ test material.}$$

* Proportion of test material in test diet.

The value 3.64 kcal/g or 15.22 kJ/g is the AME of glucose reported by Anderson et al. (1958) and is assumed to be constant under all experimental conditions.

Potter et al. (1960) used a more or less similar procedure except that they proposed alpha cellulose as the standard ingredient.

2.3.1 (b) The Assay of Sibbald and Slinger (1963a)

Instead of using semipurified materials, Sibbald and Slinger (1963a) recommended basal diets composed of practical feed ingredients as they considered the $AME_{(n)}$ values of feedstuffs thus obtained are more relevant to the formulation of practical diets. The test material is substituted for a representative portion of the basal diet and by determining the $AME_{(n)}$ values of both the diets it is possible to calculate the $AME_{(n)}$ value of the test material by difference (Sibbald et al., 1960):

$AME_{(n)}$/g test material =

$$\frac{AME_{(n)}\text{/g test diet} - [AME_{(n)}\text{/g basal diet} \times (\%\text{basal in test diet}/100)]}{\%\text{ test material in test diet}} \times 100$$

The equation is developed in the following way:

$$AME_{(n)}\text{/g basal} = AME_{(n)}\text{/g basal}$$

$$AME_{(n)}\text{/g test diet} = AME_{(n)}\text{/g basal} \times \frac{X}{100} + \frac{100 - X}{100} \times Y$$

where X = % of basal in test diet.

$AME_{(n)}$/g test diet - $AME_{(n)}$/g basal

$$= \left(AME_{(n)}\text{/g basal} \times \frac{X}{100}\right) + \left(\frac{100 - X}{100} \times Y\right) - AME_{(n)}\text{/g basal}$$

$$= AME_{(n)}/g \text{ basal } x \left(\frac{X}{100} - 1\right) + \left(\frac{100 - X}{100} \times Y\right)$$

$$= AME_{(n)}/g \text{ basal } x \left(\frac{X}{100} - \frac{100}{100}\right) + \left(\frac{100 - X}{100} \times Y\right)$$

$$= - \left(\frac{100 - X}{100}\right) \times AME_{(n)}/g \text{ basal} + \left(\frac{100 - X}{100} \times Y\right)$$

$$Y = \frac{AME_{(n)}/g \text{ test diet} - AME_{(n)}/g \text{ basal} + (100 - X)/100 \times AME_{(n)}/g \text{ basal}}{(100 - X)/100}$$

$$Y = \frac{AME_{(n)}/g \text{ test diet} + AME_{(n)}/g \text{ basal} \times [(100 - X)/100 - (100/100)]}{(100 - X)/100}$$

$$Y = \frac{AME_{(n)}/g \text{ test diet} + AME_{(n)}/g \text{ basal} \times (-X/100)}{(100 - X)/100}$$

$$Y = \frac{AME_{(n)}/g \text{ test diet} - (X/100) \times AME_{(n)}/g \text{ basal}}{(100 - X)/100}$$

$$Y = \frac{[AME_{(n)}/g \text{ test diet} - (X\%/100) \times AME_{(n)}/g \text{ basal}] \times 100}{(100 - X)\%}$$

$$= AME_{(n)}/g \text{ test material.}$$

2.3.1 (c) Assay Variations

A variation of the substitution approach was to add the test material at 2 or more levels in the test diet (Leeson et al., 1974,1977). This would serve as a check on the linearity of $AME_{(n)}$ over levels and enable determination of $AME_{(n)}$ against proportional inclusion of test materials by regression techniques. This extrapolation to a proportional inclusion of 1, gives the $AME_{(n)}$ value of the test diet when it consists wholly of the test material.

Campbell et al. (1983) derived 2 equations from those of Sibbald and Slinger (1963a) and Hill et al. (1960). They claimed that the modified equations were efficient in calculation of AME values for ingredients incorporated at low levels into a reference diet, such as ingredients of fat sources.

The 2 respective equations are:

$$AME_i = \frac{IE - [EE_t - (1 - X)EE_r]}{X}$$

and

$$AME_i = IE - [EE_g - \frac{1}{X}(EE_r - EE_t)]$$

where AME_i = AME value of the test ingredient.
IE = Gross energy of the test ingredient.
EE_t = Excreta energy voided /g test diet consumed.
EE_r = Excreta energy voided /g reference diet consumed.
EE_g = Excreta energy of glucose fed birds.
X = The level of inclusion of the test ingredient in the reference diet.

2.3.2 RAPID METHOD

2.3.2 (a) The Assay of Farrell (1978b)

Farrell (1978b) described a rapid assay for AME determination. He used adult cockerels trained to consume their daily feed requirements in 1 h. Birds were housed in individual cages. Individual feeders, designed to minimize food spillage, were placed at the front of each cage. Water was supplied ad libitum.

The cockerels were given a pelleted basal diet which consisted of normal feed ingredients. When the cockerels reached a constant body weight of about 3.5 kg they were given 110 g of a pelleted test diet for 1 h per day. The test ingredients were each mixed with an equal amount of the basal diet.

When feeders were removed from the trained birds after 1 h, a weighed plastic sheet was placed on each tray and excreta were collected for the next 24 h. Then the sheet and contents were placed in an oven at 80°C for about 20 h and thereafter were allowed to equilibrate with the atmosphere for at least 3 h. Following weighing, the excreta were removed from the sheet and stored in sealed plastic containers. Samples of excreta and of food were analyzed for gross energy. Moisture was determined by heating the food sample at 80°C for 24 h.

The excreta collection period was later extended to 32 h (Farrell, 1980a).

2.3.2 (b) The Assay of Sibbald (1975)

Sibbald (1975) employed a starvation period of 18 h. They did not train their birds to consume food placed before them rapidly. Assays were conducted directly with ingredients rather than on test materials mixed with basal diets. This may limit the application of the procedure to palatable food sources.

2.4 FACTORS AFFECTING $AME_{(n)}$ VALUES

2.4.1 Nitrogen Retention

As the proportion of dietary N excreted to that retained can be expected to affect excreta energy values either through raising urinary energy values or that of food residues, variable N retention is a factor implicated in AME variation.

Mueller et al. (1956) indicated that with the exception of a slight increase from 2 to 4 weeks of age, the percentage of dietary N retained by chicks decreased steadily with advancing age. The finding was later confirmed by Hakansson and Eriksson (1974) and Fonolla et al. (1981). It was noted that chicks fed diets from 10 to 26% protein have N retention varying between 53 and 36% (Summers et al., 1964) and with laying hens, increases in dietary protein level consistently decreased the percentage of N retained (Reid et al., 1965). Studies conducted by Miles and Featherston (1974) concluded that less N was retained by chicks fed a poor quality protein as compared with that of high quality. This agreed with the earlier finding of Solberg (1971). Working with cockerels, Swain and Farrell (1975) found that N retention increased significantly with rising temperature.

The energy characteristic used to describe the energy value of excreta N in N correction of AME has varied. The principle of N correction was early introduced to the cattle experiments of Armsby and Fries (1918). Hill and Anderson (1958) used the procedure for values obtained with poultry. They used a correction of 34.39 kJ/g (8.22 kcal/g) N retained which is the energy of uric acid. The following year Titus et al. (1959) proposed the factor of 36.51 kJ/g (8.73 kcal/g) which they claimed more closely represents the energy content of the N containing excretory products of chicken. Zelenka (1970) concluded that the AME values that were corrected by the factor 36.51 kJ/g were demonstrably more accurate than those corrected by using 34.39 kJ/g or those that were uncorrected (cited by Sibbald, 1982a). Unfortunately, both factors are being used and this has led to some of the variation among AME_n data.

The benefit of correcting AME for N has been the subject of some dispute. In a number of reports, the AME_n value was shown to be directly proportional to AME value (Sibbald and Slinger, 1962, 1963a; Proudman et al., 1970) which implies that the additional work involved in the N correction is of questionable value.

2.4.2 Quantitative Evaluation of Excreta Elimination

Sibbald et al. (1960) compared the Cr_2O_3 method with the total collection procedure. They found that data obtained with the former were more precise than the latter, but not necessarily more accurate. A comparison study by Coates et al. (1977b) showed that Cr_2O_3 method gave significantly lower but less variable AME_n values than did total collection. On the other hand, in the collaborative studies, Halloran (1972) and Carew (1978) found the difficulty of obtaining reproducible Cr_2O_3 assay data among laboratories. However, Han et al. (1976) noted that AME_n values of most poultry feedstuffs can be measured accurately by either method.

2.4.3 Level of Food Intake

Guillaume and Summers (1970) hypothesized that AME value should become smaller as food intake decreases particularly when it is below maintenance requirement level. This was also demonstrated by Sibbald (1975) and McNab and Fisher (1981).

The experimental data of Sibbald (1975,1976a) and Muztar and Slinger (1980b) were in general agreement with that hypothesized by Guillaume and Summers (1970). The relationship between food intake and AME as demonstrated by Guillaume and Summers (1970) is given in Fig. 2.1.

Wolynetz and Sibbald (1984) postulated that AME_n varies asymtotically with level of food intake but not as directly as for AME. This is shown in Fig. 2.2.

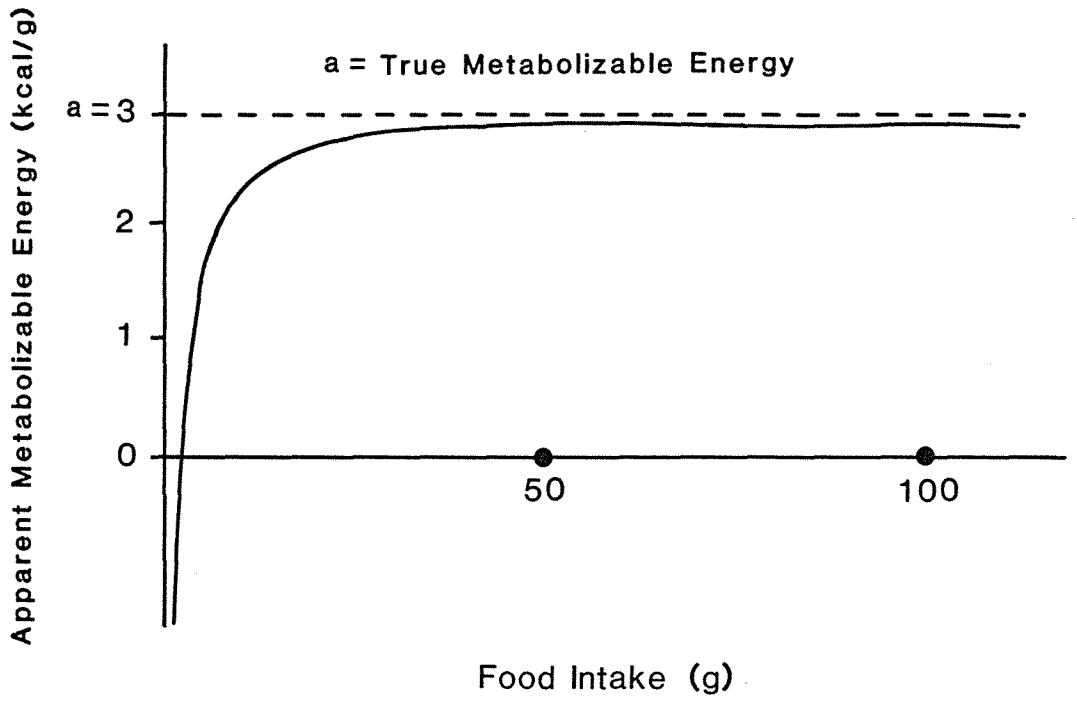


Fig. 2.1 Relationship between apparent metabolizable energy value and food intake (Guillaume and Summers, 1970).

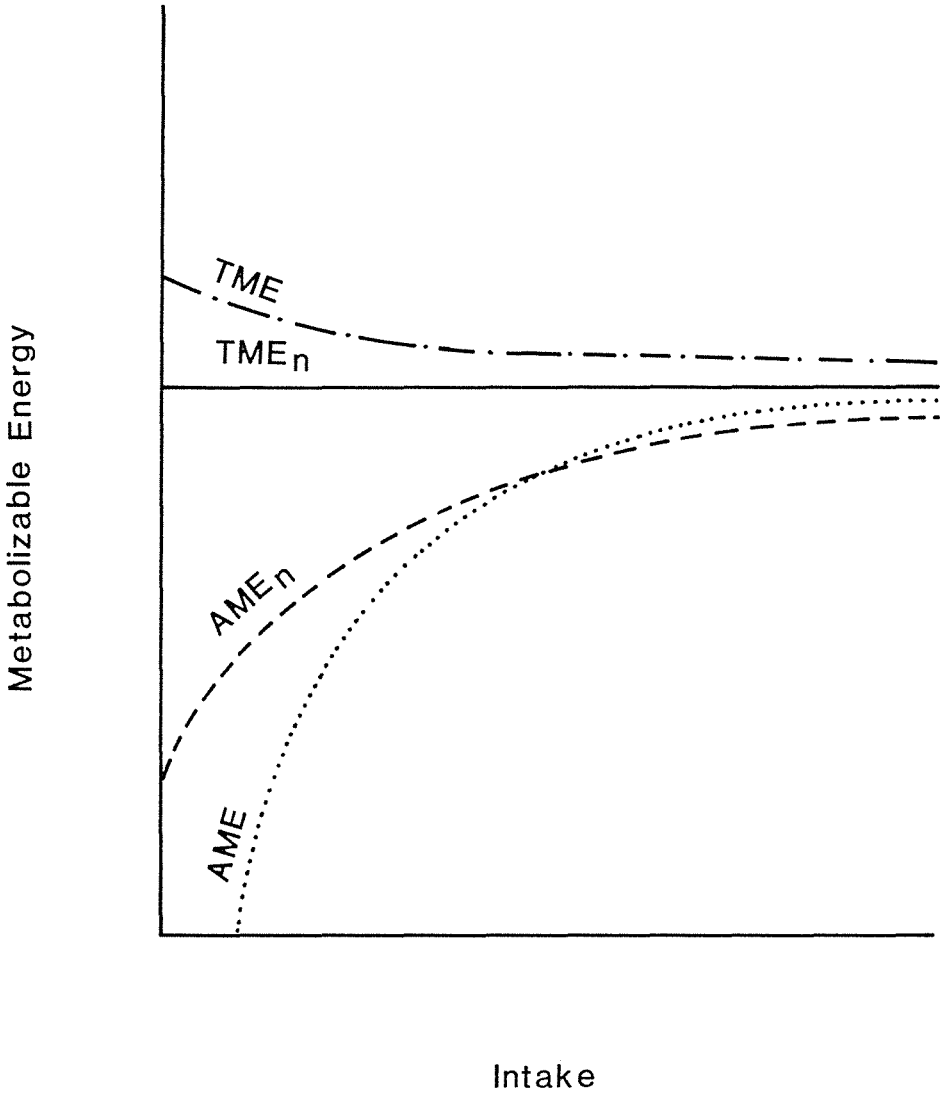


Fig. 2.2 Theoretical relationships among apparent (AME) and true (TME) metabolizable energy and their nitrogen-corrected equivalents (AME_n, TME_n) for various intake levels of a single feedingstuff (Wolynetz and Sibbald, 1984).

In this connection Hill and Anderson (1958) indicated that estimation of AME_n value was not affected by level of food intake even to as little as 30% of ad libitum. Subsequently, several reports such as those of Yoshida et al. (1964) and Han et al. (1976) supported this finding. Potter et al. (1960) restricted the food intake of chicks and observed that dietary AME_n value increased slightly as intake decreased. A similar observation was reported also by Mitchell (1942). The finding of the latter workers not only contradicts those of Hill and Anderson (1958) and others but also produces an opposite result of the hypothesis of Wolynetz and Sibbald (1984).

2.4.4 Substitution Effects

In substitution assays the level of test material in test diets has been shown to affect AME. Rao and Clandinin (1970) as cited by Vohra (1972) demonstrated a decrease in AME_n value of rapeseed meal from 1.84, 1.68 to 1.59 kcal/g as the level of substitution increased from 10 to 20 to 30%. Similar effects were observed in studies with rye (MacAuliffe and McGinnis, 1971) and dehydrated alfalfa (Miller, 1974). The effects as reported by Miller (1974) were derived by recalculating the data of Vohra and Kratzer (1970).

On the other hand, level of dietary substitution has shown no effect on AME value of soybean meal (Olson et al., 1961) and AME_n value of fish meal and fish solubles (Cuppet and Soares, 1972).

Sibbald et al. (1960) and Sibbald and Price (1975) have shown that variation associated with the AME_n value of a grain decreased as the level of dietary inclusion increased.

2.4.5 Effect of Basal Diets

The $AME_{(n)}$ content of a test material may be affected by basal diet composition. Sibbald et al. (1960) obtained varying $AME_{(n)}$ values for corn when combined with different basal diets but the value obtained for wheat was unaffected. Rao and Clandinin (1970) found that AME_n value of rapeseed meal was lower when fed with a semipurified basal diet than with a practical diet. Leeson et al. (1977) noted AME value of

rapeseed meal and wheat were influenced by the protein content of the basal diet used.

2.4.6 Effect of Nutrient Imbalances and Deficiencies

Nutrient imbalances and deficiencies may interfere with $AME_{(n)}$ value. Sibbald et al. (1962) observed effects on AME_n value in a study of the dietary interaction of riboflavin, niacin, vitamin B_{12} and methionine. In studies with chicks, Lockhart et al. (1966a,b) showed that B-vitamin deprivation decreased the AME_n value of the basal diet. This effect was observed subsequently in the studies using cockerels (Guo and Summers, 1970). Methionine deficient diets were claimed to have a greater AME_n value than methionine supplemented diets (Baldini, 1961). But, the findings of Carew and Hill (1961) and Solberg et al. (1971) failed to identify a methionine effect.

2.4.7 Species

The $AME_{(n)}$ value measured with different species of birds have been the subject of numerous investigations. Slinger et al. (1964) showed that both AME and AME_n values obtained with chicks for a high energy diet were higher than those obtained with turkeys and conversely for a diet of low energy content. Differences between $AME_{(n)}$ values determined with chicks and turkeys have also been detected in some other investigations (Bayley et al., 1968a; Leeson et al., 1974 and Coates et al., 1977b). Furthermore, variation has also been observed between bantam chickens and Blue-winged Teal (Sugden, 1974). However, that variation may diminish between species by correcting AME values to zero N retention as was indicated by Fisher and Shannon (1973).

On the other hand, a comparative study with chicken and Japanese quail indicated that there were no significant differences in their ability to metabolize dietary energy (Begin, 1968).

2.4.8 Strains

Considerable variation in $AME_{(n)}$ has been reported for bird comparisons between strains within species. White Leghorn chicks were found to give higher AME and AME_n values from high and low energy diets than did white Rocks (Sibbald and Slinger, 1963b). Slinger et al. (1964) reported that slow growing white Leghorn chicks gave higher AME and AME_n values for high energy diets than faster growing broilers.

Further evidence of differences in the $AME_{(n)}$ values of various diets for different strains and breeds have also been observed in the studies by Foster (1968), Proudman et al. (1970) and March and Biely (1971).

On the contrary, Hochstetler and Scott (1975) noted no AME_n differences when 3 broiler strains were compared. Other comparative investigations by Stutz and Matterson (1963), Begin (1967,1969) and Washburn et al. (1975) also revealed no variations.

2.4.9 Age

The $AME_{(n)}$ value of a feedstuff may be influenced by the age of the assay bird. Conflicting results however have been obtained. The results of Lodhi et al. (1969), Rao and Clandinin (1970) and March et al. (1973) showed that the AME_n value of rapeseed meal increased with advancing age. Petersen et al. (1976) reported higher AME_n values determined with adult hens than with chicks for a variety of feedstuffs. Additional evidence of age effects were also demonstrated by Fisher and Shannon (1973), Charalambous and Daghir (1976) and Coates et al. (1977b).

In a detailed study, Zelenka (1968) observed that dietary AME_n values reached a minimum between the 7th and 9th day of age, then increased until the 14th day. Thereafter, small increases continued until a peak was reached at 50 days. The initial decrease was attributed to the excretion of nutrient from the yolk and the subsequent rise to the development of the chick's ability to digest food.

The $AME_{(n)}$ values of feedstuffs are generally higher when determined with mature birds. However, Sibbald et al. (1960) found no significant differences between $AME_{(n)}$ values of corn using birds varying in age from 2 weeks to 16 months. Other investigations like those of Hochstetler and Scott (1975), Horani and Daghir (1975) and Fonolla et al. (1981) have failed to identify age effects.

2.4.10 Environmental Temperatures

Olson et al. (1970) observed that AME_n measured with chicks was higher under warmer environmental temperatures. O'Neill and Jackson (1974) using hens and cockerels have also noted this effect although the differences obtained were not significant. However, in studies with chicks, Lei and Slinger (1970) and Olson et al. (1972) noticed that AME values after correcting for N retention were not affected by alterations in environmental temperature.

With laying hens, Davis et al. (1972) reported that both AME and AME_n values were slightly higher in warm environments. The differences however were insignificant and were smaller after N correction. Swain and Farrell (1975) showed that both classical and N corrected AME values measured with cockerels were significantly higher under raised temperatures.

A seasonal effect in which AME_n values were significantly higher in summer than in winter was observed by Charalambous and Daghir (1976).

2.4.11 Stocking Density and Colony Size

The effect of stocking density and colony size on avian performance has been investigated. Studies have also been conducted on the effect of water deprivation on dietary $AME_{(n)}$. It has been shown that AME_n values measured with chicks were not affected by crowding and water restriction (Lei and Slinger, 1970) and neither were AME_n values affected by population density when determined with hens (Lei et al., 1972). This latter finding was later supported by Sibbald and Hamilton (1976).

2.4.12 Method of Calculation

There are basically 2 methods. One involves a multidose regression approach with extrapolation. The other involves single dose determination using the method of difference (Hill et al., 1960; Sibbald and Slinger, 1963a). Applying regression methods to the data of Vohra and Kratzer (1970), Miller (1974) showed that the AME of dehydrated alfalfa decreased as the proportion of test material in the test diet increased. The values obtained were 12.2, 9.1, 6.0 and 2.9 kJ/g. This effect was different to that recorded by Vohra and Kratzer (1970) who obtained corresponding values of 4.5, 7.9, 4.4 and 1.0 kJ/g. The example provided by Miller (1974) also serves to illustrate how AME may vary within a calculation method according to dose.

2.4.13 Pelleting Effects

Mash type diets are normally used in conventional AME assays. Pelleting is preferred when assays involve bulky fibrous feedstuffs or ingredients which contain heat-labile growth inhibitors or where palatability problems are expected. There is evidence however that pelleting influences the $AME_{(n)}$ values of feedstuffs. Cave et al. (1965,1968) and Bayley et al. (1968b) have observed that the $AME_{(n)}$ values of certain feedstuffs namely wheat bran, wheat shorts and wheat germ meal increased with pelleting. However, experiments by Hussar and Robblee (1962) and McIntosh et al. (1962) failed to demonstrate such effects for wheat, barley, oats and corn in the case of the latter authors and a whole diet in the case of Hussar and Robblee (1962).

2.4.14 Laboratory Handling

Preliminary excreta drying prior to analysis is important and this can be a variable source of error. Manoukas et al. (1964) reported substantial losses of energy and N ranging from 1.2 to 20.2% and -7.1 to 15.2% respectively when excreta samples were dried in a convection oven at 65°C for 24 h. The finding led to their suggestion of making determinations on fresh material. Shannon and Brown (1969) measured the effects of freeze drying, drying in a forced-air oven at 60, 100 and 120°C and drying under vacuum at 40°C and observed that losses of energy

were smallest when freeze drying was used. The average loss in energy due to freeze drying amounted to 1.3% while the loss of N was smallest when freeze drying and vacuum drying at 60°C were used (mean losses of 4.8% and 4.6% respectively). The mean energy and N losses using vacuum drying at 40°C were the largest among the various methods used.

2.4.15 Fats

The AME value of fats has been shown to be a variable quantity. Differences in AME value of fats are caused primarily by differences in their absorbability (Fuller, 1975). Young (1964) as cited by Fuller (1975) reported that factors influencing absorbability of fats include:

(i) Chain length of the fatty acids.
(ii) Degree of unsaturation.
(iii) Degree of esterification.
(iv) Position of the fatty acid on the glyceride moiety.
(v) Ratio of saturated to unsaturated fatty acids.

AME value of fats has also been shown to vary according to the age of the experimental birds. Renner and Hill (1960) fed a diet containing 17.5% tallow to chicks and showed that the AME value of fat increased with bird age reaching a plateau at about 8 weeks. The effects of bird age were also noted by Salmon (1969) and Whitehead and Fisher (1975).

There are several reports which show that dietary calcium may affect fat utilization. Yacowitz and Boyko (1962) showed that dietary limestone increased lipid excretion of chicks while Fedde et al. (1960) noticed the absorption of beef tallow decreased when dietary calcium content was increased.

There are reports which show that the AME values of some fats are greater than their gross energy values (Cullen et al., 1962; Jensen et al., 1970).

SECTION 2 TRUE METABOLIZABLE ENERGY

True metabolizable energy (TME) represents a refinement of the ME system of measurement. It is a development which seeks to remove the influence of energy of an endogenous nature which is an integral part of AME values. Since its proposal by Sibbald in 1976 it has undergone intensive scrutiny particularly in respect of a premise fundamental to its acceptance and validity concerning the accuracy with which endogenous excreta energy can be measured. Though there are a number of ways of deriving TME the methods advocated by Sibbald (1976a) or variants of it are relatively simple, though specialized, of short duration, and seldom require feed sample preparation outside of weighing. These features are advantageous particularly where routine evaluation or monitoring work is involved and in respect to the reproducibility of values. On the other hand, the approach involves feeding of the test food directly and considerable interest is evident in the literature in respect to the additivity of such values. With this in mind it is worthy of note that comment on AME values has cited differences between reference diets as potential sources of variance distorting additivity.

In this section the nature of TME is first explored, methods and evidence bearing on the central premise to the measurement presented, and other areas that have gained attention reviewed.

2.5 THE NATURE OF TRUE METABOLIZABLE ENERGY

2.5.1 Definitions and Derivations

True metabolizable energy is defined by Sibbald (1982a) and Wolynetz and Sibbald (1984) as the gross energy of the feed less the energy of feed residues emanating from the gut and the urine:

$$TME = GE - (FfE + FuE)$$

The definition is based on excreta energy comprising energy yielding components originating from the gut:

$$FE = FfE + FeE + FmE$$

and those occuring as urinary elimination products:

$$UE = FuE + UeE + UmE$$

where the definition of each component is given in section 2.1.1.

The concept of TME is founded on a linear relationship between energy or food intake and energy of excreta given as $Y = a + bGE$ where Y = energy output as excreta and on the premise that the intercept "a" corresponding to energy losses of an endogenous nature i.e. those occuring at zero food intake is a constant that is invariant to changes in level of food or energy fed. Under this premise the value of the intercept is the sum of 4 parts:

$$a = FeE + FmE + UeE + UmE$$

It follows that the balance of energy losses given as bGE arise directly from the food and on the basis of excretion components given above correspond to FfE and FuE. In this way the point perspective:

$$TME = GE - (FfE + FuE)$$ equates with the linear view:

$$TME = GE - bGE$$
$$= (1 - b)GE$$

This expression corresponds to the 2 levels approach or operational method of determining TME as proposed by Sibbald (1976a). The relationship is shown as follows:

$$Y = a + bGE$$
$$bGE = Y - a$$

Since
$$TME = GE - bGE$$
$$TME = GE - (Y - a)$$
$$= GE - [(a + bGE) - a]$$
$$TME = GE - (a + bGE) + a$$

That is, TME equals gross energy (GE) fed less total excreta energy plus energy of excreta at zero food intake as obtained from starved controls. The measures required in practice are demonstrated in the following equation:

$$TME/g\ food = GE/g\ food - (a + bGE)/g\ food + a/g\ food$$

where g food = grams of test material consumed.

The definition of TME in terms of excreta energy components as proposed by Sibbald (1982a) as shown above, may be derived as follows:

$$TME = GE - (a + bGE) + a$$

but $$a = FeE + FmE + UeE + UmE$$

and bGE = FfE and FuE for any level of GE.

Substituting:

$$TME = GE - (FfE+FuE+FeE+FmE+UeE+UmE) + (FeE+FmE+UeE+UmE)$$

$$TME = GE - (FfE + FuE)$$

King (1984) on the basis of a theoretical study of N balance and its relationship to TME proposes an addition of a term to Sibbald's (1982a) TME definition. He suggests:

$$TME = GE - (FfE + FuE + UiE)$$

where UiE is the energy of N excretion products in the urine resulting from metabolic processing of absorbed food. As this definition together with the associated N balance study form the substance of the experimental part of this thesis, the derivation of the above definition is given in full here.

Given that TME may be determined by subtracting from the gross energy of the food fed, the energy of the associated excreta, and correcting the result for endogenous energy produced by starved controls, then:

$$TME = GE - (FiE+UiE+UmE+UeE+FmE+FeE) + (UmE+UeE+FmE+FeE) \quad 2.1$$

where GE = The gross energy of the food eaten.

FiE = The energy of the food residues.

UiE = The energy of N excretion products in the urine resulting from metabolic breakdown of absorbed food.

UmE = Energy equivalent of N by-products of tissue protein catabolism of the type occuring in the urine under starvation conditions.

UeE = Energy equivalent of N by-products in the urine, resulting from the day to day wasting of N due to maintenance activity.

FmE = Energy of products of the gut specified as cells of the gut wall, bile mucous and unabsorbed digestible juices and gas.

FeE = The microflora and microbial debris voided as faeces.

and where: FmE + FeE = Fm+eE

and where in general terms: E = Energy equivalent
F = Material emanating from the gut
U = Material of the urine

and where lower case letters specify material: i = Food
m = Metabolic
e = Endogenous.

Note: The energy contribution of gas to FmE is assumed to be small (Wolynetz and Sibbald 1984) and in practice is not collected.

FiE may be partitioned into energy of N products that are absorbed across the gut wall and excreted directly in the urine without undergoing metabolic change $F_{iun}E$, energy of N containing products of the food not absorbed and passaged via the gut $F_{ign}E$, and energy of excreta of food residues remaining $F_{ir}E$, which are eliminated via the gut or the urine:

$$FiE = F_{iun}E + F_{ign}E + F_{ir}E \qquad 2.1A$$

Dividing throughout with the corresponding energy characteristic gives:

$$Fi = F_{iun} + F_{ign} + F_{ir} \qquad 2.1B$$

Note: FiE = FfE+FuE of Sibbald (1982a) and Wolynetz and Sibbald (1984).

Further GE may be partitioned into energy that will be absorbed from the gut and metabolically processed GEa and FiE:

$$GE = GEa + FiE \qquad 2.2$$

Similarly GEa may be partitioned into energy not lost as energy of N excretion products in the urine resulting from metabolism of food absorbed, GEr, and that eliminated by this process UiE:

$$GEa = GEr + UiE \qquad 2.3$$

Then

$$GEr = GEa - UiE \qquad 2.4$$

and substituting 2.4 into 2.3:

$$GEa = (GEa - UiE) + UiE \qquad 2.5$$

Thus the form of assimilation of the gross energy fed, equation 2.2 can be expressed as:

$$GE = (GEa - UiE) + UiE + FiE \qquad 2.6$$

Substituting GE of 2.6 into equation 2.1:

$$TME = [(GEa-UiE)+UiE+FiE] - (FiE+UiE+UmE+UeE+FmE+FeE) + (UmE+UeE+FmE+FeE) \qquad 2.7$$

Simplifying:

$$TME = [(GEa - UiE) + UiE + FiE] - (FiE + UiE) \qquad 2.8$$

$$TME/g = GE/g - (FiE + UiE)/g \qquad 2.8A$$

or from 2.8:

$$TME = GEa - UiE = GEr \qquad 2.9$$

Thus according to this derivation TME from equation 2.8 may be defined as the energy of the food eaten that is available to the bird after subtracting the food residues and the energy of N urinary excretion products of the food fed. Alternatively from equation 2.9 it is the energy of the food absorbed and subsequently used in metabolic processes less the energy of N urinary excretion products arising therefrom. Again from equation 2.9 it may be expressed as the energy of food material retained after assimilation but before heat increment losses.

2.5.2 The Relationship between TME and AME

The relationship between TME and AME as presented by McNab and Fisher (1981) has the following form:

$$TME = GE - bGE - a + a$$

$$TME = AME + a$$

In terms of per unit weight of food fed, this is equivalent to:

$$TME/g = AME/g + a/g \qquad 2.10$$

and

$$AME/g = TME/g - a/g \qquad 2.11$$

The derivation of TME as (1 - b)GE indicates that TME is a constant proportion (1 - b), of the gross energy level of the food fed. This implies that the value of TME remains constant over all levels of food fed. On the other hand equation 2.11 indicates that AME is a variable quantity that changes in relation to the level of endogenous excreta energy eliminated per unit of food fed. McNab and Fisher (1981) have noted this interdependency, and suggest that the two are interconvertible, provided that food intake and endogenous energy losses are specified.

2.5.3 Deviation from Linearity

However as mentioned under the section on AME (section 2.1.2), there is evidence that the excreta energy derived from fasted birds is greater than, or different from, that obtained by the intercept of the slope relating energy of excreta output to energy of food eaten at energy intake levels somewhat greater than zero (McNab and Fisher, 1981; Sibbald and Morse, 1983a,b).

It has been postulated that this effect may result from an additional component described by the slope of b_{Fm+eE} (King, 1983) or b_{UmE} (King, 1984; Wolynetz and Sibbald, 1984) or both (King, 1984) where b_{Fm+eE} and b_{UmE} are values corresponding to energy of endogenous products of gut origin increasing linearly with energy intake and energy equivalent of N by-products of tissue protein catabolism occuring in the urine and linearly related to energy intake respectively.

Wolynetz and Sibbald (1984) describe quantitatively this effect as follows:

If the unbiassed value of TME defined as $TME_u = GE - (FfE + FuE)$ is,

$$TME_u = (1 - b_u)GE$$

but the biassed value defined as $TME_b = GE - (FfE + FuE) + UmE$ is,

$$TME_b = (1 - b_b)GE$$

where UmE is the difference between the energy of endogenous excreta (EE) of TME_b and TME_u, such that $EE_b - EE_u = UmE$, then the bias or change in TME is given by:

$$TME_b - TME_u = \Delta TME$$

$$\Delta TME = (1 - b_b)GE - (1 - b_u)GE$$

$$\Delta TME = - GEb_b + GEb_u$$

$$\Delta TME = - (b_b - b_u)GE$$

Further the work of Sibbald and Morse (1983a) suggests that for high protein feedstuffs in particular the intercept a_b of TME_b may be less than that of a_u of TME_u such that:

$$a_u - a_b > 0$$

Thus the bias may be represented by the formula:

$$\Delta TME = a_{u\ -\ b} - (b_b - b_u)GE$$

Wolynetz and Sibbald (1984) postulate that such a situation could cause a slight deviation from linearity in the regression of energy of excreta output on energy input at low energy input values, and tentatively attribute it to variations in UmE between fasted and fed birds. With respect to the latter, King (1984) reaches a similar conclusion.

2.5.4 Nitrogen Corrected True Metabolizable Energy (TME_n)

For a review of the nature, purpose and manner of N correction refer to section 2.1.3. For TME the form of the correction involves adding the correction term, energy equivalent N balance, given as:

$$E_{NB}\ (kJ) = 36.51(NI - NO)$$

to the energy of excreta output recorded for each food intake level. In the 2 levels approach to TME determination, 2 corrections are consequently required, whereas in the linear approach as many are needed as there are food intake levels. For the 2 levels of food intake method the equation is:

$$TME_n = GE - (FE + UE + E_{NB}) + (Fm+eE + UeE + UmE + E_{NB})$$

$$TME_n/g\ food = GE/g\ food - (FE+UE+E_{NB})/g\ food + (Fm+eE+UeE+UmE+E_{NB})/g\ food$$

$$\text{kcal/g food} = \text{kcal/g food} - \underset{\text{(fed birds)}}{\text{kcal excreta/g food}} + \underset{\text{(control birds)}}{\text{kcal excreta/g food}}$$

As referred to in section 2.1.3, a purpose of correcting for N is to eliminate or reduce variations arising from variable energy retention resulting from differences in N utilization such as may be incurred by bird differences, levels of intake differences and dietary difference effects. On these grounds it is to be expected that N correction to zero N balance (ZNB) would reduce variation associated with TME values.

In this connection Shires et al. (1980) produced evidence of reduced variability associated with TME values measured with chicks and adult cockerels. Nitrogen correction to ZNB reduced the error mean square associated with TME values obtained for hull-less barley by 40% (Sibbald, 1982b). Sibbald and Morse (1983a) report that such corrections substantially reduced the random variation associated with

excreta energy output of fasted birds. Sibbald and Morse (1983b) studied the effect of correcting faecel plus urinary energy (FE + UE) output to ZNB. They found that N correction reduced error variance estimates of excreta energy by greater than 40% in each of 4 experiments and found that the correction improved the fit of the linear model on which the TME bioassay is based. These authors concluded that N correction of TME values is to be recommended. Sibbald and Wolynetz (1985) found N correction reduced the variation in TME estimates.

In addition to the improved precision, by which is meant reduced variation associated with N corrected TME values, Wolynetz and Sibbald (1984) conclude that the correction removes a source of bias otherwise present. In many accounts describing the relationship between energy of excreta output and energy or food intake a linear relationship was established for a variety of foodstuffs (Sibbald, 1975,1976a; Shires et al., 1980; Jonsson, 1980 as cited by McNab and Fisher, 1981; Farrell, 1981; Sibbald, 1982a; Sibbald and Morse, 1983a). In some experiments there is evidence of an elevated energy excretion at zero food intake to that obtained by the intercept of regression lines based on higher food intake values (McNab and Fisher, 1981; Sibbald and Morse, 1983a,b). Indeed as mentioned in section 2.1.2, Sibbald and Morse (1983b) indicate that 2 regression lines might better fit the data relating excreta energy output to food input, one for high and one for low intake levels. These latter, however, also concluded that N correction improved the linearity of the relationship on which TME is based.

The argument presented by Wolynetz and Sibbald (1984), is that, TME_n may be an unbiassed estimate because the linearity of the relationship between excreta energy out and energy input holds for all values of food input. If on the other hand it is a biassed estimate then the deviation from unbiassed TME, TME_u, is given by:

$$TME_n - TME_u = \Delta TME$$

$$(1 - b_n)GE - (1 - b_u)GE = \Delta TME$$

$$-(b_n - b_u)GE = \Delta TME$$

Given that the intercept of TME_n and TME_u differ, and $a_u > a_n$:

$$\Delta TME = a_{u-n} - (b_n - b_u)GE$$

As the relationship for N uncorrected excreta energy values is non linear, TME uncorrected, TME_o, is a biassed estimate. The bias is estimated as:

$$TME_o - TME_u = \Delta TME$$

$$\Delta TME = a_{u-o} - (b_o - b_u)GE \quad \text{given that } a_u > a_o$$

The authors surmise that the cause of the bias associated with N uncorrected TME may lie with changing UmE. As the energy of this component is related to its N level, correcting excreta energy values to ZNB may remove this distortion. These arguments suggest that if TME_n corresponds to TME_u then:

$$UmE = a_{u-o} - (b_o - b_u)GE.$$

The argument of King (1984) suggests that correcting TME values to ZNB remove one source of bias connected with UmE but introduces another associated with UeE. These contentions are studied in detail in Part 2.

To overcome bias arising from departure from linearity at low levels of food intake, Wolynetz and Sibbald (1984) propose an assay procedure which involves feeding the test material at 2 levels such that each lies beyond the level at which deviation occurs. The excreta energy values so obtained may be corrected back to ZNB, the advantage over non corrected values being a reduced variance, or an improvement in precision. Either procedure is designed to return the energy excreted to energy input relationship to a linear form.

An alternative approach is one which seeks to reduce tissue degradation of birds between treatments by provision of a N free, wholly digestible energy source to control birds. Without such a supplement increased catabolism might be expected to result in increased elimination of N containing products and in consequence increased energy losses. A number of investigations in this area have been conducted. Dale and Fuller (1981) fed a 50:50 mixture of glucose and corn starch to adult cockerel controls and excreta energy was reduced from 57.74 to 48.91 kJ/24 h over an administration of 0 to 25 g of mixture. McNab and Fisher (1981) using a 48 h witholding and 48 h collection period found 25 g of glucose administration reduced the mean excreta of 6 birds from 139.0$\pm$27.1 kJ/48 h to 71.1$\pm$10.1 kJ/48 h. Fisher (1982) has estimated energy excretion from birds fed 25 g of glucose. Sibbald and Morse (1983b) worked with mixtures of cornstarch, soybean oil, glucose and cellulose to supply a N free energy source to control (fasted) birds. Provision reduced body weight and N loss. They found that supplementing low food inputs with an energy source could provide acceptable data. With precision feeding reasonable TME values were obtained but free choice feeding of the energy supplement was not satisfactory due to spillage, variable intakes, and excreta contamination.

2.6 THE TRUE METABOLIZABLE ENERGY BIOASSAY

In this section the TME bioassay advocated by Sibbald (1976a) together with some of its modifications are described.

2.6.1 The Assay of Sibbald (1976a)

Adult White Leghorn cockerels are housed in individual wire cages in a room where 12 h of light are supplied. Feed and water are provided ad libitum.

After a preliminary 21 h fasting period, a bird is selected, weighed and force-fed a known quantity of test material (20 -- 25 g cold pressed pellets). Force feeding is done by administrating food through a glass tube inserted into the oesophagus of the bird with a glass plunging rod. After feeding, the bird is returned to its cage set over an excreta collection tray and the time of housing noted. A bird of similar body weight is then selected. It receives no feed and serves as an estimator of the endogenous losses. The procedures are repeated to provide the desired number of replications.

Exactly 24 h after placement the birds are weighed again and the excreta is collected quantitatively, frozen, freeze dried, equilibrated with atmospheric moisture, weighed and ground. Samples of the ground test material and excreta are assayed for gross energy using an Adiabatic Oxygen Bomb Calorimeter. The test materials are assayed for dry matter.

The TME of the test material is calculated using the following equation:

$$\text{TME (kcal/g)} = \frac{(GE \times X) - EO + EE}{X}$$

where GE = Gross energy of the test material (kcal/g).
EO = Energy voided as excreta by the fed bird.
EE = Energy voided as excreta by the unfed bird.
X = Weight of the test material fed (g).

Sibbald (1979b,1980) described additional features and modifications of the bioassay. He supports the use of dubbed adult White Leghorn cockerels which have never had access to grit. Dubbing is desirable because a large comb gets in the way when holding the bird's head during force feeding. Freedom from grit is preferable because grit may be retained in the gizzard for a long time and when voided in collected excreta may damage grinding equipment.

The feeding apparatus is described as a stainless steel funnel and plunger. The funnel, 40 cm in length and 1.3 cm in external diameter, is pushed into the crop via the oesophagus. The material is poured into the funnel and pushed into the crop with the plunger. Failure of the food to enter the crop may result in regurgitation. After feeding the funnel is removed with a rotary motion, pressure being applied to the wall of the oesophagus as the tube is withdrawn to remove any adhering feed particles.

The test material is weighed into a sealed container prior to the assay. The gross energy and dry matter of the test material are determined at the same time to avoid variation caused by subsequent changes in moisture content. Feeding is made easier if the feed is pelleted but this is not essential. Ground feeds require additional care in avoiding losses due to adherence to the funnel.

Care should be exercised during excreta collection and processing. When birds are force-fed feathers and scale are loosened and may contaminate the excreta. This problem can be eased by blowing contaminants off the trays about 1 h after feeding. At excreta collection times "contaminated" feathers should be washed clean before they are removed. Trays should also be checked for regurgitated feed and the data of offending samples discarded. Freeze-drying of excreta is advocated if amino acid analyses are to be made. If only gross energy is to be determined, Sibbald (1979d) found no difference in the effect of freeze-drying or drying in an oven at 65, 80 or 95°C on the amount of energy voided by the negative control or force-fed birds. When excreta is dry, it should be equilibrated with atmospheric moisture for 2 to 3 days before weighing, grinding and further processing. Excreta can be ground by use of mortar and pestle.

The work of Shires et al. (1979), as cited by Sibbald (1980), suggests birds should be given the same maintenance diet between assays. The maintenance diet need not be similar to the feedstuffs to be assayed (Kessler and Thomas, 1979). Water should be available at all times through the assay and afterwards birds should be returned to the maintenance regime for a minimum of 1 day though a longer rest period is preferred (Sibbald, 1978c).

The test material is usually assayed as a single ingredient but sometimes a reference diet and a blend of the reference diet with the test material is used. Fats are usually assayed together with a reference diet (Sibbald and Price, 1977a; Sibbald and Kramer, 1977,1978; Sibbald, 1978d), although corn oil was determined alone (Sibbald, 1975). The substitution approach has also been employed in the studies of rapeseed meal (Sibbald, 1977e).

The optimal amount of test material depends upon the size of the bird, its handling characteristics, and experimental error considerations (Sibbald, 1979b). Presumably the experimental error reffered to is that associated with precision of food allowance and variation in the energy of excreta of birds arising from laboratory techniques of assessment and directly from the number of birds employed. In general the greater the intake the smaller the effects of the experimental error. However, the

incidence of regurgitation increases as feed intake rises. Sibbald (1977b,c) observed that at levels of feed intake above 40g per bird there is a risk of regurgitation. Sibbald (1980) suggested that the optimum feed intake for adult White Leghorn cockerels weighing about 2 kg is 30 -- 40g of pellets or 25 -- 30g of ground feed.

2.6.2 Assay Variations

There are several variations which have been introduced into the TME bioassay as proposed by Sibbald (1976a).

A variety of species and strains of birds other than adult White Leghorn cockerels have been used in TME bioassays. Among them were turkeys (Sibbald, 1976c; Dale and Fuller, 1980; Parsons and Potter, 1980), ducks (Muztar et al., 1977), geese (Storey and Allen, 1982), laying hens and meat-type hens (Sibbald, 1976c; Kessler and Thomas, 1978), broiler chicks (Sibbald, 1978b) and dwarf White Leghorn cockerels (Boldaji et al., 1981). However, Sibbald (1979b,1980) considered that for routine assay studies, the adult White Leghorn cockerel was to be preferred. They tended to maintain a steady state, did not become obese and had good livability. He commented that meat type birds had high feed consumption between assays, tended to become obese and had poorer livability. Sub-optimal feed intake during the assay caused laying hens to produce soft shelled eggs which could break and contaminate the excreta. Chicks and growing birds needed replacement after each assay if several experiments were to be performed with birds of a uniform physiological state.

The duration of the fasting period was extended from the original 21 h to 24 h (Sibbald 1976b). Muztar and Slinger (1979a) used 30 h for the starvation period while McNab and Fisher (1981) have worked with 48 h periods.

Evidence has shown that the residues of some feedstuffs such as rapeseed meal (Sibbald 1978c, Muztar and Slinger 1979a,b), meat meal, fish meal and dehydrated alfalfa (Sibbald 1979c,e) and peanut skins (Sibbald 1979e) take more than 24 h to clear the digestive tract. In recognition of this, Sibbald (1980) recommended a collection period of 48 h but measurements reported by Sibbald and his co-workers since, were sometimes based on 24 h collections. McNab and Fisher (1981) adopted the 48 h regime in spite of their assumption that the carryover residues at the beginning of the assay would be balanced by the small loss at the end.

Originally, test fed birds were paired with control birds on the basis of body weight to obtain the endogenous correction. Since then, however, Sibbald and Price (1977a) and Sibbald (1978b,d) have used 5 or more starved, adult cockerels to obtain a single value for endogenous energy loss. Edmundson (1980) has proposed each bird as its own estimator of endogenous energy loss. He and Dale and Fuller (1981) observed that by pooling excreta samples prior to analysis, the calorimetry work can be reduced very substantially. This does not affect the TME value of the test material but can have a profound effect on the variance thereof (Sibbald and Morse, 1982).

2.7 EVIDENCE RELATED TO THE CENTRAL PREMISE OF TME

The central premise is that endogenous energy loss is constant and invariant to type or level of food fed. For unbiassed TME values it is important that the endogenous energy loss measured using starved birds is a close estimate of the actual loss of this component of fed birds. The endogenous excreta energy range has been investigated. Values ranging from 33.9 to 101.2 kJ/bird/24 h were obtained for a population of 12 birds (Patchell and Edmundson, 1977a), 25.8 to 62.9 kJ/bird/24 h for one of 48 birds (Patchell and Edmundson, 1977b), 25.0 -- 69.3 kJ/bird/24 h for 300 observations (Sibbald and Price, 1978) and from 32.5 -- 82.0 kJ/bird/24 h (Farrell, 1978b) have been reported. In a subsequent report, McNab and Fisher (1981) found a somewhat wider range, with values ranging from 47 to 238 kJ/bird/48 h. They suggest that this may be caused by additional stress imposed by the longer hours of starvation.

There are reports which show little endogenous excreta energy variation among birds within experiments. Some of the reported means and standard errors of endogenous excreta values were 40.293±0.903 kJ/24 h tested on 8 adult cockerels (Malhotra *et al.*, 1980 as cited by Sibbald, 1982a) and 70.4±2.94 kJ/24 h for 22 adult cockerels (Ranaweera and Nano, 1981). Similar low variability was noted also by Sibbald and Price (1980).

Patchell and Edmundson (1977b) and Farrell (1978a,b) provide evidence of marked variation in the day to day excreta output of the starved cockerel. However, Sibbald and Price (1980) indicated that variation in endogenous excreta is largely a characteristic of the bird.

The studies of Patchell and Edmundson (1977a,b) demonstrated that there is no significant correlation between body weight of birds and endogenous excretion. This was confirmed by Johns and Edmundson (1977), Farrell (1978b), Sibbald and Price (1978,1980), Arvat *et al.* (1980), Muztar and Slinger (1980a) and Ranaweera and Nano (1981). On the other hand, Shires *et al.* (1979) observed a body weight effect while Miski and Quazi (1981) noted age-related differences which can be explained by variability in body weight. A similar age:weight effect was reported by Sibbald (1981a). Storey and Allen (1982) in studies with geese, found

no relationship between endogenous energy losses and body weight.

The thermal environment affects endogenous losses. Dale and Fuller (1981) observed differences in endogenous losses due to seasonal change. The differences ranging from 133.9 kJ/48 h obtained during winter (mean temperature 5°C) to 75.3 kJ/48 h during summer (mean temperature 30°C). Farrell (1980b) using the data of Farrell and Swain (1977), had earlier shown a decrease in endogenous excreta as ambient temperature increased.

The endogenous energy losses of a fasted bird appears to change with the duration of fasting. Sibbald (1976b) observed that the quantity of endogenous energy excreted by adult cockerels, following an initial 24 h fast, decreased with the duration of starvation. The endogenous energy losses, in each subsequent 24 h period were 45.03±2.05, 37.04±1.63, 34.94±1.84 and 34.48±2.93. Similar effects were noted by Shires *et al.* (1979), Muztar and Slinger (1980a) and Sibbald (1981b).

2.8 ADDITIVITY AND REPRODUCIBILITY

An important feature of any system measuring available energy is that it predict dietary energy from its constituent parts. Sibbald (1977a) measured TME values with corn, wheat, soybean meal, fish meal, dehydrated alfalfa and 10 diets prepared from these ingredients. He observed TME values of the diets were not different from the values calculated using TME data of their ingredients. Tenesaca and Sell (1979) illustrated additivity using corn and oats. Additivity was observed also in studies by Sibbald (1977e,1979a), Muztar et al. (1978,1981), Dale and Fuller (1980) and Sibbald et al. (1980).

A favourable feature of the TME system is the demonstrated reproducibility of data between laboratories. Sibbald (1978a) conducted a collaborative study on AME and TME determination. He found 17 mean AME_n values for a corn sample ranged from 12.89 to 16.87 kJ/g while 9 mean TME values ranged from 16.66 to 17.37 kJ/g. Further evidence of reproducibility of TME values within and between laboratories were reported by Sibbald (1977d), Kessler and Thomas (1978), Halloran (1980) and Dale and Fuller (1981). However, in a collaborative study, Muztar et al. (1978) reported good agreement on the TME value of rapeseed meals but poor agreement for the whole seeds.

PART 2

CHAPTER 3

A THEORETICAL STUDY OF THE RELATIONSHIP BETWEEN NITROGEN EXCRETION AND TRUE METABOLIZABLE ENERGY

In sections 2.1.3 and 2.5.4, an attempt has been made to clarify the nature and purpose and effects of N correction. In the literature these issues have not, until recently, been clearly addressed. Leeson et al. (1977) referring to AME values states that "many investigators have avoided a decision about correction by quoting both corrected and uncorrected values" and adds "that despite the fundamental nature of correction, confusion and difference still exist". In their explanation of the procedure they state "a N retention of zero is assumed by addition to the heat of combustion of the urine of energy equivalent to the extra N which would have been excreted if N deposition had not occured, i.e. equivalent to the N retained". An explanation for these difficulties may reside in part with the terminology used in which N retention, N retained, N balance and net N retention have been employed often interchangeably with in some cases little regard for their shades of meaning. The perplexity has been augmented at times by empirical evidence. Sibbald and Slinger (1963a) questioned the validity of correcting to zero N balance (ZNB) suggesting it did little to improve the usefulness of AME values and questioned whether the extra work was justifed. Others for example Morgan (1972) as cited by Leeson et al. (1977) have advocated a correction related to the typical N retention of the animal rather than to zero.

More recently, Sibbald (1982a), King (1983), Sibbald and Morse (1983b) and Wolynetz and Sibbald (1984) have attempted to define and allocate functions to components involved in N balance. This has culminated in the work of King (1984) and Wolynetz and Sibbald (1984) who analyzed by different approaches the nature of N balance and its effects on TME and reached a common conclusion that UmE may well be a source of bias in TME values.

The subject of this chapter is the model developed by King (1984) to explain deviations in linearity of the relationship between N excretion (NO) and N input (NI) as it may apply to adult cockerels and the nature of the correction of TME values to ZNB. The study uses as a basis the definition of TME presented by King (1984) and given in section 2.5.1 and illustrates and discusses to what extent application of various forms of N correction may result in TME values conforming to the basis.

3.1 A MODEL OF NITROGEN BALANCE AND ITS RELATIONSHIP TO TME

The symbols employed if not explained in the text have been specified in section 2.5.1.

3.1.1 Analysis of the Excreta Energy Slope of TME

A proposition on which TME is based, is that the slope b of the line relating energy excreted (EO) to energy input (EI), is the same as the sum of the slopes b_{FiE} representing EO of food residues, FiE, and b_{UiE} representing the slope of energy of NO products of metabolically degraded absorbed food, UiE. That is:

$$b = b_{FiE} + b_{UiE} = b_{FiE + UiE} \qquad 3.0$$

In fact the slope may contain other components, such as one arising from Fm+eE, b_{Fm+eE} or, a component resulting from the effect of GEr on bird metabolism, b_{UmE} where UmE is the energy equivalent of N by-products of tissue protein catabolism of the type occuring in the urine under starvation conditions. That is:

$$b = b_{FiE} + b_{UiE} + b_{Fm+eE} + b_{UmE} \qquad 3.1$$

and $$b_{FiE + UiE} = b - (b_{Fm+eE} \text{ and } b_{UmE}) \qquad 3.2$$

and $b_{FiE + UiE} = b$ where b_{Fm+eE} and b_{UmE} each equal or sum to zero.

Graphically this is shown in Fig. 3.1.

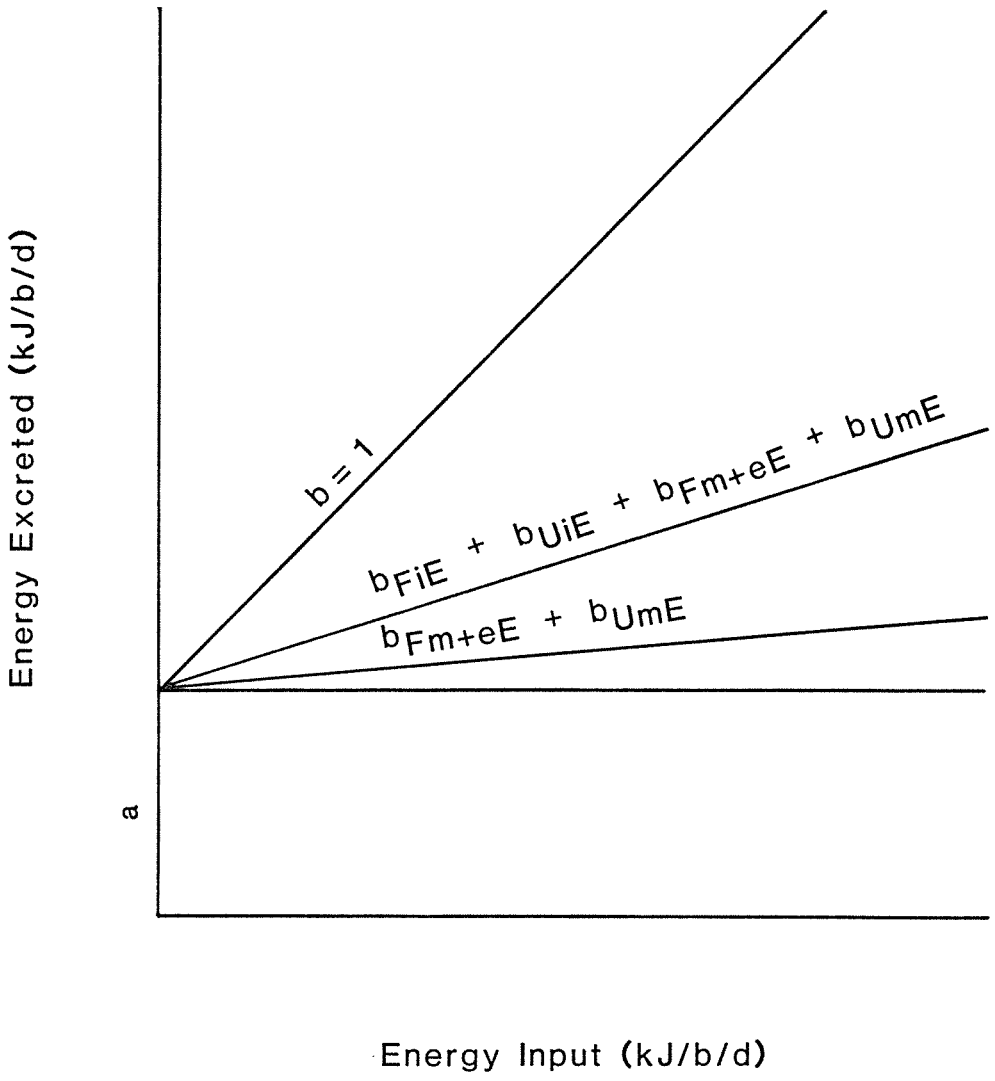


Fig. 3.1 Slope components of energy excretion (King, 1984).

Given that TME is based on a linear relationship between EO and EI as expressed by the relation:

$$Y = a + bGE \qquad 3.3$$

such that

$$TME = (1 - b)GE \qquad 3.4$$

$$= GE - bGE \qquad 3.5$$

or

$$TME = GE - (a + bGE) + a \qquad 3.6$$

as derived previously (see section 2.5.1), then equations 3.3, 3.4, 3.5 and 3.6 may be re-expressed in terms of equation 3.1 as follows:

$$Y = a + (b_{FiE + UiE} + b_{Fm+eE} + b_{UmE})GE \qquad 3.7$$

$$TME = GE - (b_{FiE + UiE} + b_{Fm+eE} + b_{UmE})GE \qquad 3.8$$

$$TME = GE - [a + (b_{FiE + UiE} + b_{Fm+eE} + b_{UmE})GE] + a \qquad 3.9A$$

Estimates of TME based on these forms deviate from the value as defined by an amount $(b_{Fm+eE} + b_{UmE})GE$. If $(b_{Fm+eE} + b_{UmE})GE > 0$ the derived value is less than the defined value.

The strongest evidence for a slope component of a measurable magnitude, one additional to that of $b_{FiE + UiE}$, comes from work concerned with variation of NO with levels of food fed (Ishibashi, 1973; Sibbald and Morse, 1983b). It can be argued that during periods of starvation by-products of tissue protein breakdown, add to the energy of urine giving rise to a change in the NO slope and resulting in TME values different from those obtained under less severe conditions of feeding. That is TME may be:

$$TME = GE - [a + (b_{FiE + UiE} + b_{UmE})GE] + a \qquad 3.9B$$

3.1.2 A Model of Nitrogen Balance

The nature of the model was based on the form of the NO to NI relationship obtained by reconstructing N balance data of Ishibashi (1973) and as obtained for a broiler grower type diet of 21.16% crude protein (analyzed) and 3255 kcal/kg TME (determined) using adult egg type cockerels and detailed in the experimental section to come. The relationship is described in Fig. 3.2. Its form describes one slope change at a NI between zero and the value at which ZNB is achieved.

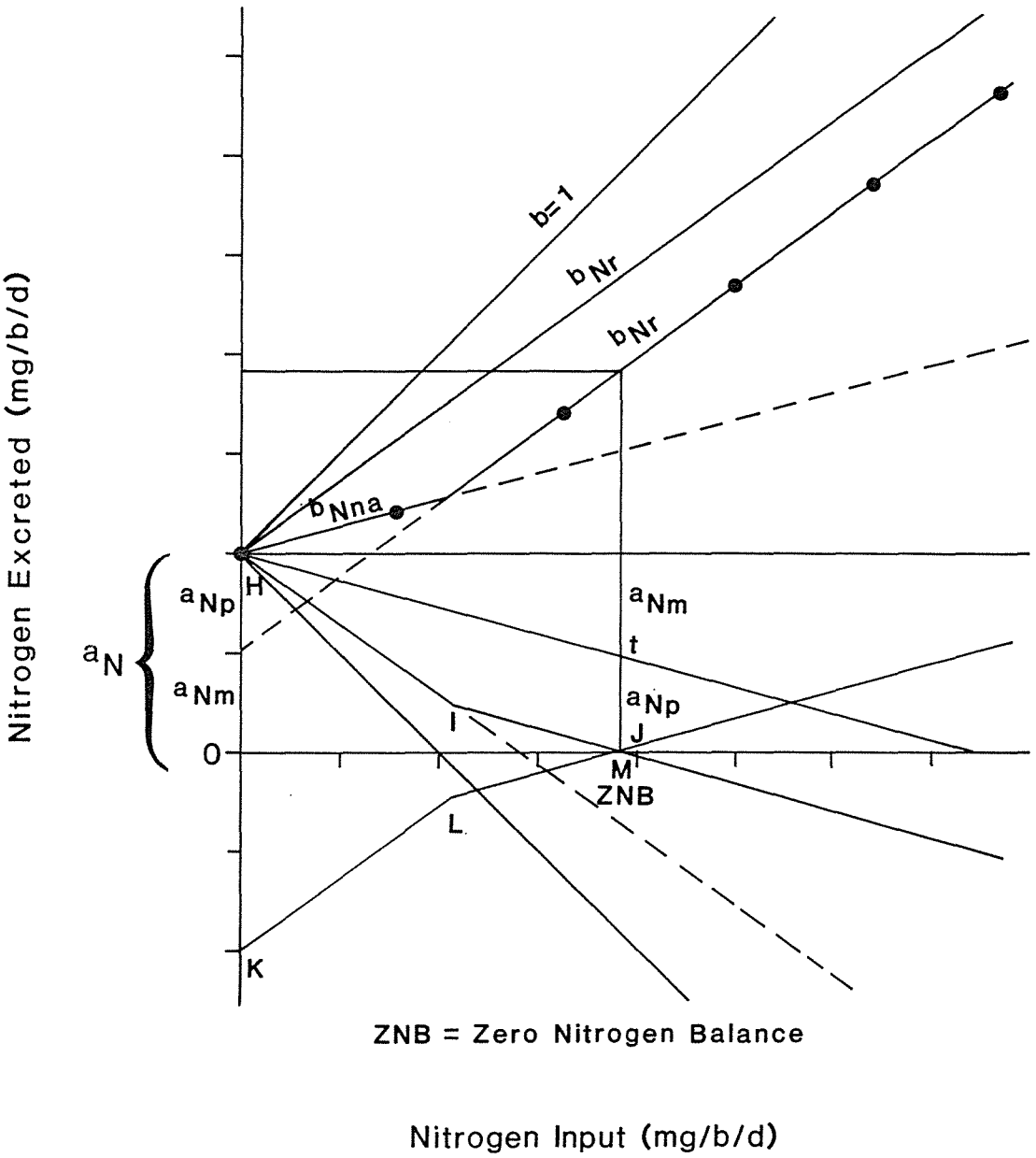


Fig. 3.2 Relationships between nitrogen input, nitrogen excreted and zero nitrogen balance (King, 1984).

The model states that at fasting, adult cockerels have a NO given by the intercept a_N of the slope for NO on NI. It is postulated that the value a_N derives from 2 processes:

(i) Tissue protein catabolism, a_{Np}, that takes place for the purpose of supplying energy needs and takes place during starvation.

(ii) Day to day wasting of N from the body that results from normal maintenance activity (Nm). This results in a NO constant, a_{Nm}, which is body weight related and independent of the food fed.

With the supply of food 3 processes operate:

(i) A proportion of NI, $b_{Nna}NI$, equivalent to $F_{iun} + F_{ign}$ is not metabolized and is eliminated directly. The remaining proportion, $(1 - b_{Nna})NI$, equivalent to the N contained in GEa is metabolically processed. Of this:

(ii) A proportion, $(1 - b_{Nr})NI$, equivalent to the N contained in GEr is retained and serves to offset loss of body N due to N wasting of normal maintenance activity.

(iii) A remaining proportion, $(b_{Nr} - b_{Nna})NI$, equivalent to the N contained in UiE is catabolized to supply energy which results in a yield of N containing by-products in the urine. At initial values of NI i.e. values of NI less than or equal to that at the slope change (see Fig. 3.2), this process spares an equal quantity of tissue protein N from loss. Hence $(b_{Nr} - b_{Nna})NI$ in Fig. 3.2 appears initially as an amount of N retained.

For levels of NI above that defined by the level of NI at which a_{Np} is completely spared, referred to hereafter as subsequent values of NI, a change in slope to that of b_{Nr} occurs.

Nitrogen excretion is thus defined by 2 lines :

$$NO_i = a_N + b_{Nna}NI \text{ for initial (i) values of NI} \qquad 3.10$$

and $$NO_s = a_{Nm} + b_{Nr}NI \text{ for subsequent (s) values of NI} \qquad 3.11$$

Over the initial range of NI the amount of NI appearing to be retained is given by:

$$(1 - b_{Nna})NI = [(1 - b_{Nr}) + (b_{Nr} - b_{Nna})]NI \qquad 3.12$$

For subsequent values of NI the amount retained is $(1 - b_{Nr})NI$.

The estimate of the amount of protein tissue N catabolized directly for purposes of energy supply at zero food intake, a_{Np}, is given by:

$$a_{Np} = (b_{Nr} - b_{Nna})NI \qquad 3.13$$

where NI is the value of NI at the point of change of slope.

The estimate of the NO constant, a_{Nm}, is given by:

$$a_{Nm} = a_N - a_{Np} \qquad 3.14$$

NI at the change of slope point, is given by:

$$NI = \frac{a_N - a_{Nm}}{b_{Nr} - b_{Nna}} = \frac{a_1 - a_2}{b_2 - b_1} \qquad 3.15$$

where a_1 and a_2 are intercepts of the initial and subsequent regression lines of NO regressed on NI and b_1 and b_2 are the corresponding slopes.

The amount of a_N remaining to be spared and offset to achieve ZNB, is given by the line HIM, whilst the line KLM gives the amount of N to be spared and retained by which achievement of ZNB is short. Each is defined by the appropriate regression equations. For initial values of NI, for the line KLM:

$$Y_i = -a_N + (1 - b_{Nna})NI \qquad 3.16$$

which when partitioned into sparing and offsetting effects, has the form:

$$Y_i = -a_N + (b_{Nr} - b_{Nna})NI + (1 - b_{Nr})NI \qquad 3.17$$

For subsequent values of NI:

$$Y_s = -a_{Nm} + (1 - b_{Nr})NI \qquad 3.18$$

The corresponding equations for the line HIM are:

$$Y_i = a_N - (1 - b_{Nna})NI \qquad 3.19$$

$$= a_N - (b_{Nr} - b_{Nna})NI - (1 - b_{Nr})NI \qquad 3.20$$

and

$$Y_s = a_{Nm} - (1 - b_{Nr})NI \qquad 3.21$$

Zero N balance is achieved at:

$$NO = NI = a_{Nm} + b_{Nr}NI$$

$$(1 - b_{Nr})NI = a_{Nm}$$

$$NI = \frac{a_{Nm}}{1 - b_{Nr}} \qquad 3.22$$

This interpretation states that, at zero food intake, there is a contribution to excreta via the urine of N containing products arising from catabolism of tissue protein estimated as a_{Np}, and metabolism associated with normal maintenance activity estimated as a_{Nm}. With food intake, the contribution to urinary N from tissue protein breakdown decreases at a rate given by $(b_{Nr} - b_{Nna})$, until, at a food intake level equivalent to a sparing effect on tissue protein breakdown of a_{Np}, catabolism of tissue protein and its contribution to metabolic NO ceases. To this point, and following this point, excretion of metabolic N from maintenance activity continues at a constant rate. Its loss is offset by an amount given by the N retention slope $(1 - b_{Nr})$. It is the combination of the sparing effect and N retention effect that results in ZNB being achieved.

3.1.3 Correction of TME for UmE and for UmE + UeE

For estimates of TME to conform to its definition i.e. TME=GE-(FiE+UiE), contributions to EO from UmE, UeE and Fm+eE should be removed from the basis i.e. from equation 2.1:

$$-(FiE+UiE+UmE+UeE+Fm+eE) + (UmE+UeE+Fm+eE) = -(FiE + UiE)$$

The energy contribution arising from NO products of protein catabolism, UmE, is given for initial values of NI by:

$$UmE = Ea_{Np} - E(b_{Nr} - b_{Nna})NI \qquad 3.23$$

in which E = the energy equivalent of metabolic N (taken as 36.51 kJ/gN from Titus et al., 1959). Note that Ea_{Np} = UmE at zero food intake. In addition urinary N contains the component of maintenance activity, a_{Nm}, which being independent of food intake is constant for all values of NI. In its energy equivalent form it has the value Ea_{Nm} = UeE.

Now if NI is written in terms of gross energy such that:

$$NI_{ge} = \frac{NI}{GE}$$

and $$NI_{ge} \times GE = NI$$

then: $$UmE = Ea_{Np} - [E(b_{Nr} - b_{Nna})NI_{ge}]GE$$

is related to the gross energy fed.

The operational expression encompassing these components has the general form of that of equation 3.9B in which "a" incorporates Ea_{Np} and Ea_{Nm} in its value, viz.,

$$a = Ea_{Np} + Ea_{Nm} + [a - (Ea_{Np} + Ea_{Nm})] \qquad 3.24$$

Thus for initial values of NI, TME determination by the operational method incorporates the terms:

$$\begin{aligned} TME = GE - [&(Ea_{Np} + Ea_{Nm} + (a - (Ea_{Np} + Ea_{Nm}))) \\ &+ (b_{FiE} + UiE - E(b_{Nr} - b_{Nna})NI_{ge})GE] \\ &+ [Ea_{Np} + Ea_{Nm} + (a - (Ea_{Np} + Ea_{Nm}))] \end{aligned} \qquad 3.25$$

For subsequent values of NI, because tissue protein breakdown has ceased, UmE no longer is a component of the energy of urine and TME is expressed by the relation (composition of terms by the operational method):

$$\begin{aligned} TME = GE - [&(Ea_{Nm} + (a - (Ea_{Np} + Ea_{Nm}))) + (b_{FiE} + UiE^{GE})] \\ &+ [Ea_{Np} + Ea_{Nm} + (a - (Ea_{Np} + Ea_{Nm}))] \end{aligned} \qquad 3.26$$

For initial values of NI, TME may be corrected by subtracting the contribution of UmE from the energy excretion of the fed birds

$$- UmE \text{ (refer to equation 3.23)}$$

and subtracting Ea_{Np} from the EO of control birds or alternatively by subtracting (UmE + Ea_{Nm}) from fed birds and (Ea_{Np} + Ea_{Nm}) from control birds. The result indicates an estimate of TME in concordance with the definition, equation 2.8 and 2.8A (p. 58):

$$TME_{Ni} = GE - [(a - (Ea_{Np} + Ea_{Nm})) + (b_{FiE + UiE}GE)] + [a - (Ea_{Np} + Ea_{Nm})] \quad 3.27$$

$$TME_{Ni} = GE - b_{FiE + UiE}GE = (1 - b_{FiE + UiE})GE \quad 3.28$$

$$TME_{Ni}/g = GE/g - (FiE + UiE)/g \quad 3.29 = 2.8A$$

For subsequent values of NI, TME is corrected by subtracting Ea_{Np} from the EO of starved controls or alternatively $(Ea_{Np} + Ea_{Nm})$ from control birds and Ea_{Nm} from fed birds. The result is equation 3.27 but equal to TME_{Ns}:

$$TME_{Ni} = TME_{Ns} = TME_{N_{UmE}} \text{ or } TME_{N_{UmE + UeE}} \quad 3.30$$

according to whether Ea_{Np} or $(Ea_{Np} + Ea_{Nm})$ are corrected. As the definition of TME is $TME = (1 - b_{FiE + UiE})GE$ the regression line for subsequent values of NI of EO regressed on EI as obtained from equation 3.26 is:

$$EO_s = [Ea_{Nm} + (a - (Ea_{Np} + Ea_{Nm}))] + b_{FiE + UiE}GE \quad 3.31$$

This is equivalent to a TME value of $TME_{N_{UmE}} = (1 - b_{FiE + UiE})GE$.

If this line is corrected for N by subtracting Ea_{Nm} for all values of EI then:

$$EO_s = [a - (Ea_{Np} + Ea_{Nm})] + b_{FiE + UiE}GE \quad 3.32$$

and $TME_{N_{UmE + UeE}} = (1 - b_{FiE + UiE})GE$.

Thus the line obtained by regressing EO on EI over subsequent values of NI gives an estimate of TME, $TME_{N_{UmE}}$. Further correction using Ea_{Nm} (subtraction) results in an estimate of TME, $TME_{N_{UmE + UeE}}$. Both conform to TME as defined.

3.1.4 Correction of TME to Zero Nitrogen Balance

Most studies involving correction of TME for N equivalent energy have corrected to ZNB. The form of this correction in relation to the nature of N balance as presented, is as follows :

For initial values of NI, the quantity of a_{Np} remaining to be spared is:

$$Ya_{Np} = a_{Np} - (b_{Nr} - b_{Nna})NI \qquad 3.33$$

and the quantity of a_{Nm} remaining to be offset by an equal quantity of N retention to achieve ZNB is given by:

$$Ya_{Nm} = a_{Nm} - (1 - b_{Nr})NI \qquad 3.34$$

Thus for initial values, the amount of absorbed N available for metabolic activity required to spare and offset is given by the sum of the equations 3.33 and 3.34:

$$Ya_{Np} + Ya_{Nm} = (a_{Np} + a_{Nm}) - [(b_{Nr} - b_{Nna}) + (1 - b_{Nr})]NI \qquad 3.35$$

$$= a_{Np + Nm} - (1 - b_{Nna})NI \qquad 3.36$$

This is represented by the line HI, Fig. 3.2.

At subsequent values of NI, when a_{Np} has been completely spared the quantity of a_{Nm} remaining to be offset is given by equation 3.34. This is represented by the line IM, Fig. 3.2.

To obtain TME_{ZNB}, excreta ouput is corrected by subtracting the energy equivalent of the amount of $a_{Np + Nm}$ remaining to be spared and offset as given by the line HIM, or adding the energy equivalent of the amount of N to be spared and retained by which achievement of ZNB falls short as given by the line KLM. That is, for the initial slopes:

$$-E[(a_{Np} + a_{Nm}) - (1 - b_{Nna})NI] = E[- (a_{Np} + a_{Nm}) + (1 - b_{Nna})NI] \quad 3.37$$

and for subsequent slopes:

$$-E[a_{Nm} - (1 - b_{Nr})NI] = E[- (a_{Nm}) + (1 - b_{Nr})NI] \quad 3.38$$

This is the equivalent of adding E(NI - NO) to EO as in the expression of Sibbald (1983):

$$\text{Corrected EO} = \text{EO} + (\text{NI} - \text{NO})36.51$$

Over initial values of NI, TME has the form given by equation 3.25 and for subsequent values the form as given by equation 3.26. Subtracting equation 3.37 from 3.25 and 3.38 from 3.26 gives in each case:

$$TME_{ZNB} = GE - [(a - (Ea_{Np} + Ea_{Nm})) + (b_{FiE+UiE} + E(1 - b_{Nr})NI_{ge})GE] + [a - (Ea_{Np} + Ea_{Nm})] \quad 3.39$$

Alternatively:

$$TME_{ZNB} = [1 - (b_{FiE} + UiE + E(1 - b_{Nr})NI_{ge})]GE \qquad 3.40$$

and is defined by the line:

$$Y_{TME_{ZNB}} = a - (Ea_{Np} + Ea_{Nm}) + [b_{FiE} + UiE + E(1 - b_{Nr})NI_{ge}]GE \qquad 3.41$$

The endogenous quantity $[E(1 - b_{Nr})NI_{ge}]GE$ contributing to the EO indicates TME_{ZNB} does not conform to TME as defined and results in values lower than TME by $[E(1 - b_{Nr})NI_{ge}]GE$. The cause of the effect is the form of the correction which suggests that N retained, spares an equal amount of metabolic N arising from maintenance activity. This rationale would mean that over the range of food intake from zero to ZNB the value of N loss due to maintenance activity would decline from a_{Nm} to zero. This does not seem sensible and cannot occur under this model as a_{Nm}, by definition, is a constant.

There appears then, to be 2 methods of deriving TME which result in estimates that conform to the definition as set out at the beginning of this study. One corrects for the energy of urinary N arising from catabolism of tissue protein to give 1 set of TME estimates, the other in addition, subtracts the energy of urinary N arising from wasting of N by maintenance activity to give a second set of TME estimates. A problem is that the regression techniques involve relatively complex experimental procedures and are demanding in terms of labour, time and birds. This problem may be overcome if there were found to be a high correlation between a_{Nm} and body weight which would then allow a return to the "operational" procedures of Sibbald involving fed birds at 1 level and starved controls with an expectation of a reduction in bias.

In summary the approach explores the effect on TME of a deviation from linearity in the relationship between NO and NI. The model postulates a causal component for a deviation whose energy value is estimated by the relation:

$$UmE = E(Ya_{Np}) = E[a_{Np} - (b_{Nr} - b_{Nna})NI]$$

It is postulated that its contribution to TME is described by the equation:

$$TME = GE - [a + (b_{FiE + UiE} + b_{UmE})GE] + a$$

The study indicates a method by which an estimate of EYa_{Np} = UmE can be obtained and a method by which TME corrected for UmE may be determined.

The study suggests that correcting TME to ZNB, whilst removing the bias caused by UmE introduces a secondary distortion $[E(1 - b_{Nr})NI_{ge}]GE$ related to the partial correction of UeE given as $E(Ya_{Nm})=[a_{Nm}-E(1-b_{Nr})NI_{ge}]GE$.

CHAPTER 4

EXPERIMENTAL

In Chapter 2 evidence has been presented, and in Chapter 3 a hypothesis developed, to support the view that the energy of food residues and by-products of metabolized food per unit of energy fed "b" is greater at high food intakes than low. Graphically this is shown in Fig. 4.1.

This indicates that 2 measures of TME are possible according to whether food levels result in "low" or "high" "b" values:

$$TME_h = GE - b_{high}GE$$

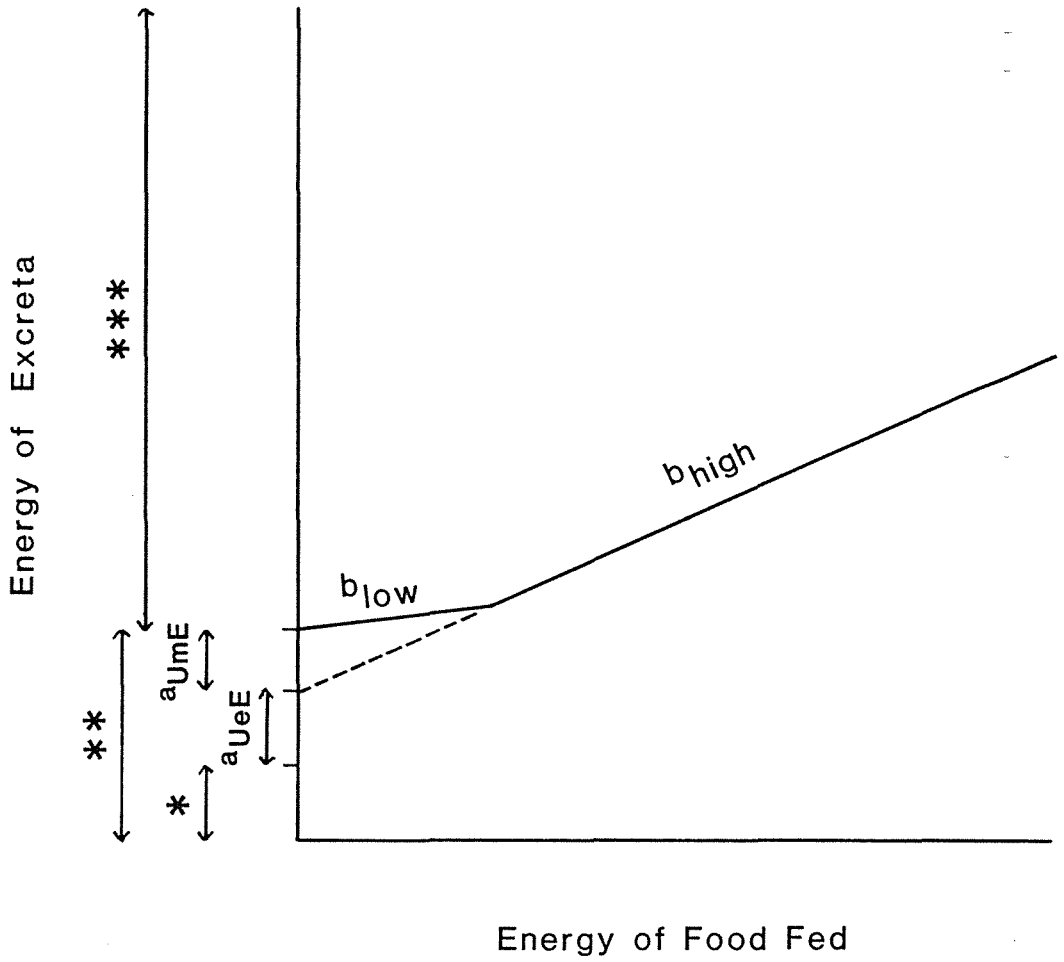
$$TME_l = GE - b_{low}GE$$

The cause has been attributed to an increase in endogenous excreta energy at low food intake levels. This, it is postulated, results from the burning of tissue protein for energy with release of energy containing by-products [urinary metabolic energy (UmE)] into the excreta via the urine, so raising excreted energy.

One consequence is that if we use the equation:

TME=GE - (Excreta energy of fed birds - Excreta energy of control birds)

to determine the TME of foods fed at "high" levels, the value will vary with the level of food fed. Diagramatically this is shown in Fig. 4.2.



* $a - a_{UmE} - a_{UeE}$

** Endogenous excreta energy = a

*** Energy of food residues and metabolic by-products of food

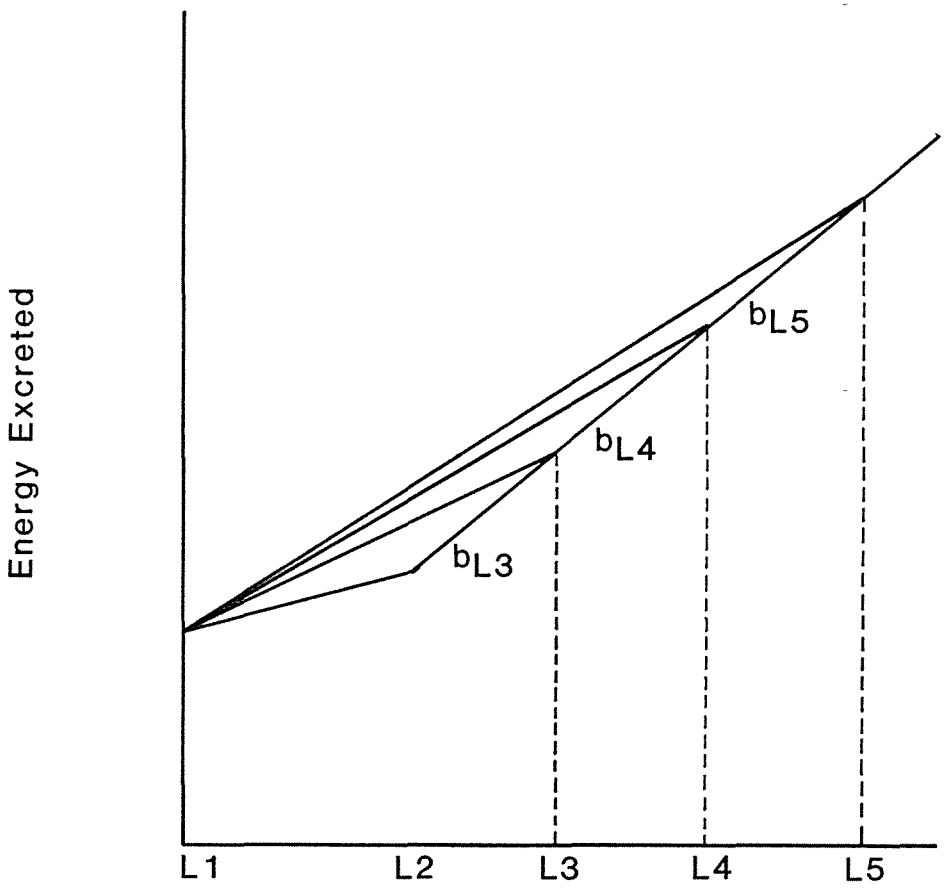
Components of "a":

a_{UmE} = Urinary metabolic energy

a_{UeE} = Urinary endogenous energy

$a - a_{UmE} - a_{UeE}$ = Remaining endogenous excreta energy.

Fig. 4.1 Components of excreta energy.



$$TME_{L3} = GE - b_{L3}GE$$

$$TME_{L4} = GE - b_{L4}GE$$

$$TME_{L5} = GE - b_{L5}GE$$

Fig. 4.2 The relationship between endogenous excreta energy and levels of energy fed on TME.

It has been demonstrated experimentally that correcting TMEs to zero N balance (ZNB) reduced variation associated with energy of excreta and TME values. It has been postulated that this correction may remove the component thought to be responsible for the increase in endogenous excreta energy at low food intake levels, UmE, so giving rise to what is referred to as unbiassed TME, TME_u. This value is defined by the slope, b_{high}, and has an intercept or actual endogenous excreta energy component equal to $a - a_{UmE}$:

$$TME_u = TME_{high} = GE - b_{high}GE$$

In fact theoretical considerations (Chapter 3) suggest correction to ZNB removes an additional component of endogenous excreta energy, termed urinary endogenous energy, UeE, a product of maintenance activity associated with day to day loss of body N. Under this hypothesis TME corrected to ZNB has a "b" value that intercepts the Y axis at $a-a_{UmE}-a_{UeE}$. This is shown in Fig. 4.3.

Under this hypothesis TME corrected to ZNB gives rise to biassed values.

The reasoning presented suggests that 4 measures of TME are possible:

$$TME_{low} = GE - b_{low}GE$$

$$TME_{high} = GE - b_{high}GE = TME_u$$

$$TME_{L3..} = GE - b_{L3..}GE$$

$$TME_{ZNB} = GE - b_{ZNB}GE.$$

This chapter deals with 2 experiments, LN 202 and LN 204. The first was set up to examine the effect on TME of correcting to ZNB. The second studies the impact of diet and assay procedure on TME of meat and bone meal (M & B) and the effect of assay procedure on a whole diet.

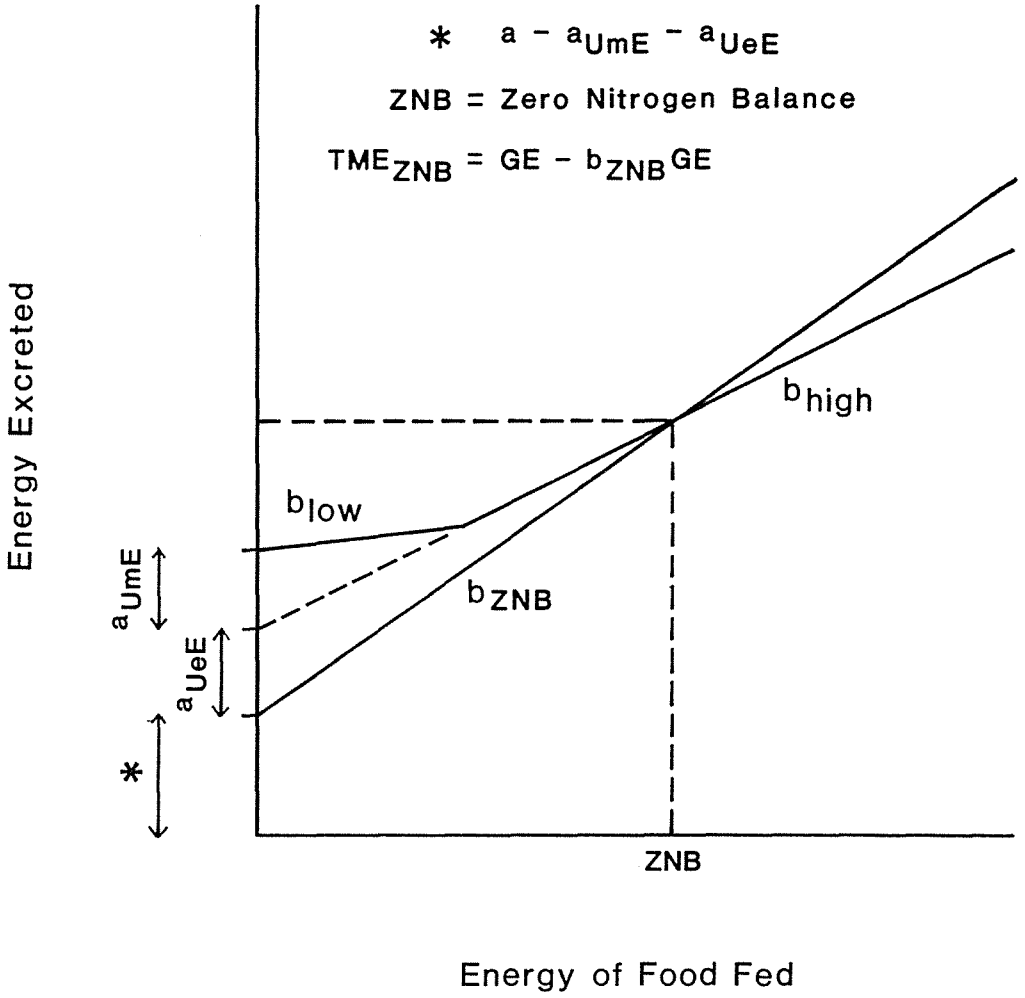


Fig. 4.3 The effect on TME of correcting to zero nitrogen balance.

4.1 **EXPERIMENT 1 — LN 202**

4.1.1 OBJECTIVES

(a) To examine the variability of N excretion (NO) of adult cockerels between days and for a range of food intakes.

(b) To examine the effect on the regression relationship obtained for adult cockerels, energy of excreta output (EO) to energy input (EI), of correcting to zero N balance (ZNB).

(c) To estimate the N and TME requirements of adult cockerels at maintenance.

4.1.2 MATERIALS, METHODS AND TREATMENTS

Sykes strain egg-type cockerels, 36 in number, and 64 weeks of age (WOA) were arranged in cockerel cages at 2 birds per cage on Thursday afternoon 1 March 1984 and given ad libitum the Poultry Research Centre layer cage mash until Friday 23/3/84, when they were transferred on to a high density pelleted diet of 3350 kcal/kg TME (calculated) and 20% crude protein (calculated) which subsequently formed the test diet (Tables A5 and A6, see Appendix). On Friday 2 March 1984, they were leg banded and individually weighed. From Thursday morning 8/3/84 starting at 10 a.m. through to Wednesday 28/3/84 the cockerels were trained to consume their daily food allowance in 1 h. Their time of access to food was reduced progressively from 5 h to 1 h per day by the use of hardboard feed covers. They were allowed 5 days at each of 4 feed access periods, 5 h per day, 4, 3 and 2 h per day. From Wednesday 28 March 1984 access time to feed was 1 h per day given between 9 and 10 a.m. This was changed progressively to operate between 1 p.m. and 2 p.m. in line with the proposed start and end times of the assay.

Individual body weights were taken immediately following return of food covers on the last day of each feeding period and once weekly after the return of food covers through the 1 h access to feed period until stable body weights were achieved.

On 6/4/84 birds were transferred to the assay quarters and housed 1 bird per cage in holding cages until the assay start on 10/4/84 when they were rehoused in single bird assay cages. During the period in assay quarters, the birds received daily, 14 h of artificial light and 10 h of darkness.

Allocation to treatment groups was organized so as to achieve 6 groups of 6 birds with matching group mean body weights. This was achieved by ranking birds by weight and distributing them accordingly. The body weight allocations to treatments are shown in Table 4.1. Body weights were determined by weighing birds individually using a Salter 5 kg suspended weigher.

The assay cages were in 8 cage 2 tier batteries with cages numbered 1 to 36. These had individual feeders and a Hart water cup was shared between birds of 2 adjacent cages. Each feeder had a label attached marked with the legband of the bird destined for the cage.

Paper bags were used to store bird daily feeding amounts. The excreta of each bird was individually collected off dropping trays in preweighed plastic pots.

There were 6 treatments. Each was a daily allocation of food. The treatments, their cage location and feeding, body weighing and excreta collection schedules for the assay period are shown in Table 4.2.

Table 4.1

The Body Weight Allocations to Treatments for Experiment LN 202

	Treatment					
	A	B	C	D	E	F
	kg					
Body weight	3.45	3.47	3.59	3.49	3.52	3.49
	3.57	3.41	3.50	3.41	3.29	3.40
	3.39	3.27	3.38	3.35	3.45	3.36
	3.28	3.25	3.16	3.14	3.24	3.13
	3.12	3.31	3.12	3.09	3.07	3.21
	2.82	2.77	2.78	3.08	3.13	2.99
$\bar{X}$	3.27	3.25	3.26	3.26	3.28	3.26

Table 4.2

Timetable of Assay Procedures for Experiment LN 202

	Treatment					
	A	B	C	D	E	F
Cages	1-6	7-12	13-18	19-24	25-30	31-36
Test period allocation (g/b/d)	0	20	40	60	80	100
Tue. (10/4)	H	H	H	H	H	H
Wed. (11/4)	hr	hr	hr	hr	hr	hr
Thu. (12/4)	hr	hr	hr	hr	hr	hr
Fri. (13/4)	hr	hr	hr	wh	wh	wh
Sat. (14/4)	hr	hr	wh	WF	WF	WF
Sun. (15/4)	wh	wh	WF	WCF	WCF	WCF
Mon. (16/4)	W	WF	WCF	WCF	WCF	WCF
Tue. (17/4)	WC	WCF	WCF	WCF	WCF	WCF
Wed. (18/4)	WCTF	WCTFF	WCF	WCF	WCF	WCF
Thu. (19/4)	FF	FF	WCTFF	WCTFF	WCTFF	WCTFF

H = House in assay cages.

hr = Provide 1 h of access to food.

wh = Witholding period of 24 h beginning from start of assay i.e. about 1 p.m.

W = Weigh birds individually (before excreta collection and feeding).

C = Collect excreta.

*F = Feed (present) the treatment amount of feed for 1 h.

T = Transfer to holding cages when convenient.

FF = Give the birds access to food 24 h a day.

* In the case of Treatments E and F the feeders were returned 3 h later for half an hour for the birds to consume the remainder. The time periods employed were the same from day to day.

Pots containing the daily collections of each bird's excreta were freeze dried, ground, cleared of contaminants and some weeks later their air dry weight obtained using a Mettler H3C160C balance. Samples of about 1 g of air dry excreta for each bird day were analyzed for N content. Samples of air dry excreta, on an individual bird basis, were pooled for gross energy determination by weighting daily excreta weights. For the latter only the final 3 days excreta collections were used for Treatment C, final 4 days for Treatments D, E and F, whilst each of the 2 days collections pertaining to Treatments A and B were used. Gross energy, N content and amino acid of the assay diet were also determined.

Gross energies were determined using a Gallenkamp Automatic Adiabatic Bomb Calorimeter CB-100 in accordance with the procedure manual.

Nitrogen analyses were made using the Tecator, Digestion System 6 and Kjeltec System 1002 Distilling Unit in accordance with the procedure manuals.

Amino acid was conducted using a Waters High Pressure Liquid Chromatograph (HPLC) amino acid analysis system.

Regression procedures were conducted using the statistical computing system "Minitab Release 82.1" (1982), copyright of The Pennsylvania State University. Analysis of variance for estimating proportional contribution of components to the total variance and other statistical techniques employed were taken from Snedecor and Cochran (1980).

4.1.3 RESULTS

The treatment mean body weights (kg) recorded over the days of the assay are given in Table 4.3. The raw data of which this table is a summary is given in Table A1 (see Appendix).

The treatment mean food intakes (g) for feed days of the assay are given in Table 4.4. The individual data from which this table is obtained is shown in Table A2.

The N content and gross energy of the assay diet was 3.386% and 16.709 kJ/g (3990.85 kcal/kg). Treatment mean dietary N intake (NI) (g), excreta N (NO) (g) and gross energy of air dry excreta (kJ) per bird per day are given in Tables 4.5, 4.6 and 4.7 respectively. The individual bird data from which Tables 4.6 and 4.7 have been obtained are given in Tables A3 and A4 respectively. Results of bird number 9 (Treatment B) are not included (see next paragraph).

The treatment mean NO per $kg^{0.67}$ body weight (BW) per bird associated with each daily food allocation are given in Fig. 4.4. In Treatments C, D, E and F excreta N associated with the first days feeding was relatively low. On subsequent days NO was fairly uniform though in Treatment F a peak was evident on day 4. Consequently day 1 results for both NO and energy excreted (EO) were considered unrepresentative of stable conditions and were not employed in subsequent calculations. Inspection of individual bird results showed that NO of bird coded as 9 of Treatment B was well in excess of the normal range. Consequently data of this bird was not used in subsequent analyses.

Analysis of variance was conducted separately on 3 groups, Treatments D, E and F, a second group, Treatment C, and on a third group, Treatments A and B, using daily bird NO data per $kg^{0.67}$ BW to investigate the day and bird contributions to treatment NO variance. The analysis of variance tables are given in Table A10. The estimate of bird contribution to the total variance for the 3 groups was respectively 83.1, 86.4 and 88.1%, with days contributing relatively little, estimated as between 17 and 12% of the total variance in each group.

Table 4.3

Treatment Mean Body Weights by Days

	A	B	C	D	E	F
	kg					
Before						
1st feeding	3.015	2.940	2.992	2.992	2.945	2.923
2nd "	2.943	2.890	2.967	2.992	2.958	2.955
3rd "	-	-	2.952	2.985	2.953	2.957
4th "	-	-	2.932	2.980	2.947	2.952
5th "	-	-	-	2.963	2.938	2.952
At final excreta collection	2.882	2.856	*	2.950	2.933	2.948

* The birds were inadvertently not weighed.

Table 4.4

Treatment Mean Food Intakes by Days

Feeding	A	B	C	D	E	F
	g					
1st	-	18.87	37.56	58.06	74.79	88.67
2nd	-	18.87	38.58	58.28	74.73	90.36
3rd	-	-	38.71	58.57	76.38	91.01
4th	-	-	38.80	58.79	76.36	92.56
5th	-	-	-	58.57	75.77	88.17
$\bar{X}$	-	18.87	38.41	58.45	75.61	90.15

Table 4.5

Treatment Mean Dietary Nitrogen Intake (g/b/d)

Feeding	A	B	C	D	E	F
1st	-	0.639	1.272	1.966	2.532	3.002
2nd	-	0.639	1.306	1.973	2.530	3.060
3rd	-	-	1.311	1.983	2.586	3.082
4th	-	-	1.314	1.991	2.586	3.134
5th	-	-	-	1.983	2.566	2.985
$\bar{X}$	-	0.639	1.301	1.979	2.560	3.053

Table 4.6

Treatment Mean Excreta Nitrogen (g/b/d)

Day	A	B	C	D	E	F
1	0.771	0.973	1.234	1.659	2.030	1.994
2	0.824	0.982	1.379	1.855	2.154	2.446
3	-	-	1.356	1.904	2.334	2.605
4	-	-	1.357	1.923	2.334	2.915
5	-	-	-	1.841	2.300	2.597
$\bar{X}$	0.798	0.978	1.332	1.836	2.230	2.511

Table 4.7

Gross Energy of Air Dry Excreta Based on Samples Weighted for Days (kJ/b/d)

	A	B	C	D	E	F
Weighted (Days)	2	2	3	4	4	4
Birds						
1	38.493	105.663	148.962	223.415	275.534	289.216
2	43.919	70.031	156.255	206.056	249.732	322.096
3	52.479	-	140.860	202.450	283.814	300.583
4	51.433	87.489	150.172	201.350	258.968	336.282
5	38.759	88.146	143.361	220.091	267.783	297.420
6	52.425	98.136	138.400	210.568	251.073	311.531
$\bar{X}$	46.251	89.893	146.335	210.655	264.484	308.688

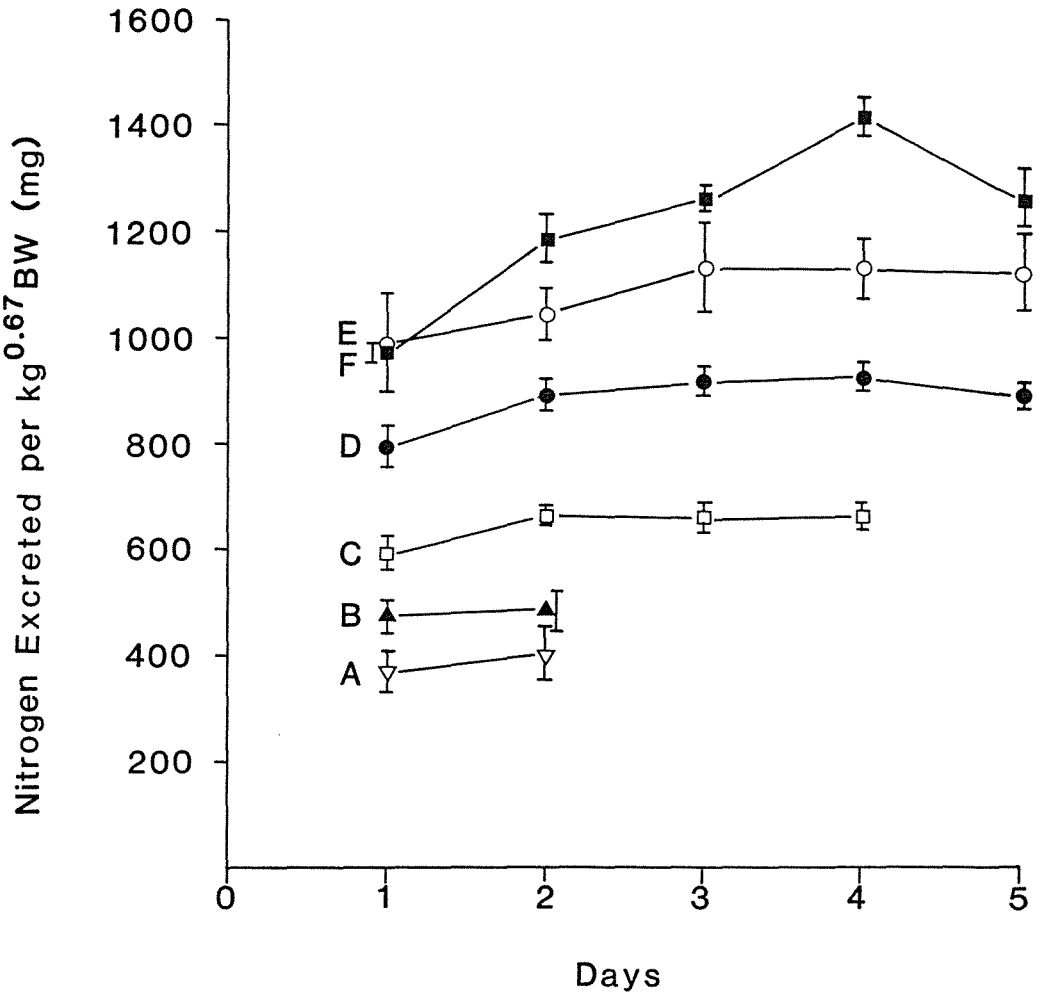


Fig. 4.4 Treatment 24 hour nitrogen excreted per kg $^{0.67}$ BW associated with each day's feeding schedule.

The relationship between NO (Y) and NI (X) on a per bird per day basis in which observations are bird means (over days) is shown in Fig. 4.5. Two regression equations describe the relationship. Units are milligrams.

$$Y_1 = 795.4 + 0.2883X_1 \qquad r = 0.493 \quad df = 9$$

$$Y_2 = 415.2 + 0.7288X_2 \qquad r = 0.958 \quad df = 22$$

At low levels of food intake the N retained per g of NI given as 1 - slope (b_1) was 0.7117 g, but at greater levels of food intake, N retention (1 - b_2) lessened, it being 0.2712 g. The 95% confidence limits for b_1 = 0.2883 were - 0.095 to 0.671 and those for b_2 = 0.7288 were 0.633 to 0.842. Zero N balance (ZNB) was achieved at a NI of 1.531 g/b/d ($NI = aY_2/1 - b_2$).

Given that N balance may be defined by:

$$NB = NI - NO$$

and energy retention may be estimated from the relation:

$$\text{Energy retention} = \Delta\,BW/b/d\ (g) \times 5\ (kcal/g\ \Delta\,BW) \times 4.1868\ (kJ/kcal)$$

the relationship between bird mean NI (X) and N balance (Y) using as units g/b/d and that between determined bird mean TME intake (X) in kJ/b/d and bird mean energy retention (Y) were assessed (Fig. 4.6 refers). Two linear regression equations were used to describe each relationship. For N balance:

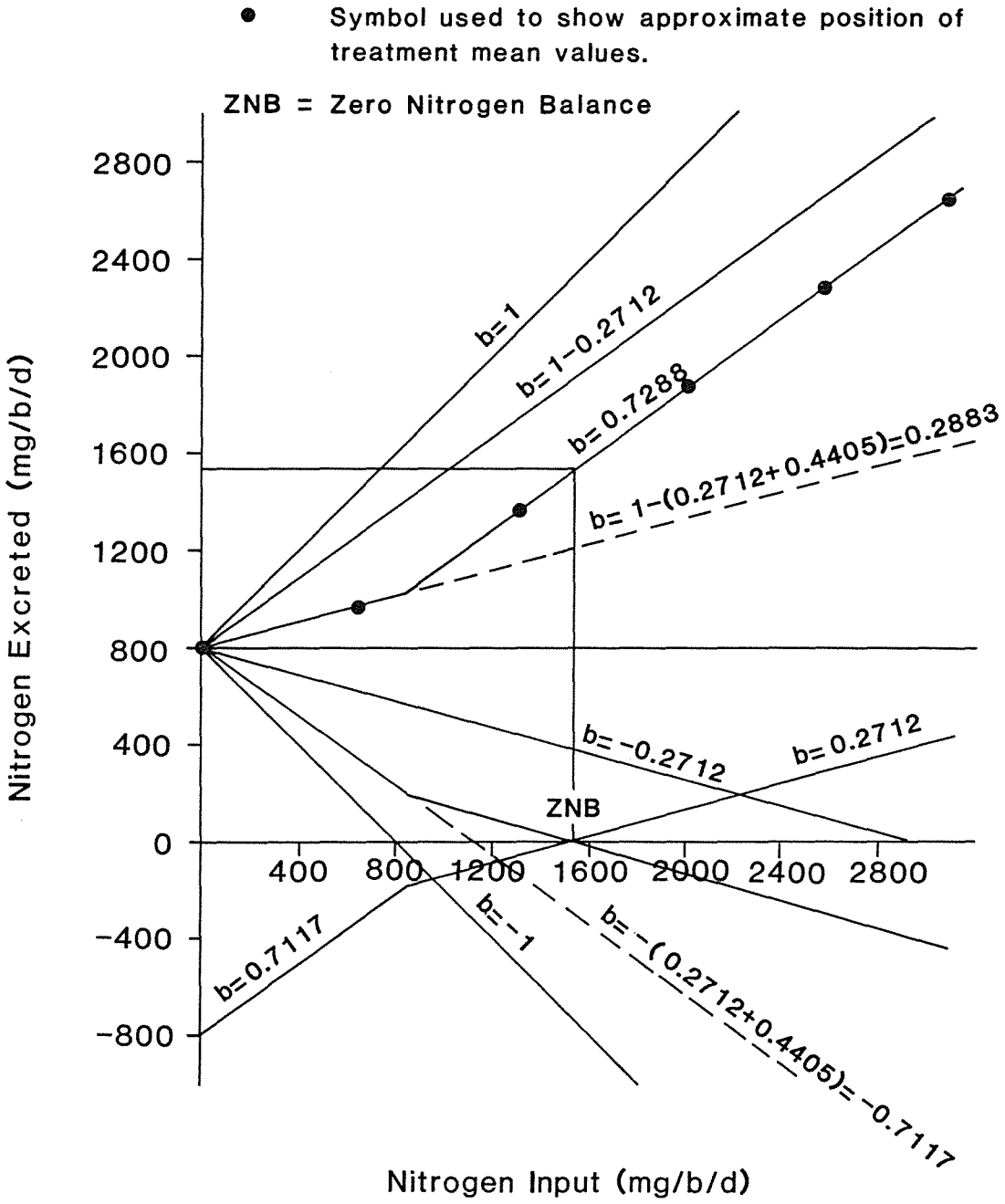


Fig. 4.5 The relationship between nitrogen input and nitrogen excreted.

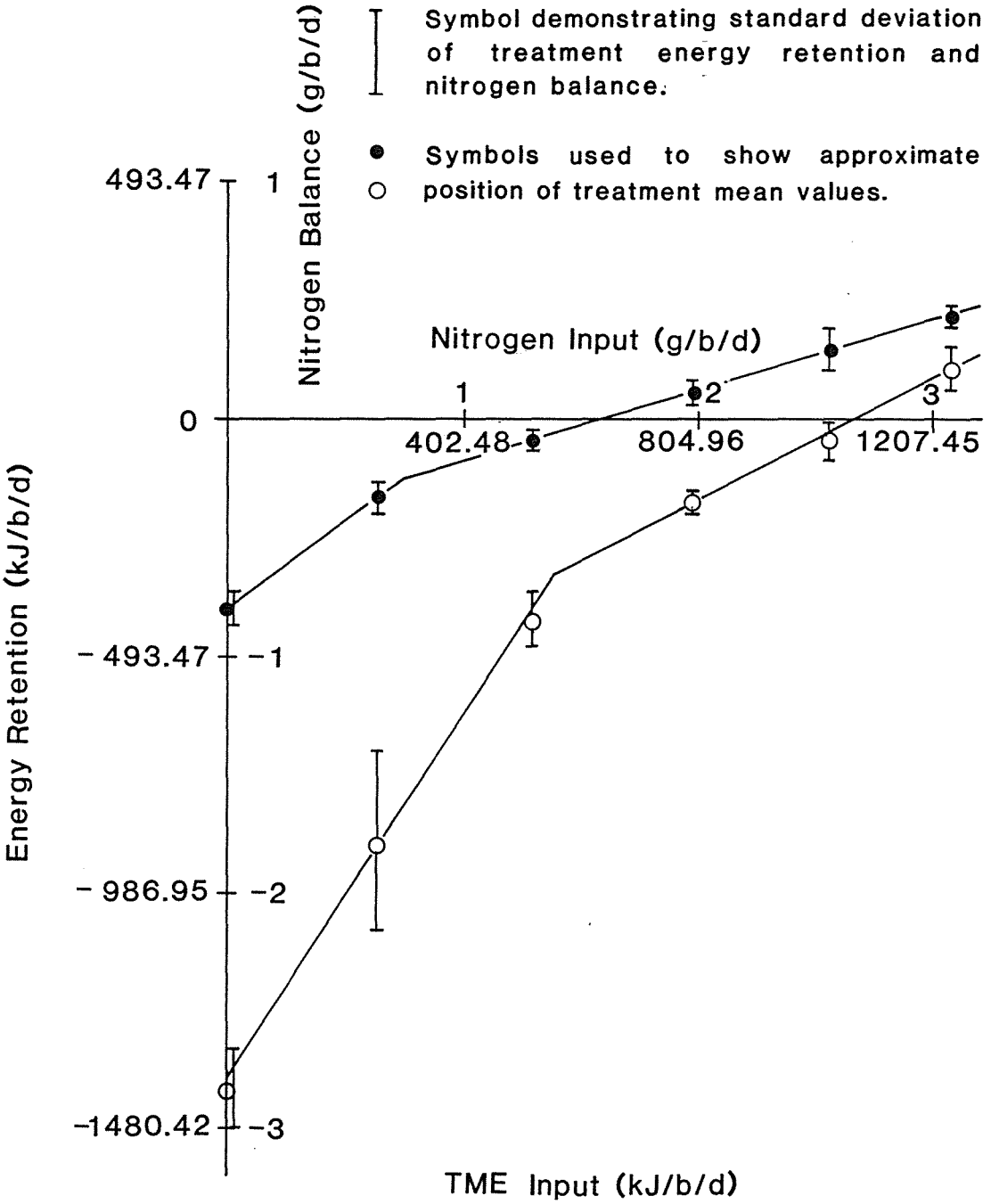


Fig. 4.6 Relationship between nitrogen input and nitrogen balance (upper line) and between TME input and energy retention (lower line).

$Y_1 = -0.7954 + 0.7117X$ df = 9 $S_b = 0.1695$ $r^2 = 0.662$ $0 \leq X \leq 0.7725$

$Y_2 = -0.4152 + 0.2712X$ df = 22 $S_b = 0.0463$ $r^2 = 0.609$ $X \geq 0.7725$

and for energy retention:

$Y_1 = -1380.2 + 1.8384X$ df = 15 $S_b = 0.2949$ $r^2 = 0.722$ $0 \leq X \leq 577.12$

$Y_2 = -682.4 + 0.6293X$ df = 16 $S_b = 0.1175$ $r^2 = 0.642$ $X \geq 577.12$

Note: $Y_1 = Y_2$ when $X = \dfrac{a_1 - a_2}{b_2 - b_1}$

Whereas on the basis of the regression line $Y_2 = -0.4152 + 0.2712X$, ZNB was achieved at 1.531 g NI/b/d which was equivalent to an intake of 1.531 x 402.482 = 616.2 kJ TME/b/d, zero energy retention occurred at 1084.4 kJ TME/b/d and 2.694 g NI. Put more directly, when zero body weight change was being experienced at an intake of 1084.4 kJ TME/b/d and 2.694 gN, body N was accumulating at $Y_2 = -0.4152 + 0.2712 \times 2.694$ = 0.315 g/b/d and at ZNB a steady loss of body weight amounting to Y_2 = (682.4 + 0.6293 x 616.2)/20.934 = 14.07 g/b/d was occurring.

The opportunity was taken to use the results to hand to determine the energy of endogenous excreta associated with each unit of endogenous excreta N eliminated per day. This was achieved by regressing bird mean EO on bird mean NO as obtained for birds of Treatment A (starved controls). The slope of the regression equation:

$Y = 19.29 + 33.81X$ $r^2 = 0.889$ $S_b = 5.96$ df = 4

indicated 33.81 kJ was attributable to each g of endogenous excreta N output. This compares with 36.51 kJ/gN from Titus *et al.* (1959).

A series of regression equations were obtained for estimating TME and TME_n (TME_{ZNB}):

(i) Uncorrected (Unc.). Bird mean EO was regressed on bird mean EI.

(ii) Nitrogen correction (N). Bird mean N balance multiplied by 36.51 i.e. [36.51(NI - NO)] was regressed on bird mean EI.

(iii) Corrected (C). Bird mean EO corrected to ZNB was regressed on bird mean EI. Corrected values were obtained by adjusting each bird mean EO value according to the following expression:

Corrected EO = EO + 36.51(NI - NO).

From each of the above relationships 2 slopes were produced. One obtained by using data of birds of Treatments A and B, and the other from using data of birds of Treatments B to F. In addition a further slope was generated by employing all 6 treatments A to F. TME and TME_n values were calculated using the expression $TME=(1-b)GE$ and $TME_n=(1-b_n)GE$ respectively. TME value obtained from the initial slope (Treatments A and B) was represented by the expression $TME_{in}=(1-b_{in})GE$, whilst for the subsequent slope (Treatments B to F), the expression $TME_s=(1-b_s)GE$ was represented. The corresponding linear regression equations, df, r^2, 95% confidence intervals of the slopes and the various TME and TME_n values are given in Table 4.8. Fig. 4.7 provides a graphical representation. The corresponding analysis of variance tables are given in Table A11.

Table 4.8

Summary of Regression Determinations and TME/TME_n Estimations

Code	Intercept a	St.dev. S_a	Slope b	St.dev. S_b	95% C.L. S_b	df	r^2	---TME (kJ/g)---	95% C.L.	TME (kcal/kg)	95% C.L.
Unc.											
T. A + B	46.328	4.250	0.1379	0.0200	0.0927 0.1831	9	0.841	14.406	13.650 15.160	3441	3260 3621
T. B to F	29.845	4.487	0.1844	0.0043	0.1757 0.1931	27	0.986	13.628	13.482 13.773	3255	3220 3289
T. A to F	39.118	3.132	0.1764	0.0033	0.1697 0.1830	33	0.989	13.762	13.651 13.874	3287	3314 3261
a= A + B, b= B to F*	46.328	-	0.1844	-	-	-	-	14.179	-	3387	-
N											
T. A + B	-29.041	2.667	0.0527	0.0125	0.0244 0.0810	9	0.662	-	-	-	-
T. B to F	-18.708	2.477	0.0228	0.0024	0.0180 0.0276	27	0.777	-	-	-	-
T. A to F	-24.589	1.912	0.0279	0.0020	0.0239 0.0320	33	0.856	-	-	-	-
C											
T. A + B	17.287	3.213	0.1906	0.0151	0.1564 0.2248	9	0.946	13.524	12.953 14.096	3230	3094 3367
T. B to F	11.137	4.054	0.2073	0.0039	0.1993 0.2153	27	0.991	13.245	13.112 13.379	3164	3132 3195
T. A to F	14.529	2.488	0.2043	0.0026	0.1990 0.2096	33	0.995	13.295	13.207 13.384	3176	3154 3197
a= A + B, b= B to F*	17.287	-	0.2073	-	-	-	-	13.451	-	3213	-

Notes: The expression GE - bGE has been used to calculate all TME/TME_n values except in rows marked with an *.

*The TME/TME_n in these rows has been calculated using the operational expression GE-EO+EE/g in which g = 29.924 and GE = 500 kJ. EE = the intercept given. EO is based on the slope given.

Unc. = TME values not corrected to zero N balance.

N = The results of regression of (NI - NO)36.51 on energy input.

C = TME values corrected to zero N balance.

T. A + B, T. B to F, T. A to F= Regression employs data from birds of Treatments A + B or B to F etc. as stated. The data of bird coded as number 9 of T. B was not used in the above calculations.

The analyses of variance associated with the regressions are given in Table A11.

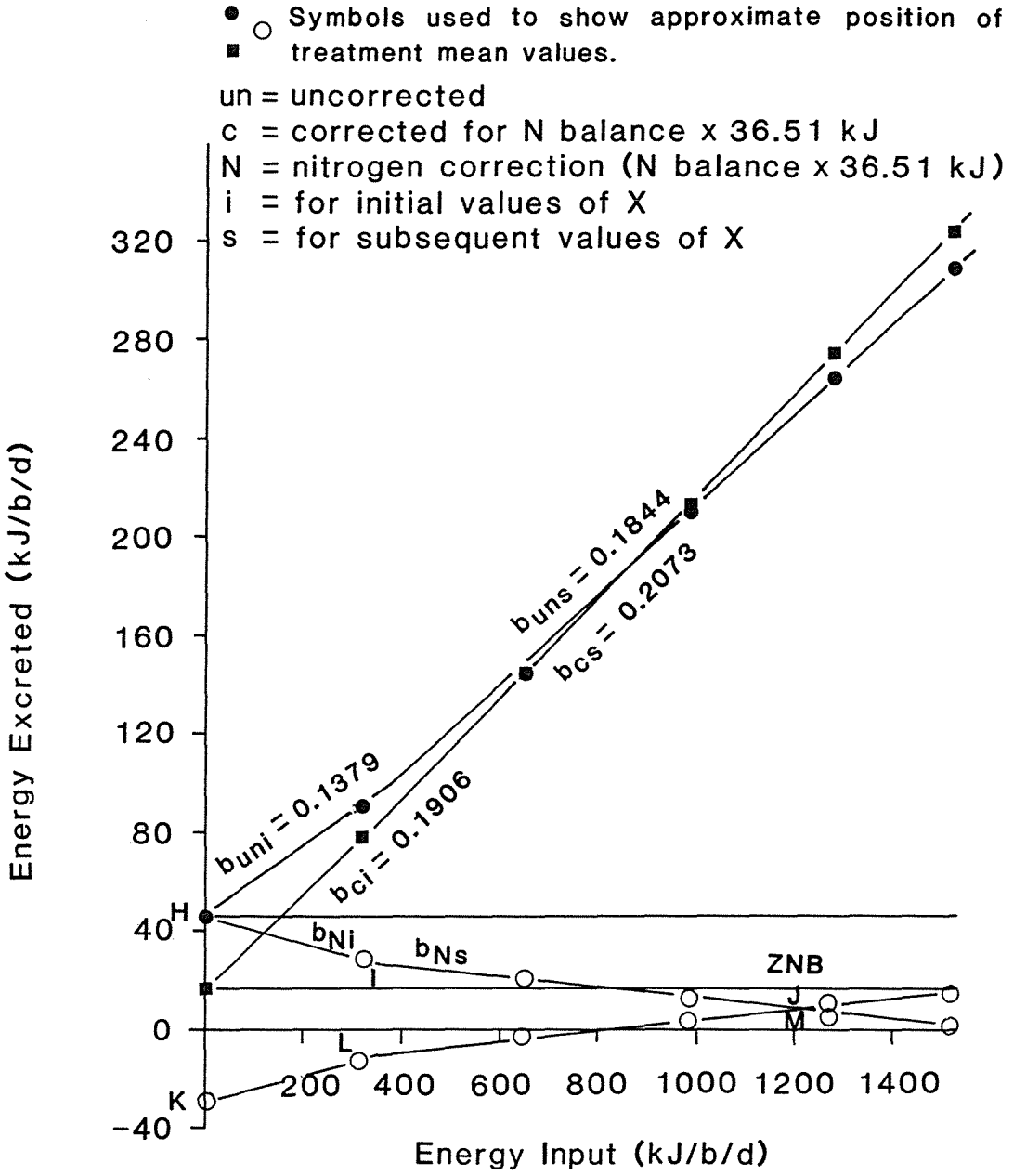


Fig. 4.7 Regression relationships for energy excreted on energy input, nitrogen balance x 36.51 kJ on energy input and energy excreted corrected for nitrogen balance x36.51 kJ on energy input.

The TME and TME_n were also calculated using the operational expressions:

$$TME = \frac{GE - EO + EE}{g} \qquad 4.1$$

and

$$TME_n = \frac{GE - EO_n + EE_n}{g} \qquad 4.2$$

in which in equation 4.1 EO was obtained using the slope from uncorrected Treatments B to F and endogenous excreta energy (EE) using the intercept obtained from uncorrected Treatments A and B. In equation 4.2, EO_n was obtained by using the slope of corrected Treatments B to F and EE_n by using the intercept of the slope of corrected Treatments A and B. TME and TME_n values obtained in this way are given in Table 4.8.

The TME and TME_n/TME_{ZNB} results from the various measurements are summarized as follows. Values are kcal/kg.

Quantity Measured	TME	TME_{ZNB}	TME_u
$(1 - b_{in})GE^*$	3441	3230	-
$(1 - b_s)GE^*$	3255	3164	3255
$GE - EO + EE^*$	3387	-	-
$GE - EO_n + EE_n$	-	3213	-

* Refers to column headed TME

The slopes between pairs of consecutive treatments, B and C, C and D, D and E and E and F were obtained for the relationships, uncorrected (Unc.), N corrected (N) and corrected (C) as referred to above. The mean and standard deviation (S) of the values of each relation was obtained and examined against the slopes of the corresponding relationships generated for values of Treatments A and B. The results are set out as shown in Table 4.9. Values are slopes.

The slopes fitted to data of Treatments A and B differs from the mean of slopes fitted between consecutive pairs of treatments other than A and B by more than 2 standard deviations for the relationship "uncorrected" and "energy equivalent nitrogen balance" but is within 2 standard deviations for the relationship "corrected" for Treatment pairs EF, DE and BC and is only just outside that for Treatment pairs C and D.

Table 4.9

The Slopes Obtained by Regressions of Energy Excreted on Energy Input (Uncorrected), Energy Equivalent Nitrogen Balance on Energy Input and Energy Excreted Corrected for Energy Equivalent Nitrogen Balance on Energy Input (Corrected) for Various Combination of Treatments

	Regression		
Derived from Treatments	Uncorrected	Energy Equivalent Nitrogen Balance [36.51(NI - NO)]	Corrected
B and C	0.1695	0.0268	0.1963
C and D	0.1936	0.0215	0.2151
D and E	0.1854	0.0174	0.2028
E and F	0.1798	0.0168	0.1967
$\bar{X}$	0.1821	0.0206	0.2027
S	0.0101	0.0046	0.0088
A and B	0.1379	0.0527	0.1906

4.1.4 DISCUSSION

The slopes of the 2 regression lines describing the relation between NI and NO of b_1 = 0.2883 and b_2 = 0.7288 are taken to indicate that NO varies over the range of NI showing less excretion of N per unit NI at low intake values of N. This suggests that the N retained per unit of NI at low values of NI exceeds that at high values. The cause is attributed, according to the experimental model, to a dietary induced sparing effect on tissue protein breakdown. It also suggests that the rate at which ZNB is achieved is greater at low values of NI. This is shown in Fig. 4.5 by the line KLM in which ZNB is approached initially from a negative balance position at a rate of 0.7117 and subsequently at a rate of 0.2712 units of NO per unit NI.

It should be pointed out that the ZNB point of 1.531 g/b/d applies to N balance associated with N of excreta only. It does not take into account N loss arising from scale and feather loss. In this sense it is an underestimation of the actual ZNB point and is thought to account for the apparent incompatability between the occurence of ZNB at a TME intake of 616.2 kJ/b/d and the occurence of zero energy retention at 1084.4 kJ/b/d TME intake (Fig. 4.6 refers).

Energy value attributed to endogenous NO has been taken to be 36.51 kJ/gN (Sibbald and Morse, 1983b) and the value estimated in this trial was 33.81 kJ/gN. Using the "taken" value the regression lines relating energy due to N balance i.e. (NI - NO)36.51 to EI is shown in Fig. 4.7. This illustrates that the energy contributing to EO from N balance changes more rapidly at low values of EI (b_1 = 0.0527 versus b_2 = 0.0228). When for this relationship the mean and standard deviation of the slopes between consecutive pairs of treatments for "high" values were compared with the slope for Treatments A and B (Table 4.9), that of the latter lay beyond 2 standard deviations of the mean. From these results it seems reasonable to expect that the EO to EI slope would differ between small and large values of EI.

In consideration of the above, 2 regression lines were fitted to the EO to EI data with slopes of b_1 = 0.1379 and b_2 = 0.1844. Though the 95% confidence intervals of the slopes overlap the evidence of NO and energy

of N balance strongly support a deviation from linearity over the EI range. In addition when the mean and standard deviation of the slopes between consecutive pairs of treatments for "high" EI values for this relationship were compared with the slope for Treatments A and B, that of the latter lay beyond 2 standard deviations of the mean.

To correct to ZNB, EO values for each EI level were adjusted by an amount given by the N balance operating x 36.51 kJ/gN. Alternatively the expression for the slope and intercept obtained by regressing EO on EI can be corrected to ZNB by adding to it the slope and intercept obtained for the relation N balance x 36.51 kJ/gN regressed on EI, see Fig. 4.7. When this is done the initial slope sums to 0.1379 + 0.0527 = 0.1906 and the subsequent slope to 0.1844 + 0.0228 = 0.2072.

Regression lines were fitted to the N corrected data of Treatments A and B, Treatments B to F and to Treatments A to F. The resulting slopes, which respectively, were 0.1906, 0.2073 and 0.2043, were in closer agreement than the corresponding uncorrected slopes of 0.1379, 0.1844 and 0.1764. The closeness of values is illustrated in Fig. 4.7. In addition when the mean and standard deviation of the slopes between consecutive pairs of treatments for the "corrected" relations were compared with the slope for Treatments A and B, that of the latter lay within 2 standard deviations of the mean. The result suggests that the slopes between consecutive pairs of treatments for the relation EI to EO corrected to ZNB belongs to 1 population and that 1 slope satisfactorily represents the relationship.

The theoretical model of Chapter 3 suggested that for TME estimated by the regression approach, values obtained for feed intakes defining subsequent slopes:

$$TME = (1 - b_s)GE$$

result in unbiassed estimates, TME_u. On this basis the slope relating to TME_u is given by that obtained for uncorrected Treatments B to F i.e. 0.1844. The model also suggests that correcting TME to ZNB, whilst

removing the bias caused by UmE introduces a secondary distortion, $[E(1-b_{Nr})NI_{ge}]GE$ related to the partial correction of UeE whose value is given as $E(Ya_{Nm}) = [a_{Nm}-E(1-b_{Nr})NI_{ge}]GE$. On this basis the distortion in the slope $[E(1-b_{Nr})NI_{ge}]GE$ is estimated as 0.2072 - 0.1844 = 0.0228 units of EO per unit of EI.

According to the theoretical study the slope characteristic of unbiassed TME is 0.1844 which equates with a TME_u of 13.628 kJ/gN or 3255 kcal/kg.

4.2 **EXPERIMENT 2 — LN 204**

4.2.1 OBJECTIVES

(a) To evaluate the TME of meat and bone meal (M & B) when assayed directly and as an ingredient of a whole diet.

(b) To examine the effect on these values of correcting to zero N balance (ZNB).

(c) To derive the TME and TME_n of the high protein diet assayed in Experiment 1 using the force feeding technique.

4.2.2 MATERIALS, METHODS AND TREATMENTS

The method for objectives (a) and (b) was to obtain the excreta output of individuals of 3 treatments of 6 birds fed 0, 20 and 40 g of basal and again when fed mixtures as follows:

Basal	40	40	40
M & B	0	8	16
	40	48	56 g of compound diet.

The M & B contribution to TME, N excreted and weight of excreta would be estimated by regression techniques. To a further group of 3 x 6 cockerels M & B was force fed at 0, 8 and 16 g using a 24 h withholding and collection period. Using the same procedure this latter group of cockerels was used in a subsequent period to meet objective (c).

Thirty-six leg banded Sykes cockerels aged 73 weeks of age (WOA) on Wednesday 9 May 1984 were ranked according to body weight and sorted into 6 treatments of 6 birds so as to give matching mean treatment weights. The procedure of birds having access to food for 1 h per day

was pursued with feed presented between 1 and 2 p.m. The feed used to maintain the birds between experiments was a pelleted low energy diet. This diet is detailed in Table A9 (see Appendix).

The dietary and nutrient composition of the basal treatment diet and the basal plus M & B at the 8 g per bird level and the 16 g level and also the nutrient composition of M & B are given in Tables A7 and A8. These diets together with the single ingredient M & B were pelleted at the Feed Processing Unit. The high protein LN 202 diet was in pellet form and it was from the same batch used in Experiment 1. Its composition is given in Table A6.

Feed time for the assay started at 1 p.m. daily. The basal was fed to Treatments D, E and F at 0, 20 and 40 g/b/day respectively for 1 day for Treatments D and E and for 4 days for Treatment F by presenting weighed amounts of food. The basal plus M & B at levels of 8 and 16 g/b/day M&B were fed to Treatments D and E respectively for 4 days commencing on the day following feeding of the basal. During the assay birds of appropriate treatments were allowed up to 1.75 h to consume their allocation.

For Treatments A, B and C forced feeding with a 24 h withholding and collection period was employed. The Treatments A, B and C received 0, 8 and 16 g of M & B per bird on 1 day in the first assay and in the second by using the same technique groups received 0(A), 12(B) and 24(C) g of LN 202 diet per bird. The design of the assay is shown in Table 4.10.

A sample of all diets fed were taken for amino acid, N and gross energy determination.

Pots containing the daily collections of each bird's excreta were freeze dried, ground, cleared of contaminants and later their air dry weight obtained. Samples of about 1 g of air dry excreta for each bird day for Treatments A, B and C were analyzed for N content. In the case of Treatments D, E and F, only D Mon. and E Mon. were N analyzed on an each bird basis. For Treatments D, E and F, Wed. through to Fri., samples of air dry excreta on an individual bird basis were drawn for N determination by weighting for daily excreta weights. Samples of air

Table 4.10

Timetable of Assay Procedures for Experiment LN 204

	Treatment					
	D	E	F	A	B	C
Cages	49-54	55-60	61-66	67-72	73-78	79-84
Sat. (12/5)	Basal	Basal	Basal			
	wk	wk	wk	hr	hr	hr
Sun. (13/5)	DF(0)	DF(20)	DF(40)	wk	wk	wk
Mon. (14/5)	Basal +	Basal +		M & B	M & B	M & B
	M & B	M & B				
	CF(48)	CF(56)	DF(40)	DF(0)	DF(8)	DF(16)
Tue. (15/5)	DF(48)	DF(56)	CF(40)	CFF	CFF	CFF
Wed. (16/5)	CF(48)	CF(56)	CF(40)	FF	FF	FF
Thu. (17/5)	CF(48)	CF(56)	CRFF	FF	FF	FF
Fri. (18/5)	CRFF	CRFF	-	hr	hr	hr
Sat. to Tue.						
(19 -- 22/5)	-	-	-	hr	hr	hr
Wed. (23/5)	-	-	-	wk	wk	wk
Thu. (24/5)	-	-	-	HP	HP	HP
	-	-	-	DF(0)	DF(12)	DF(24)
Fri. (25/5)	-	-	-	CRFF	CRFF	CRFF

hr = Provide 1 h of access to food.

wk = Withholding period of 24 h beginning from start of assay i.e. about 1 p.m.

D = Dispose of manure and clean collecting tray for subsequent collection.

C = Collect excreta.

F = Feed by presenting the treatment amount for 1 h or by forced feeding.

R = Return birds to holding cages.

FF = Give the birds access to food 24 h a day.

HP = LN 202 diet.

dry excreta on an individual bird basis for appropriate treatments were drawn for gross energy determination by weighting for daily excreta weights.

Amino acid, N and gross energy determination procedures have been described earlier (p. 102). Statistical procedures utilized the "Minitab" statistical programme referred to in section 4.1.2.

4.2.3 RESULTS

The analyzed N and gross energy of the treatment diets of LN 202 and LN 204 are shown in Table 4.11.

The regression relationships between treatment combinations for N excreted (NO) regressed on N input (NI), energy excreted (EO) regressed on energy input (EI), EO corrected for energy equivalent N balance regressed on EI and energy equivalent N balance regressed on EI are given for M & B by basal diet, M & B by force feeding and diet LN 202 in Tables 4.12, 4.13 and 4.14 respectively.

Table 4.15 gives the food intake, energy, N and N balance data means over the final 3 days for Treatments F, D and E meat and bone meal. Tables 4.16 and 4.17 give the energy, N and energy equivalent N balance data of Treatments A, B and C meat and bone meal and A, B and C diet LN 202 respectively. Data of birds 10 and 18 of Treatments A, B and C meat and bone meal and data of bird 7 of Treatments A, B and C LN 202 diet were discarded because they deviated greatly from the treatment mean.

Table 4.11

Analyzed Nitrogen and Gross Energy of Treatment Diets of LN 202 and LN 204

Diet	Mean Nitrogen Over 2 Analyses (%)	Gross Energy kJ/g	Gross Energy kcal/kg
Basal + 0 M & B	1.557	15.4542	3691.17
Basal + 8 M & B	2.668	16.0565	3835.03
Basal + 16 M & B	3.603	16.3509	3905.35
M & B	8.284	18.4511	4406.97
LN202 Diet	3.386	16.7089	3990.85

Table 4.12

Meat and Bone Meal -- By Basal -- Regression Relationships

Measure* & Treatments	a	St.Dev. S_a	b	St.Dev. S_b	df	r^2	TME kJ/g	TME kcal/kg
NO on NI(mg)								
FDE	535.9	87.6	0.869	0.063	16	92.7	-	-
FD	278.8	77.8	1.190	0.082	10	95.9	-	-
DE	1045.4	196.6	0.578	0.118	10	72.7	-	-
FE	468.6	65.4	0.874	0.045	11	97.4	-	-
EO on EI(kJ)								
FDE	-17.9	11.6	0.306	0.015	16	96.4	12.798	3057
FD	-59.8	10.5	0.371	0.016	10	98.4	11.602	2771
DE	49.0	25.4	0.227	0.031	10	85.9	14.259	3406
FE	-20.5	9.8	0.306	0.013	11	98.2	12.807	3059
Corrected EO on EI(kJ)								
FDE	-47.5	5.1	0.328	0.007	16	99.4	12.399	2961
FD	-57.4	3.2	0.343	0.005	10	99.8	12.122	2895
DE	-29.4	13.9	0.307	0.017	10	97.4	12.796	3056
FE	-47.9	5.7	0.328	0.008	11	99.5	12.405	2963
(NI-NO)x0.03651(kJ) on EI								
FDE	-29.6	8.3	0.022	0.011	16	20.3	-	-
FD	2.5	8.9	-0.028	0.013	10	33.4	-	-
DE	-78.4	18.0	0.079	0.022	10	59.6	-	-
FE	-27.5	5.9	0.022	0.008	11	43.8	-	-

*NO on NI = Nitrogen excreted regressed on N input.

EO on EI = Energy excreted regressed on energy input.

Corrected EO on EI = Energy excreted corrected for energy equivalent N balance regressed on energy input.

(NI-NO)x0.03651 on EI = Energy equivalent N balance in kJ regressed on energy input.

Table 4.13

Meat and Bone Meal -- Force Feeding -- Regression Relationships

Measure* & Treatments	a	St.Dev. S_a	b	St.Dev. S_b	df	r^2	TME kJ/g	TME kcal/kg
NO on NI(mg)								
ABC	833.8	77.7	0.599	0.094	15	74.4	-	-
AB	814.3	92.6	0.699	0.207	10	55.8	-	-
BC	950.9	217.1	0.493	0.207	9	41.4	-	-
AC	814.3	76.1	0.596	0.085	10	84.5	-	-
EO on EI(kJ)								
ABC	48.2	2.8	0.262	0.015	15	95.8	13.619	3253
AB	48.0	3.4	0.267	0.034	10	87.3	13.534	3233
BC	49.4	7.8	0.257	0.034	9	88.0	13.709	3274
AC	48.0	2.7	0.262	0.014	10	97.7	13.621	3253
Corrected EO on EI(kJ)								
ABC	17.8	0.9	0.328	0.005	15	99.7	12.405	2963
AB	18.3	0.9	0.316	0.009	10	99.2	12.622	3015
BC	14.7	2.5	0.340	0.011	9	99.2	12.174	2908
AC	18.3	1.0	0.328	0.005	10	99.8	12.397	2961
(NI-NO)x0.03651(kJ) on EI								
ABC	-30.44	2.8	0.066	0.015	15	56.7	-	-
AB	-29.73	3.4	0.049	0.034	10	19.0	-	-
BC	-34.72	7.9	0.083	0.034	9	42.9	-	-
AC	-29.73	2.8	0.066	0.014	10	71.5	-	-

*NO on NI = Nitrogen excreted regressed on N input.

EO on EI = Energy excreted regressed on energy input.

Corrected EO on EI = Energy excreted corrected for energy equivalent N balance regressed on energy input.

(NI-NO)x0.03651 on EI = Energy equivalent N balance in kJ regressed on energy input.

Table 4.14

LN 202 Diet -- Force Feeding -- Regression Relationships

Measure* & Treatments	a	St.Dev. S_a	b	St.Dev. S_b	df	r^2	TME kJ/g	TME kcal/kg
NO on NI(mg)								
ABC	832.8	85.6	0.536	0.161	16	42.4	-	-
AB	850.9	73.3	0.385	0.268	10	18.7	-	-
BC	727.7	244.3	0.688	0.370	10	27.7	-	-
AC	851.0	106.0	0.536	0.185	11	45.8	-	-
EO on EI(kJ)								
ABC	51.0	4.8	0.134	0.018	16	78.5	14.463	3455
AB	52.5	4.5	0.109	0.033	10	54.2	14.896	3558
BC	42.1	13.0	0.160	0.040	10	64.4	14.029	3351
AC	52.5	5.7	0.134	0.020	11	81.9	14.463	3455
Corrected EO on EI(kJ)								
ABC	20.6	2.1	0.169	0.008	16	96.8	13.890	3318
AB	21.4	2.1	0.154	0.015	10	91.7	14.136	3376
BC	15.5	5.4	0.184	0.017	10	93.1	13.643	3259
AC	21.4	2.4	0.169	0.009	11	97.5	13.890	3318
(NI-NO)x0.03651(kJ) on EI								
ABC	-30.4	3.1	0.034	0.012	16	35.5	-	-
AB	-31.1	2.7	0.046	0.020	10	37.0	-	-
BC	-26.6	8.9	0.023	0.027	10	7.3	-	-
AC	-31.1	3.9	0.034	0.014	11	38.7	-	-

*NO on NI = Nitrogen excreted regressed on N input.

EO on EI = Energy excreted regressed on energy input.

Corrected EO on EI = Energy excreted corrected for energy equivalent N balance regressed on energy input.

(NI-NO)x0.03651 on EI = Energy equivalent N balance in kJ regressed on energy input.

Table 4.15

Food Intake, Energy, Nitrogen and Nitrogen Balance Data Means over Final 3 Days of Treatments F, D and E Meat and Bone Meal

T	Bird	gFood/b/d	(EI) kJ/b/d	(EO) kJ/b/d	(NI) mg/b/d	(NO) mg/b/d	(NI-NO) x0.03651 kJ/g	(NI-NO) x0.03651 + EO kJ/g
F_3*	1	38.85	600.4	163.1	604.7	997.8	-14.353	148.7
	2	39.16	605.2	164.0	609.5	999.4	-14.233	149.8
	3	39.29	607.1	164.4	611.5	1000.0	-14.186	150.2
	4	38.70	598.0	162.7	602.3	997.0	-14.411	148.2
	5	38.84	600.3	163.1	604.6	997.8	-14.355	148.7
	6	38.95	601.9	163.4	606.3	998.3	-14.314	149.1
D_3*	7	46.62	748.5	224.9	1243.5	1862.0	-22.581	202.3
	9	47.16	757.3	218.5	1258.1	1735.3	-17.424	201.1
	10	45.80	735.4	219.0	1221.8	1903.8	-24.899	194.1
	11	46.99	754.5	215.8	1253.5	1667.0	-15.095	200.7
	12	47.14	757.0	216.6	1257.5	1653.5	-14.457	202.2
E_3*	13	54.99	899.1	246.3	1981.3	2088.1	- 3.900	242.4
	14	54.53	891.6	260.2	1964.7	2248.8	-10.372	249.8
	15	54.18	885.8	260.4	1952.0	2416.6	-16.963	243.4
	16	54.38	889.2	252.7	1959.3	2224.6	- 9.686	243.0
	17	55.26	903.5	243.1	1990.9	2033.5	- 1.555	241.6
	18	51.06	834.9	238.7	1839.7	2017.6	- 6.495	232.2

* Subscripts refer to final 3 days collections.

Table 4.16

Energy, Nitrogen and Energy Equivalent Nitrogen Balance Data of Treatments A, B and C Meat and Bone Meal

Treatments	Bird*	(EI) kJ/b/d	(EO) kJ/b/d	(NI) mg/b/d	(NO) mg/b/d	(NI-NO) x0.03651 kJ/g	EO+(NI-NO) x0.03651 kJ/g
A	1	0	44.2	0	605.2	-22.10	22.14
	2	0	37.3	0	603.7	-22.04	15.28
	3	0	53.5	0	952.8	-34.79	18.77
	4	0	48.1	0	802.0	-29.28	18.90
	5	0	47.2	0	805.0	-29.39	17.83
	6	0	57.7	0	1117.1	-40.70	16.91
B	7	147.6	101.1	662.7	1695.4	-37.70	63.40
	8	147.6	79.4	662.7	1055.7	-14.35	65.13
	9	147.6	79.3	662.7	1091.3	-15.65	63.73
	11	147.6	83.9	662.7	1222.5	-20.44	63.52
	12	147.6	92.9	662.7	1321.4	-24.05	68.91
C	13	295.2	129.5	1325.4	1634.3	-11.28	118.29
	14	295.2	130.5	1325.4	1852.7	-19.25	111.31
	15	295.2	119.4	1325.4	1467.9	- 5.20	114.24
	16	295.2	128.4	1325.4	1635.7	-11.33	117.08
	17	295.2	118.6	1325.4	1427.5	- 3.73	114.85

* Data of birds 10 and 18 were discarded.

Table 4.17

Energy, Nitrogen and Energy Equivalent Nitrogen Balance Data of Treatments A, B and C LN 202 Diet

Treatments	Bird*	(EI) kJ/b/d	(EO) kJ/b/d	(NI) mg/b/d	(NO) mg/b/d	(NI-NO) x0.03651 kJ/g	EO+(NI-NO) x0.03651 kJ/g
A	1	0	52.1	0	772.9	-28.22	23.84
	2	0	31.7	0	551.9	-20.15	11.58
	3	0	64.2	0	1110.8	-40.56	23.64
	4	0	60.5	0	1026.2	-37.47	23.01
	5	0	46.4	0	754.8	-27.56	18.83
	6	0	60.1	0	888.6	-32.44	27.68
B	8	200.5	86.9	406.3	1183.9	-28.39	58.55
	9	200.5	76.2	406.3	1012.5	-22.13	54.06
	10	200.5	60.2	406.3	778.0	-13.57	46.63
	11	200.5	73.7	406.3	1008.5	-21.99	51.70
	12	200.5	74.2	406.3	1053.2	-23.62	50.57
C	13	401.0	114.2	812.6	1506.0	-25.32	88.89
	14	401.0	112.4	812.6	1518.1	-25.76	86.63
	15	401.0	128.4	812.6	1562.0	-27.36	101.07
	16	401.0	105.7	812.6	1306.3	-18.02	87.66
	17	401.0	89.5	812.6	997.6	- 6.75	82.76
	18	401.0	88.2	812.6	830.2	- 0.64	87.54

* Data of bird 7 was discarded.

4.2.4 DISCUSSION

It was assumed on the evidence of Experiment 1 that force feeding of diet LN 202 to upper levels of 24 g would result in EO/EI slopes representative of initial slopes in the EO and EI 2 slope relationship. A similar assumption was made for the energy relationships of M & B force fed. For regressions involving Treatments D, E and F concerned with M & B mixed with basal, the slope was assumed to constitute the subsequent slope. TMEs given in Tables 4.12 to 4.14 were calculated using the equation:

$$TME/g = (1 - b)GE/g$$

where b was either assumed to measure initial (in) or subsequent (s) slopes.

As mentioned in the theoretical model of Chapter 3 and also according to King (1985), TME values may be defined in terms of initial slopes, $(1-b_{in})GE$, measuring the quantity GE-(FiE+UiE)+(1-X)UmE where X = proportion of UmE being excreted. (1-X)UmE is the quantity that gives rise to the slope change and which is a source of bias. TMEs are also defined in terms of subsequent slopes, $(1-b_s)GE$, a measure of unbiassed TME, TME_u, equal to GE-(FiE+UiE) and slopes corrected to energy equivalent ZNB, measuring the quantity TME_u + (a bias quantity related to UeE) and given as $[1-(b_s+E(1-b_{Nr})NI_{ge})]GE$.

In terms of these measurements the TME results are summarized and related to Experiment 1 (LN 202) results as shown in Table 4.18. The M & B assays resulted in TME values that differed, 3253 versus 3057 kcal/kg. This difference is attributed to the assertion that each is a measure of a different quantity. Correction to TME_{ZNB} resulted in values in close agreement i.e. 2963 versus 2961. The hypothesis proposed in explanation is that correction resulted in the same quantity being measured. Given this reasoning, the basal diet seemingly had no affect on the TME_{ZNB} of M & B.

Table 4.18

TME Measurements of Meat and Bone Meal and Diet LN 202

Material	Trial	TME Quantity Measured	TME kJ/g	TME kcal/kg	TME_{ZNB} kJ/g	TME_{ZNB} kcal/kg	TME_u kJ/g	TME_u kcal/kg
M & B	LN 204	$(1-b_{in})GE^*$	13.62	3253	12.41	2963	-	-
		$(1- b_s)GE^{**}$	12.80	3057	12.40	2961	12.80	3057
LN202								
Diet	LN 202	$(1-b_{in})GE^*$	14.41	3441	13.52	3230	-	-
		$(1- b_s)GE^{**}$	13.63	3255	13.25	3164	13.63	3255
	LN 204	$(1-b_{in})GE^*$	14.47	3455	13.89	3318	-	-

* Refers to column headed TME.

** Refers to column headed TME and TME_u.

The TME values of the whole diet obtained in the previous experiment in terms of initial and subsequent slopes were 3441 and 3255 respectively. In this trial, TME by force feeding procedures was 3455. It is postulated that similar values 3441 and 3455 were obtained because the same quantity was measured. However, correction to ZNB increased divergence, the values 3230 and 3318 are markedly different. The divergence was caused by different NO. This suggests that although total EO per unit of energy fed was the same between assay treatments, the proportions of energy yielding excreta components differed fundamentally and specifically in respect to N. It suggests that correcting to a N base line may not always work to improve precision.

In view of the results with respect to LN 202 diet, the discrepancy of the values of TME_{ZNB} may be attributed, as alluded in the introduction chapter, to the difference in assay procedures and environment. As also evidence presented in section 2.4, factors such as environmental temperature, stock and age may interfere with AME_n values. These factors are however likely to influence TME_n values.

The assay procedures employed in Experiment LN 202 and LN 204 varies. The former used trained cockerels as in the Farrell rapid bioassay techniques, whilst the latter employed force feeding procedures. Both trials used the same cockerels but separated in time by 4 weeks (April, May).

However the variation in assay procedures does not appear to influence the TME_{ZNB} values as evidence in the results of the M & B determination. Thus, the divergence caused by difference in NO in TME_{ZNB} of diet LN 202 could be attributed to ambient temperature that was not standardized.

CHAPTER 5

SUMMARY AND CONCLUSIONS

An objective of this thesis has been to examine the quantitative nature of N excretion (NO) from adult cockerels fed a complete diet and to determine the effect of such excretion on TME values. Experiment 1, LN 202 indicated that NO per unit of N fed was markedly less at "low" intake values than at "high". King (1984) proposed a model to explain the form of this NO profile. The model introduced a term for NO arising directly from metabolized food. The model states that at low N intakes (NI) dietary protein spares catabolism of tissue protein which results in a lesser excretion slope than at greater intakes. The model attributes differences in the TME recorded over the range of intakes to this feature. It demonstrates that by attributing an energy value to urinary N, the slope, energy excreted (EO) on energy input (EI) (on which TME is based), contains an additional component at "low" EI levels which is a source of bias. In the model correcting for this component results in a slope measuring the same quantity as that produced at "high" energy intakes. According to the model correction to zero N balance (ZNB) over corrects for this component and introduces a bias.

In LN 202 the relations NO regressed on NI, energy equivalent N balance regressed on EI, EO regressed on EI and EO corected for energy equivalent N balance regressed on EI were evaluated. For each of the first 3 relations 2 slopes were evident. Correction of EO slopes for various degrees of energy equivalent N excretion resulted in changes in slope that corresponded in magnitude and direction with those predicted in the model. TME values obtained were "low intake" or "initial" TME, "high intake" or "subsequent" TME and TME corrected to ZNB.

In LN 204 the TME values of meat and bone meal (M & B) as determined by dietary inclusion and by direct supply were compared and assessed in terms of the model. Correction of the TMEs to ZNB resulted in a single value. TME assessment of a whole diet by 2 different assays resulted in similar values when the values, according to the model, estimated the same quantity and different values when the quantities measured were, as predicted by the model, different. Correction to ZNB caused like values

to deviate and unlike values to come closer together.

True metabolizable energy according to the model and the experimental evidence presented is a measure of the dietary energy available for partition to heat increment, maintenance and production functions. Alternatively it is a measure of the dietary energy available for heat production and storage of energy as protein, fat and carbohydrate in tissue or product. According to the model it is defined as:

$$TME = GE - (FiE + UiE)$$

and

$$TME = (1 - b_{FiE+UiE})GE.$$

The measure of the slope defining TME at "low intake" or "initial" values of EI was found to deviate from $b_{FiE+UiE}$ by an amount estimated as $-E(b_{Nr} - b_{Nna})Ni_{ge}GE$.

This value is the quantity by which the EO resulting from the food, $b_{FiE+UiE}GE$, is diminished over "low" input energy levels. This diminution arises, not through a reduction in feed energy excreted per unit of energy fed, but from a saving in the amount of tissue protein catabolism taking place. The deviation in the slope reflects improved utilization of available dietary energy at low intakes. On the other hand, the slope misrepresents the amount of available energy per unit of energy fed over both low and high levels of intake.

True metabolizable energy based on "subsequent" or "high intakes" of energy define the quantity $TME = (1 - b_{FiE+UiE})GE$. This is an unbiassed estimate of the dietary energy available over all levels of intake. For TME_{ZNB} the bias, induced by the nature of the correction:

$$E(1 - b_{Nr})Ni_{ge}GE$$

operates equally at all values of EI. For the whole diet of LN 202 it was found to be approximately half that operating for TME based on

"initial" EI levels.

For purposes of feed formulation the correlation between the assayed TME of diets and the sum of the TMEs contributed by the dietary components provides a measure of additivity. Though Sibbald (1977a) has brought together some such data for TMEs obtained using the 2 levels force feeding approach, there is inadequate information available for approaches expected to result in "subsequent" TME values and ZNB corrected values to compare on the grounds of correlation the additivity of the 3 measures. Indeed for methods producing "initial" and ZNB corrected TMEs such correlations do not as such indicate how well summing constituent values in either system would correlate with corresponding whole diet assayed values in the unbiassed system. Ultimately it is this latter relationship that would provide an illustration of how closely the system of measurement would be likely to provide for a prescribed quantity.

Working from the data of Sibbald (1977a), referred to above, the correlation between assayed dietary TME and calculated values was found to be 89%. This compares with 79% for Poultry Research Centre (PRC) data set out in Table 1.1. In these relations calculated values understated assayed whole diet values by a factor given by the slope of the regression line of approximately 0.82. For the PRC data the TME values used in deriving calculated diet values were not specifically those obtained from batches of ingredients used to form the whole diets whereas in the case of Sibbald (1977a) they were.

This point raises issues concerning the contribution made by regular monitoring of ingredients and the standardization of assays to additivity in sets of values. In the ME system of measurement values need updating as new batches of material come into use to take account of variation in quality. Ideally such updating should be based on assay conditions and methods that remain constant with time.

These factors, regular and standardized monitoring, together with the additive characteristics of the quantity measured, would seem to be major factors governing levels of additivity achieved. The Sibbald 2 levels force feeding approach meets requirements of simplicity and

economy of time and effort in respect to assay and laboratory procedures, important in long term monitoring work. It does on the other hand appear to result in biassed values. It is presumed that the level of bias operating is characteristic of the feedstuff as it is a measure of the degree to which the material spares tissue protein catabolism. If this sparing effect is additive then there is little reason to expect such biassed values to depart from additivity anymore than those of unbiassed values. In this case the merit of unbiassed values is that they estimate a quantity that is directly usable in other areas such as the partition of energy.

To obtain unbiassed values however requires another approach which in its unmodified form is based on regression methods in an assay that runs over several days and which involves more birds, more feed and feed preparation and increased excreta material and laboratory operations. The absence of TMEs based on this method is in part a reflection of the scope of and the time and effort involved in such procedures. This is not to say that the approach could not be simplified to more acceptable levels but the simplification procedures have yet to be evaluated and in the mean time the size of the procedure is too large for it to be conducted in general on a routine basis.

Assays for TMEs corrected to ZNB take the same form as the 2 levels force feeding approach. Laboratory analysis of N content is required on the food sample and on treatment excreta and excreta energy values require N based adjustments. The value obtained is biassed but the level of bias would appear to be characteristic of the feedstuff as it is a function of the rate at which N retained of the material replaces N lost through maintenance activity.

Again if it is assumed that the rate of N replacement is additive there seems little reason for supposing that such values will be any less additive than unbiassed values. The size of bias in such values may be somewhat less than that operating in the non corrected biassed approach.

Of the 3 quantities outlined the preceding rationale suggests that at present, complexities in assay technique preclude routine monitoring for unbiassed values. Of the 2 techniques resulting in biassed values, if

it is assumed that the bias operating is additive, then in terms of additivity neither is advantaged over the other and neither is disadvantaged relative to unbiassed values. The assay involving force feeding and 2 feeding levels without correction to ZNB is the simplest overall TME procedure but it is expected to result in larger bias and bias in an opposite direction to that of ZNB corrected values.

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APPENDIX

Table A1
Experiment LN 202: Individual Bird Body Weights at the Assay Start and at 24 Hour Intervals

Treatments & Birds	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	kg					
A 1	3.26	3.19	3.14	-	-	-
2	3.20	3.13	3.07	-	-	-
3	3.23	3.15	3.06	-	-	-
4	2.99	2.91	2.85	-	-	-
5	2.81	2.75	2.70	-	-	-
6	2.60	2.53	2.47	-	-	-
B 7	3.18	3.10	3.07	-	-	-
8	3.10	3.06	3.04	-	-	-
9	2.78	2.71	2.61	-	-	-
10	2.93	2.86	2.79	-	-	-
11	2.90	2.88	2.86	-	-	-
12	2.59	2.55	2.52	-	-	-
C 13	3.31	3.28	3.27	3.24	*	-
14	3.24	3.21	3.20	3.17	*	-
15	3.15	3.11	3.09	3.06	*	-
16	2.83	2.81	2.80	2.78	*	-
17	2.86	2.85	2.83	2.82	*	-
18	2.56	2.54	2.52	2.52		-
D 19	3.14	3.15	3.13	3.12	3.10	3.08
20	3.16	3.16	3.15	3.15	3.13	3.13
21	3.04	3.04	3.04	3.04	3.02	3.00
22	2.91	2.90	2.89	2.89	2.87	2.85
23	2.90	2.90	2.90	2.89	2.88	2.87
24	2.80	2.80	2.80	2.79	2.78	2.77
E 25	3.23	3.25	3.24	3.23	3.21	3.20
26	3.13	3.14	3.14	3.12	3.11	3.10
27	2.82	2.84	2.82	2.83	2.81	2.80
28	2.86	2.86	2.86	2.86	2.84	2.84
29	2.81	2.83	2.83	2.82	2.83	2.84
30	2.82	2.83	2.83	2.82	2.83	2.82
F 31	3.13	3.16	3.15	3.16	3.13	3.10
32	2.91	2.98	2.97	2.96	2.96	2.95
33	3.03	3.09	3.09	3.10	3.07	3.08
34	2.87	2.89	2.89	2.88	2.88	2.89
35	2.84	2.82	2.84	2.82	2.87	2.87
36	2.76	2.79	2.80	2.79	2.80	2.80

* Birds were inadvertently not weighed.

Table A2
Experiment LN 202:
Individual Bird Food Intake for Days of the Assay

Treatments & Birds		Day 1	Day 2	Day 3	Day 4	Day 5
		g				
A	1	0	0	-	-	-
	2	0	0	-	-	-
	3	0	0	-	-	-
	4	0	0	-	-	-
	5	0	0	-	-	-
	6	0	0	-	-	-
B	7	19.26	18.98	-	-	-
	8	18.77	18.71	-	-	-
	9	19.02	18.87	-	-	-
	10	19.53	19.60	-	-	-
	11	19.09	19.19	-	-	-
	12	17.69	17.89	-	-	-
C	13	38.16	38.06	39.27	39.04	-
	14	36.90	38.00	38.44	38.14	-
	15	39.18	39.12	39.40	39.43	-
	16	35.19	39.42	39.51	39.03	-
	17	38.37	38.58	37.32	38.64	-
	18	37.53	38.29	38.31	38.53	-
D	19	58.74	58.54	59.09	58.85	58.93
	20	58.91	58.64	58.77	58.93	58.26
	21	57.80	58.27	58.97	59.48	59.20
	22	58.14	58.21	58.42	58.58	58.21
	23	58.27	59.12	58.92	58.83	58.42
	24	56.47	56.88	57.23	58.06	58.40
E	25	78.12	78.64	78.41	78.28	77.62
	26	75.73	75.19	74.50	75.06	74.55
	27	77.30	75.18	78.01	77.48	76.91
	28	76.20	77.41	77.69	77.81	78.03
	29	76.15	76.05	77.85	77.84	77.84
	30	65.21	65.90	71.83	71.67	69.67
F	31	77.93	82.26	84.09	87.79	85.74
	32	97.48	97.59	97.61	93.75	97.94
	33	92.65	90.74	93.13	87.46	85.92
	34	93.38	94.16	93.23	95.72	94.60
	35	81.71	86.42	85.07	96.45	69.19
	36	88.86	90.99	92.95	94.17	95.62

Table A3

Experiment LN 202: Nitrogen of Excreta per Bird Day (g)* and Nitrogen of Excreta per $kg^{0.67}$ BW per Bird Day (mg)**

T&B	-- Day 1 ---		-- Day 2 ---		-- Day 3 ---		-- Day 4 ---		-- Day 5 ---	
	*	**	*	**	*	**	*	**	*	**
A 1	0.597	270.6	0.597	274.4	-	-	-	-	-	-
2	0.669	306.9	0.728	338.9	-	-	-	-	-	-
3	0.886	403.7	0.964	446.8	-	-	-	-	-	-
4	0.879	422.1	0.862	421.2	-	-	-	-	-	-
5	0.600	300.4	0.648	329.2	-	-	-	-	-	-
6	0.993	523.6	1.146	615.4	-	-	-	-	-	-
B 7	1.068	492.1	1.065	498.9	-	-	-	-	-	-
8	0.733	343.7	0.720	340.3	-	-	-	-	-	-
9	2.293	1155.6	3.714	1904.6	-	-	-	-	-	-
10	1.142	555.5	1.239	612.8	-	-	-	-	-	-
11	1.041	509.8	0.890	437.9	-	-	-	-	-	-
12	0.879	464.8	0.997	532.4	-	-	-	-	-	-
C13	1.351	605.6	1.477	666.5	1.390	628.5	1.305	593.8	-	-
14	1.155	525.3	1.534	702.1	1.448	664.0	1.628	751.7	-	-
15	1.341	621.8	1.363	637.2	1.346	631.9	1.344	635.1	-	-
16	1.457	725.6	1.493	747.2	1.483	744.1	1.436	723.7	-	-
17	0.984	486.9	1.239	614.2	1.118	556.7	1.174	585.9	-	-
18	1.116	594.5	1.166	624.2	1.350	727.0	1.252	674.2	-	-
D19	1.799	835.9	2.094	970.9	2.092	973.9	2.182	1017.8	2.028	950.4
20	1.523	704.6	1.856	858.5	1.924	891.8	1.862	863.3	1.748	813.9
21	1.697	805.5	1.773	841.9	1.740	826.1	1.789	849.1	1.859	886.4
22	1.950	953.3	2.013	986.1	2.052	1007.9	2.043	1003.5	1.889	932.1
23	1.410	690.9	1.639	802.9	1.753	858.8	1.819	893.3	1.662	818.0
24	1.574	789.6	1.755	880.6	1.862	934.0	1.844	927.1	1.862	938.6
E25	2.097	956.0	2.284	1037.0	2.529	1150.5	2.527	1152.1	2.376	1087.7
26	1.479	688.4	2.022	939.3	2.012	934.6	1.961	914.9	1.883	880.6
27	2.756	1376.1	2.510	1247.1	2.703	1349.7	2.602	1295.8	2.825	1413.8
28	2.204	1089.9	2.190	1083.2	2.402	1188.0	2.538	1255.3	2.490	1237.5
29	1.912	957.0	2.122	1057.1	2.680	1334.8	2.294	1145.4	2.238	1114.5
30	1.734	865.7	1.793	893.1	1.679	836.2	2.083	1039.9	1.984	988.4
F31	2.102	978.5	2.191	1013.6	2.686	1245.0	2.900	1341.4	2.656	1236.6
32	2.037	996.0	2.372	1141.2	2.593	1250.2	3.033	1465.7	2.735	1321.8
33	1.931	918.8	2.444	1147.6	2.673	1255.2	2.748	1287.5	2.376	1120.8
34	1.867	921.1	2.688	1320.2	2.563	1258.8	2.802	1379.2	2.904	1429.6
35	2.023	1005.4	2.410	1203.3	2.371	1178.0	3.063	1529.3	2.174	1072.7
36	2.003	1014.7	2.573	1294.0	2.748	1378.3	2.945	1480.7	2.739	1374.0

Table A4
Experiment LN 202: Air Dry Excreta Output per Bird per Day

Treatments & Birds		Day 1	Day 2	Day 3	Day 4	Day 5
		g				
A	1	3.934	3.346	-	-	-
	2	3.834	3.654	-	-	-
	3	4.792	5.162	-	-	-
	4	4.784	4.627	-	-	-
	5	3.232	3.776	-	-	-
	6	4.535	5.442	-	-	-
B	7	7.913	8.909	-	-	-
	8	5.784	6.261	-	-	-
	9	11.561	18.199	-	-	-
	10	7.621	7.948	-	-	-
	11	7.645	6.853	-	-	-
	12	6.317	8.714	-	-	-
C	13	10.609	13.416	11.711	11.490	-
	14	9.230	13.719	11.749	13.143	-
	15	11.623	11.657	11.610	12.441	-
	16	11.489	12.329	12.656	12.367	-
	17	9.278	12.476	10.788	11.219	-
	18	10.028	10.288	12.232	12.265	-
D	19	15.196	18.666	17.788	19.706	17.808
	20	14.130	18.070	17.616	16.777	16.323
	21	14.909	16.986	16.651	17.247	16.867
	22	15.538	18.017	17.569	17.070	15.769
	23	13.795	16.401	17.166	19.308	15.917
	24	14.015	15.728	18.508	16.594	17.386
E	25	19.785	21.632	24.156	22.444	22.226
	26	14.082	22.541	20.782	19.474	17.718
	27	23.200	21.377	25.007	22.881	25.800
	28	20.254	20.661	22.386	22.051	21.788
	29	17.804	20.766	25.187	21.281	22.219
	30	15.593	18.148	17.579	22.567	19.531
F	31	19.424	20.927	22.232	25.959	24.479
	32	21.112	24.554	25.619	29.132	25.873
	33	20.073	24.684	25.579	25.752	22.525
	34	18.019	29.539	25.127	26.431	28.249
	35	17.532	24.271	24.092	28.816	19.673
	36	19.854	25.578	25.230	27.622	24.542

Table A5

Constraints Used In Experiment LN 202 Diet

Item	Type	
Weight	=	1*
Energy	=	335**
		-- g/100g --
Crude Protein	=	20
Calcium		1.00 -- 2.00
Available Phosphorus		0.50 -- 1.10
Sodium		0.15 -- 0.30
Chlorine		0.11 -- 0.25
Potassium		0.45 -- 1.20
Premix	=	0.20
Arginine	>	0.98
Histidine	>	0.30
Isoleucine	>	0.65
Leucine	>	1.40
Lysine	>	1.08
Methionine + Cystine	>	0.70
Tyrosine + Phenylalanine	>	1.30
Threonine	>	0.65
Tryptophan	>	0.20
Valine	>	0.93
Linoleic Acid	>	0.80
Soyabean meal	=	4.00
Synthetic Methionine	<	100.00

* The unit is "proportion of intake".

** The unit is "kcal/intake proportion".

Table A6

Ingredient, Calculated and Determined Nutrient Composition of Experiment LN 202 Diet

--Ingredient Composition--		---------- Nutrient Composition ----------		
Item	g/kg	Item	Calculated	Determined
Maize	779.5	TME (kcal/kg)	3350	3255*
Meat & Bone	79.9			
Blood	30.0		g/kg	g/kg
Fish	67.4			
Soyabean meal	40.0	Crude Protein	200.0	211.6
Salt	1.2	Calcium	10.7	-
Premix	2.0	Available Phosphorus	7.2	-
Synthetic Methionine	-	Sodium	1.5	-
		Chlorine	1.7	-
		Potassium	5.3	-
		Fat	40.7	-
		Fibre	4.7	-
		Linoleic Acid	13.4	-
		Arginine	11.7	11.9
		Histidine	5.8	7.4
		Isoleucine	6.6	6.0
		Leusine	19.4	18.9
		Lysine	11.8	11.2
		Methionine + Cystine	7.0	3.7
		Tyrosine+Phenylalanine	16.3	15.5
		Threonine	8.9	8.4
		Tryptophan	2.1	0.0
		Valine	11.1	10.4

* The value determined from the equation $TME = (1 - b_s)GE$.

Table A7

Ingredient Composition of Experiment LN 204 Diets

Item	Ingredient Composition		
	Basal + 0 M & B	Basal + 8 M & B	Basal + 16 M & B
	---- g/40g ----	---- g/48g ----	----- g/56g ----
Barley	28.897	28.897	28.897
Maize	8.635	8.635	8.635
Brewers Grains	0.372	0.372	0.372
Salt	0.047	0.047	0.047
Limestone	1.951	1.951	1.951
Premix	0.098	0.098	0.098
M & B	-	8.000	16.000

Table A8

Calculated and Determined Nutrient Composition of Experiment LN 204 Diets

Item	Nutrient Composition							
	Basal+0M&B		Basal+8M&B		Basal+16M&B		M & B	
	*Cal.	Det.	Cal.	Det.	Cal.	Det.	Cal.	Det.
TME (kcal/kg)	3027	-	3036	-	3042	-	3078	-
	-- g/kg --		-- g/kg ---		-- g/kg ---		-- g/kg ---	
Crude Protein	93.2	97.3	164.3	166.7	215.2	225.2	520.0	517.8
Calcium	16.5	-	25.4	-	31.7	-	69.7	-
Available Phosphorus	2.5	-	8.7	-	13.2	-	40.2	-
Sodium	0.6	-	1.4	-	2.0	-	5.5	-
Chlorine	1.9	-	1.6	-	1.4	-	0.2	-
Potassium	5.6	-	5.4	-	5.2	-	4.2	-
Linoleic Acid	7.9	-	7.3	-	6.9	-	4.2	-
Arginine	5.2	4.5	10.8	10.7	14.7	15.2	38.6	84.8
Histidine	2.5	3.9	3.5	5.2	4.2	5.3	8.6	9.8
Isoleucine	3.8	3.4	5.9	5.7	7.4	6.9	16.2	15.1
Leucine	7.3	7.7	12.5	12.4	16.1	15.2	38.1	34.0
Lysine	4.4	3.5	8.9	6.4	12.2	9.7	31.5	26.6
Methionine + Cystine	3.5	1.5	5.1	2.6	6.2	3.1	13.0	7.6
Tyrosine+Phenylalanine	9.9	7.9	13.7	12.2	16.5	15.0	32.9	27.9
Threonine	4.2	3.4	7.5	7.1	9.8	13.1	23.6	18.4
Tryptophan	1.4	0.0	1.6	0.0	1.8	0.0	2.7	0.0
Valine	5.7	4.6	9.0	8.2	11.4	9.7	25.7	21.2

* Cal. = Calculated values.
Det. = Determined values.

Table A9

Ingredient and Calculated Nutrient Composition of the "Low Density" Maintenance Diet Used before and between Experiments

--Ingredient Composition--		------- Nutrient Composition --------	
Item	g/kg	Item	Calculated
Barley	668.2	TME (kcal/kg)	2800
Bran	34.8		
Pollard	87.0		g/kg
Lucerne	43.5		
M & B	71.6	Crude Protein	160.7
Brewers Grains	60.9	Calcium	18.9
Salt	1.6	Available Phosphorus	7.2
Limestone	30.7	Sodium	1.5
Premix	1.7	Chlorine	2.6
		Potassium	8.3
		Arginine	9.2
		Histidine	3.3
		Isoleucine	5.3
		Leucine	9.8
		Lysine	7.3
		Methionine + Cystine	5.1
		Tyrosine + Phenylalanine	11.9
		Threonine	6.0
		Tryptophan	1.5
		Valine	7.9

Table A10

Analysis of Variance Tables for Estimating Proportional Contribution of Components of the Total Variance of Nitrogen Excreted in mg per kg BW per Day

Treatment C

Source	df	SS	MS	Components of Variance
Days (Total)	17	60484	-	
Between Birds	5	46697	9339.4	$\sigma^2 + n\sigma^2B$
Within Birds	12	13787	1148.9	σ^2

n = 3 , b = 6. The estimate of σ^2B, S^2B = (MSBB - MSWB)/n = 2730

S^2B/b + MSWB/nb = 455 + 63.83 = 519

Treatments A and B

Source	df	SS	MS	Components of Variance
Days (Total)	21	2738344	-	
Treatments	1	425435	425435	$\sigma^2 + n\sigma^2B + nb\sigma^2A$
Birds	10	2445260	244526	
Bet. Birds Wit. Treats.	9	2019824	224425	$\sigma^2 + n\sigma^2B$
Bet. Days Wit. Birds	11	293084	26644	σ^2

n = 2, b = 6, a = 2. The estimate of σ^2B, S^2B = (MSBBWT - MSBDWB)/n = 98890

MSBDWB/nb + S^2B/b = 2220 + 16482 = 18702

Treatments D, E and F

Source	df	SS	MS	Components of Variance
Days (Total)	71	2786603	39248	
Treatments	2	1690069	845035	$\sigma^2 + n\sigma^2B + nb\sigma^2A$
Birds	17	2371291	139488	
Bet. Birds Wit. Treats.	15	681222	45415	$\sigma^2 + n\sigma^2B$
Bet. Days Wit. Birds	54	415312	7691	σ^2

n = 4, b = 6, a = 3. MS estimates $\sigma^2 + n\sigma^2B$; S^2B = (MSBBWT - MSBDWB)/n
= 9431
MSBDWB/nb + S^2B/b = 320 + 1572 = 1892

Note: n = number of days.
b = number of birds.
a = number of treatments.
df = degree of freedom.
SS = sum of square.
MS = mean square.

Table A11

Sums of Squares of Regression Determinations Associated with Data of Table 4.8

	Unc.			N			C		
	T. A + B	T. B to F	T. A to F	T. A + B	T. B to F	T. A to F	T. A + B	T. B to F	T. A to F
Regression	5164	174110	303387	754	2662	7608	9866	219831	407079
Residual	976	2509	3437	384	765	1281	558	2048	2169
Total	6140	176619	306823	1139	3427	8889	10424	221879	409247
n	11	29	35	11	29	35	11	29	35

Notes: Unc. = Data employed not corrected to zero N balance.

C = Data employed corrected to zero N balance.

N = Results of regression of (NI − NO) 36.51 on energy input.

T. A + B, T. B to F, T. A to F = Regression employs data from birds of the treatments indicated.

The data of bird coded as number 9 of T. B was not used in the above calculations.