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The Net Energy Value

For Commonly Used Plant Ingredients for Poultry In Australia

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Foreword

Energy is by far the most expensive part of a poultry diet, and potential improvement in production efficiency is large if dietary energy is accurately measured. The net energy (NE) bioassay is one system that reflects the true availability of energy to the bird. The current project examined the most common ingredients for their NE values in broilers and layers for a comparison of performance of birds fed diets formulated on NE or AME values.

This publication contains data on the net energy values of some cereal grains and vegetable protein sources that are commonly used in the Australian broiler and layer industries, and presents preliminary comparative results on performance of broilers fed diets formulated using NE and AME values.

This project was funded jointly by the Chicken Meat Program of the RIRDC and the Australian Egg Corporation Limited (formerly the Egg Industry Program of RIRDC), which is matched by funds provided by the Australian Government.

This report, an addition to RIRDC's diverse range of over 1000 research publications, forms part of our R&D program for the Established Industries, which aims to enhance the competitiveness of the poultry industries by provision of up to date data on net energy values of common feedstuffs.

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Simon Hearn
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Abbreviations

AME = apparent metabolisable energy
ME = metabolisable energy
FCR = feed conversion ratio
HI = heat increment
HP = heat production
NE = net energy
RQ = respiratory quotient

Contents

Executive Summary

This pilot project was initiated to examine whether formulating poultry diets based on net energy (NE) values would give an advantage over those formulated on energy values obtained using the current default system of energy measure – the apparent metabolisable energy (AME). Due to the extreme difficulty in measuring NE, only the most commonly used Australian raw materials were assayed.

The NE values (MJ/kg) of wheat, barley, sorghum, millrun, sweet lupin, soybean meal (48% CP), canola meal and meat meal for broilers were: 11.89, 10.64, 13.18, 8.75, 3.87, 7.74, 5.28 and 7.44. The NE values (MJ/kg) of wheat, barley, sorghum, millrun, sweet lupin, soybean meal (48% CP), canola meal, meat meal and oats for layers were: 10.31, 10.26, 12.25, 6.73, 12.90, 11.71, 9.27, 15.75, and 11.44, respectively.

Broiler diets formulated on NE values gave a clear advantage in feed conversion efficiency over those formulated using the AME values, resulting in savings of over 80 grams of feed per kg liveweight gain over a 35-day growth period.

There is no doubt that the use of NE value for feed formulation is clearly advantageous, however, due to the tedious nature of the NE system the establishment of a NE database for practical feed formulation is still a long way to go.

Introduction

The heat loss in birds fed different feedstuffs is not proportional to their AME values. Therefore the amount of energy available to the bird for maintenance and production is not just a matter of applying a correction factor to the AME values (Choct, 1999). The net energy value of feedstuffs reflects the true availability of energy to the bird. The system for energy measurement for poultry has been a topic of discussion for many years. Farrell (1996), while reviewing Emmans' "effective energy system", emphasized the need for a more practical type of energy system other than the AME bioassay. This project is to examine the most common cereal grains for their NE values in broilers and layers for a comparison of performance of birds fed diets formulated on NE or AME values.

The Australian poultry industry has a total farmgate value of $2.8 billion and it is estimated to employ some 30,000 directly and 60,000 people in related enterprises. Feed, by far, is the component of production costs (65-75% of production cost) and the poultry industry uses approximately 23% of all compound feed produced in Australia, amounting to 2.2M tonnes per annum. An improvement in feed efficiency by 2.5% means that the industry will save about $17M per annum. A 2.5% improvement in FCR is equivalent to reducing FCR from 1.75 to 1.71 in broilers and from 2.50 to 2.44 in layers. With more accurate energy figures in the formulation, these reductions can be achieved.

Chapter One

Background and Literature Review

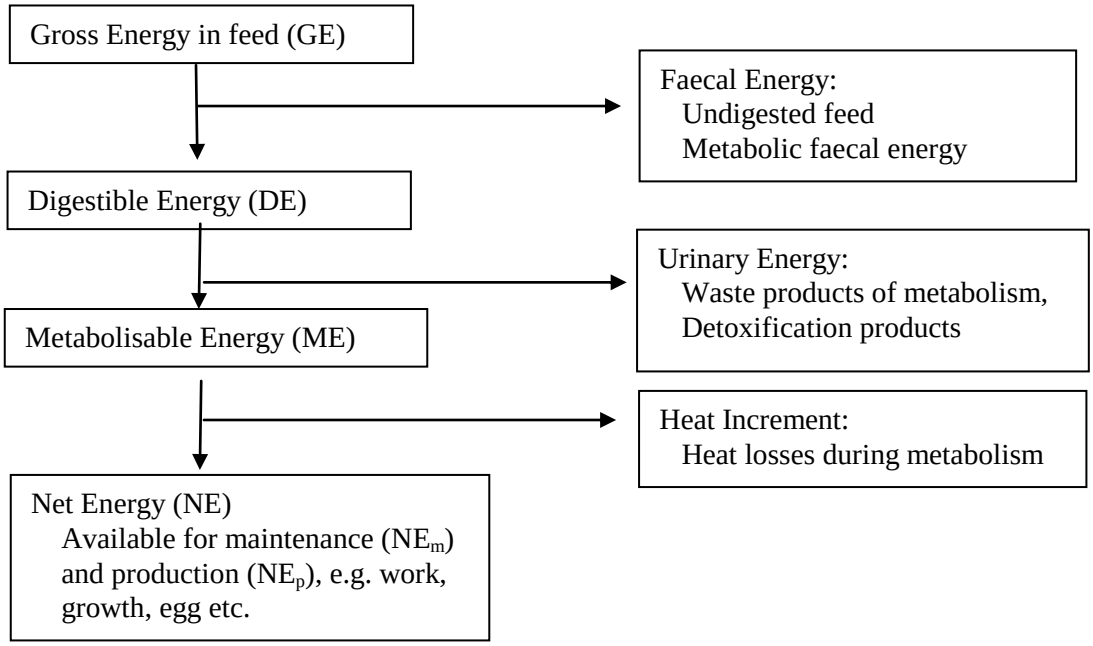
1.1 The Partitioning of Dietary Energy

Effective feed evaluation systems are required in order to predict the performance of farm animals fed different diets. Current systems of feed evaluation are based upon the requirement of the animal for the nutrient in question and on the ability of the feed or combination of dietary ingredients to meet this requirement (Close, 1990; van der Honing and Steg, 1990). It has been generally assumed that energy yielding components are the most limiting dietary ingredients and that the extent that animals convert feed into usable products is basically dependent upon the efficiency of dietary energy utilization (Bickel, 1988; Close, 1990). It follows, therefore, that considerable attention has been directed towards the development of systems for expressing both the energy requirements of animals and the energy value of feeds.

The information generated is vital in the formulation of diets of optimum quality to achieve the desired animal performance and of greater significance wider issues of agricultural policy are addressed (Van der Honing and Steg, 1990). Among these are the efficient utilisation of national feed resources, thereby reducing adverse side-effects to the environment, and planning future alternatives in animal production as a result of changing public opinion and development of consumer markets. The rapidly increasing intensification of poultry production during recent decades, and the subsequent improvement of feed conversion efficiency, has led to significant progress in the evaluation of the availability of energy in feedstuffs for broilers (Vogt and Zoiopoulos, 1988). With the advancement of knowledge in feed composition and nutrient utilisation, the prediction of energy value has gained in accuracy.

The partition of GE into its various components is outlined in Figure 1.1.

Figure 1.1. The partition of dietary energy in poultry.



Source: McDonald *et al*. (1995)

The ultimate way to calculate the feeding value of an ingredient or compound feed is by means of feeding trials, using the class of stock to which it is intended to feed the test materials (Feltwell and Fox, 1978). Rations, however, must be formulated before they are fed to chickens. Therefore, some satisfactory way of predicting the potential energy-producing content of an ingredient must be developed. In the ideal situation the energy of the diet should equal the sum of the energy values of its constituents. Gross energy (GE) is the only such measure. Gross energy can be estimated by bomb calorimetry, and deducting the physiological energy losses in the transformation processes enables the calculation of levels of digestible energy (DE) and metabolisable energy (ME) in the feed (de Boer and Bickel, 1988). But, GE has no practical applicability because not all of this energy is available to the animal.

As faeces and urine are voided together by poultry, DE cannot be measured without surgically altering the digestive tract by an appropriate technique to allow faeces to be collected separately from urine (Richardson *et al*., 1960; Ivy *et al*., 1968). But the primary difficulty encountered with, for example, the exteriorised rectum type of modification, has been failure to secure healing between the mucosa of the intestine and the skin (Richardson *et al*., 1960). In addition, the same experimenters found that feeds containing considerable levels of fibre (>5%) resulted in faeces that would dry in the cannula, clog it up and result in its expulsion, causing serious health and welfare issues. An alternate technique to surgical modification involves determining the amount of uric acid in the mixed excrement and calculating the total

urinary nitrogen (Vogt and Zoiopoulos, 1988), but this is costly in terms of time, labour and money. As a consequence of such difficulties, DE values are not generally employed in poultry-feed formulation.

ME is the system used predominantly as the feed evaluation parameter in most countries and is thus the basis of most feeding systems. It is expressed as apparent metabolisable energy (AME) or true metabolisable energy (TME). The energy metabolised is termed apparent because, of the energy in the faeces, only part has been derived directly from the food; thus only part consists of undigested and unmetabolised dietary residues (Guillaume and Summers, 1970; McNab, 1990; Sibbald, 1989; North and Bell, 1990). Part of the excreta is of endogenous origin, having been derived from the bird and the energy in this portion is known as the endogenous energy loss (EEL). In further clarification, part of the EEL is faecal in origin and is generally reported as consisting of sloughed-off gut lining, bile excretions and unabsorbed enzymes and the other part is urinary and consists of the excretory products of nitrogen metabolism (Sibbald, 1975a; 1976; McNab, 1990). Sibbald (1976) developed a rapid method for measuring TME, in which the EEL was determined directly, and argued that the energy of feedstuffs should be expressed in terms of their TME. Vogt and Zoiopoulos (1988) agreed that the TME for poultry is the GE of the feed minus the GE of the excreta of feed origin. This definition implies that a correction should be made for EEL, and this introduces complications. Due to the difficulty of its measurement, the TME method has not been adopted in the European countries (Vogt and Zoiopoulos, 1988). Furthermore, Härtel (1986) established that the Sibbald procedure delivered incorrect TME values due to the use of starved birds, which Härtel found to have led to misleading coefficients in the regression equations used to calculate energy excretion from feed intake.

Hence, the current preferred method of measurement is the AME system. The accurate measurement of AME requires estimates of losses in the form of methane and volatile fatty acids (VFAs) (McDonald *et al*., 1988, 1995). Vogt and Zoiopoulos (1988) suggested that under practical conditions, the energy lost through methane is negligible. However, variable amounts of VFAs are produced in all segments of the digestive tract (i.e.: proventriculus and gizzard, duodenum, jejunum, ileum, caeca, and colon) of birds with the caeca being the major site (Annison *et al*., 1968; McDonald *et al*., 1995; Choct *et al*., 1996).

Although several authorities have mentioned that the caeca are not essential for the fowl, it has been substantiated that some strains of bacteroides are associated with the mucosal surface of the caeca (Annison *et al*., 1968; McNab, 1973; Hume *et al*., 1992; McDonald *et al*., 1995)

and that peristaltic activity mixes these with the digesta, leading to production of VFAs (mainly acetate, propionate and butyrate) as the major end-products of fermentation (Annison *et al*., 1968; Salanitro *et al*., 1978; Choct, 1995; McDonald *et al*., 1995). McDonald *et al*. (1995) also stated that although the cellulose present in cereal grains is not broken down by microbial activity to a great extent during its passage through the fowls' digestive tract, some hemicellulose breakdown does in fact occur. Annison *et al*. (1968) demonstrated that the absorption of caecal VFAs into the portal system was a basis for an evaluation of their significance as an energy source to the fowl. However, arguments have been advanced that caecectomy or ligation of the caeca does not alter crude fibre digestibility, and hence that it is unlikely that the VFAs make a large contribution to satisfying the energy requirement of poultry (McDonald *et al*., 1995). The limitation in similar reports was the failure to relate different diets to the amount and proportions of VFAs found in the caeca or to establish the rates of fermentation (Annison *et al*., 1968; Hume *et al*., 1992).

It is well established that ingestion of feed by an animal is followed by losses of energy, not only as chemical energy in its solid, liquid and gaseous excreta, but also as heat. Such energy losses may be expressed in absolute terms (MJ/kg DM), or relatively as a proportion of ME. Therefore, in order to substantiate the extent to which the ME of the feed is utilised by the bird, it is necessary to measure the bird's heat production and ultimately its energy retention or net energy (NE) (Farrell, 1974b; Kleiber, 1975; Reid *et al*., 1980; McDonald *et al*., 1995). Heat production can be estimated from the respiratory exchange of the bird and an approach based on indirect colorimetry with a respiration chamber is normally used (MacLean and Tobin, 1987; McDonald *et al*., 1995).

The next section reviews the relative merits of the existing methods of measuring ME as well as highlighting the productive (Davidson *et al*., 1957; Hill and Anderson, 1958; Farrell, 1974b), effective (Emmans, 1994; Farrell, 1996) and NE (Close, 1990; MacLeod, 1994) systems. Metabolisable energy values are defined and discussed, assay procedures are described and compared, and attention is directed towards the problems of ME measurement and application. The review underscores AME as the default system of energy estimation in the poultry industry and it focuses on the issues that relate to the experimental section.

Formulation of diets to a specified ME is of major importance for the productivity of broiler chickens more so than for laying hens (Johnson, 1987). Although Begin (1969) found no significant differences among the breeds of chicken and their ability to metabolise energy, there was some indication that the egg-laying breeds metabolise less energy per unit of feed

than the heavier, faster growing breeds. It is generally known that increasing dietary ME for layers causes a decrease in feed intake (FI) and feed conversion ratio (FCR: g feed/g egg mass) and an increase in body weight, but has little or no effect on egg production or egg weight. From this, Johnson (1987) deduced that dietary ME concentration has not been found to be a major factor that influences egg production or egg weight. However, previous work had shown that this does not preclude the possibility that ME estimation for layers may lead to profit maximisation (de Groote, 1972; McDonald, 1984). Johnson's (1987) review further indicated that ME estimation has a greater effect on production for broilers than for laying hens.

Although it is generally accepted that the AME system has played an invaluable role in poultry nutrition, it has proved to have several shortcomings. These include poor correlation with feed conversion and poor prediction of growth rate in broilers (Härtel, 1986; 1987). Therefore, there is an urgent need for re-evaluation of the system for energy measurements for poultry by estimating the NE value of feedstuffs, which includes energy losses as heat increment and through the production of VFAs; in order to improve the accuracy and usefulness of estimates in least cost ration formulation for the broiler industry.

1.2. Energy Evaluation

1.2.1. The Systems of Energy Evaluation

Information on dietary energy content is vital for the formulation of diets of specified energy concentrations for broilers. This is to allow the inclusion of other nutrients relative to the energy concentration and to provide adequate energy intake to achieve production targets (Batterham, 1990). McNab (1990) and Leeson and Summers (1991) stated that energy intake is implicated in the physiology of appetite and satiety and in the control of feed consumption rather than cues related to specific nutrients. With reference to this, McDonald *et al*. (1988) added that there must be a means of designating the potential energy concentration of formulated diets to ensure that the desired specifications have been supplied.

A number of systems have been used in the past to assess the energy value of raw materials and diets. Examples of these include Total Digestible Nutrients (TDN), Starch Equivalents (SE) and Scandinavian Feed Units (SFU). These systems have gradually become obsolete and have been replaced by the direct measurement of energy in feeds and its expression in terms of gross, digestible and metabolisable energy (GE, DE, ME), respectively. Ultimately, calorimetric research with poultry has yielded data resulting in general agreement on the

desirability of the use of ME in the evaluation of the energy concentration of poultry feedstuffs and of the energy requirements of different classes of birds (DeBoer and Bickel, 1988). These data have been based on the definition of ME as the difference between the gross energy of the food eaten and the gross energies of the faeces and urine, and are represented by the equation:

ME = GE of feed - (GE of faeces +GE of urine + GE of fermentation gases)

The establishment of such a relationship has improved substantially the precision with which the ME value of poultry diets and feedstuffs has been assessed (McNab, 1990). Four types of ME value exist: the apparent and true metabolisable energy (AME and TME, respectively) and the nitrogen corrected apparent and true metabolisable energy (AME_n and TME_n, respectively) (McNab, 1990; Sibbald, 1989; Farrel *et al*., 1991). Sibbald (1989) commented that, although the four types of ME value exist, it is not always apparent which type is being reported and additional confusion occurs because values of a particular type may vary depending on the assay procedures used in their derivation.

1.2.2. ME values of feedstuffs

Introduction

ME values assigned to particular ingredients vary considerably. Much of the variation has been attributed to differences in the chemical composition of the test samples and differences in the methods of ME determination (Reid *et al*., 1980; Rajaguru and Ravindran, 1985; Standing Committee on Agriculture (SCA) (1987). Furthermore, factors such as feed processing (Cave *et al*., 1965; Bayley *et al*., 1968), feed composition (Rajaguru and Ravindran 1985; Choct *et al*., 1996), level of feeding (McDonald *et al*., 1995), nitrogen-retention (Sibbald and Slinger, 1963; Davidson *et al*., 1964; Miller, 1974; Sibbald, 1989), age (Zelenka, 1968; Guirguis, 1976; Peterson *et al.*, 1976; Kussaibati *et al*., 1982; Sibbald, 1982), strain and species (Begin, 1969; Proudman *et al*., 1970; Pym and Farrell, 1977) and environment (Osbaldiston, 1966; Olson *et al*., 1972; Miller, 1974) have been proved to contribute to the variation. Elaboration of each factor will follow.

Factors influencing the ME values

Nitrogen retention. The values of ME may be expressed on a 90% dry matter or air-dry weight basis with or without correction to eliminate variations arising from variable nitrogen retention (NR) (Farrell, 1980; 1981; Muztar and Slinger, 1981; Sibbald, 1982; 1989; Sibbald

and Morse, 1983b; Wolynetz and Sibbald, 1984; Rajaguru and Ravindran, 1985; Farrell *et al*., 1991). Miller (1974) wrote that part of the ME is the energy stored as protein during growth and the proportion of this dietary protein nitrogen (protein N) which is retained in the body is high in the young chick, but falls as the rate of growth reduces. NR of 10-d-old chicks receiving a commercial diet was 56-58% (Miller, 1974) while Davidson *et al*. (1964) showed that over the age span of broiler chickens, it varied between 35 and 39%. Since there is variation in the amount of protein N consumed in diets, the amount retained will depend on the quality of the feed protein as well as the stage of growth of the test bird (Farrell, 1977).

Proponents of N correction argue that since body nitrogen is excreted as energy containing products after it has been catabolised, it is desirable to bring AME data to a basis of nitrogen equilibrium. Consequently, ME values of feeds, corrected to N equilibrium, are often calculated on the assumption that all the nitrogenous end products are in the form of uric acid. This development has been in use for a long time and Hill and Anderson (1958), working with chicks, introduced a correction factor of 34.4 MJ/kg of retained nitrogen (RN) representing the GE value of uric acid which is the major N excretion product of poultry. In a subsequent study, Titus *et al*. (1959) proposed a correction factor of 36.5 MJ/kg of N which describes more accurately the GE of the N constituents of chicken urine. Unfortunately, the use of both factors has contributed to some of the variation among AME_n and TME_n data. Moreover, all excreted urine N is not present as uric acid but may appear in variable amounts as other chemical compounds (McDonald *et al*., 1988; 1995).

The merits of nitrogen correction are open to debate. Vohra (1972), Din *et al*. (1979) and Sibbald and Price (1978) argued that since there is no general consensus to this correction, the ME values should remain uncorrected to give a classical ME value. Din *et al*. (1979) further indicated that correction should also be made for nitrogen lost in the shedding of scales and feathers during the measurement period. Siregar and Farrell (1980) discovered some nitrogen loss as gaseous ammonia, but noted that this is not normally collected and corrected for. Farrell's (1974a) work had earlier shown that there was a likelihood of reducing ME estimates by a greater amount than warranted because of overestimation of the actual nitrogen stored. Baldini (1961) and Shannon (1982) have also argued that protein storage is characteristic of growth and egg production and that it is difficult to justify the penalty from a diet that permits N retention (and hence, protein) which is the objective of animal production. Shannon (1982) stated that if the function of ME is to obtain a measure of the energy value of a feed, rather than a measure of its propensity to induce protein synthesis, N corrections could be justified. Unfortunately, it is difficult to measure precisely the NR of birds (Davidson and Williams,

1968). Hence, Shannon (1982) and Härtel (1986) noted that the same feed when given to different birds might have a different ME value because of differences in the amount of feed protein that is retained. To make ME values a characteristic of the feed rather than of the conditions under which the ME value is determined, many authors have agreed to correct the value to what it might have been under standard conditions. On the other hand, some scientists have argued that N-correction is unnecessary since whatever is lost through the faeces or excreta is influenced by the nature of the feed, the physiological state of the test bird, as well as its age (SCA, 1987).

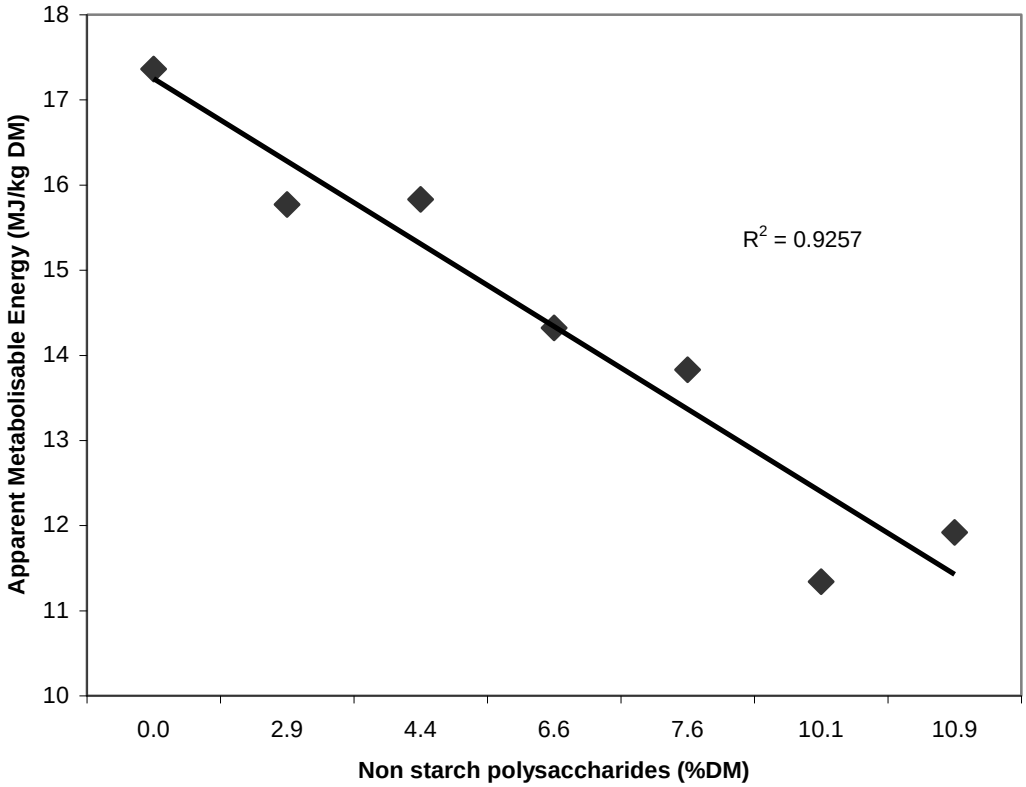
Feed composition. Feed composition is one of the main factors that affects the ME of a feed and also influences the digestibility (Janssen and Carré, 1989; Classen and Bedford, 1991; McDonald *et al*., 1995; Annison *et al*., 1997). The latter effect is closely related to its chemical composition and in feeds such as maize, which vary relatively little in composition from one sample to another, little variation will occur in digestibility and ME (McDonald *et al*., 1995).

McDonald *et al*. (1995) stated that the fibre fraction of a feed has the greatest influence on its digestibility and that both the amount and chemical composition of the fibre are influential. Rajaguru and Ravindran (1985) recognised the fact that high fibre levels led to lower ME_n values. For example, the latter researchers found that only 53.8% and 52.8% of the energy in rough rice and wheat flour, respectively, is metabolisable by chicks and that this low value was attributed to their high fibre contents. Several workers have established that NSPs for example, β-glucans and arabinoxylans (pentosans), are the major components of dietary fibre and are known to possess important nutritional activities in broilers (Classen and Bedford, 1991; Choct, 1992; Annison *et al*., 1992, 1996; Choct *et al*., 1996). Although the soluble NSPs represent a small proportion of the total fibre component in cereals, their influence on the nutritive value can be large through depression of performance, impairment of nutrient digestion, and reduced litter quality (sticky droppings and wet litter), particularly in very young broiler birds. Figure 2.1 illustrates that with increasing NSP levels in different types of cereal, an associated decrease in AME occurs.

High amounts of soluble arabinoxylans in rye and β - glucans in barley, for example, are responsible for the poor nutritive values of those cereals in poultry (Antoniou *et al*., 1981; Campbell *et al*., 1989; Choct and Annison, 1990; Annison, 1990, 1991, 1992; Friesen *et al*., 1992; Bedford, 1996). Choct and Annison (1990) established that there was a strong negative correlation (r=-0.95, P<0.001) between the total content of pentosans in a feed and the AME

content (Figure 1.1). When water and alkaline extractable pentosans were added to a commercial-type broiler diet, the AME, nitrogen-retention, feed utilisation, and growth of broilers were significantly (P<0.001) depressed. In addition, when the sum of soluble β - glucans and arabinoxylans was examined, the relationship was even stronger (*r*=-0.98, *P*<0.001).

Figure 1.1: The relationship between energy metabolisability (AME/g energy) of cereals and their NSP composition (r=-0.97, P<0.001)



Source: Choct and Annison (1990)

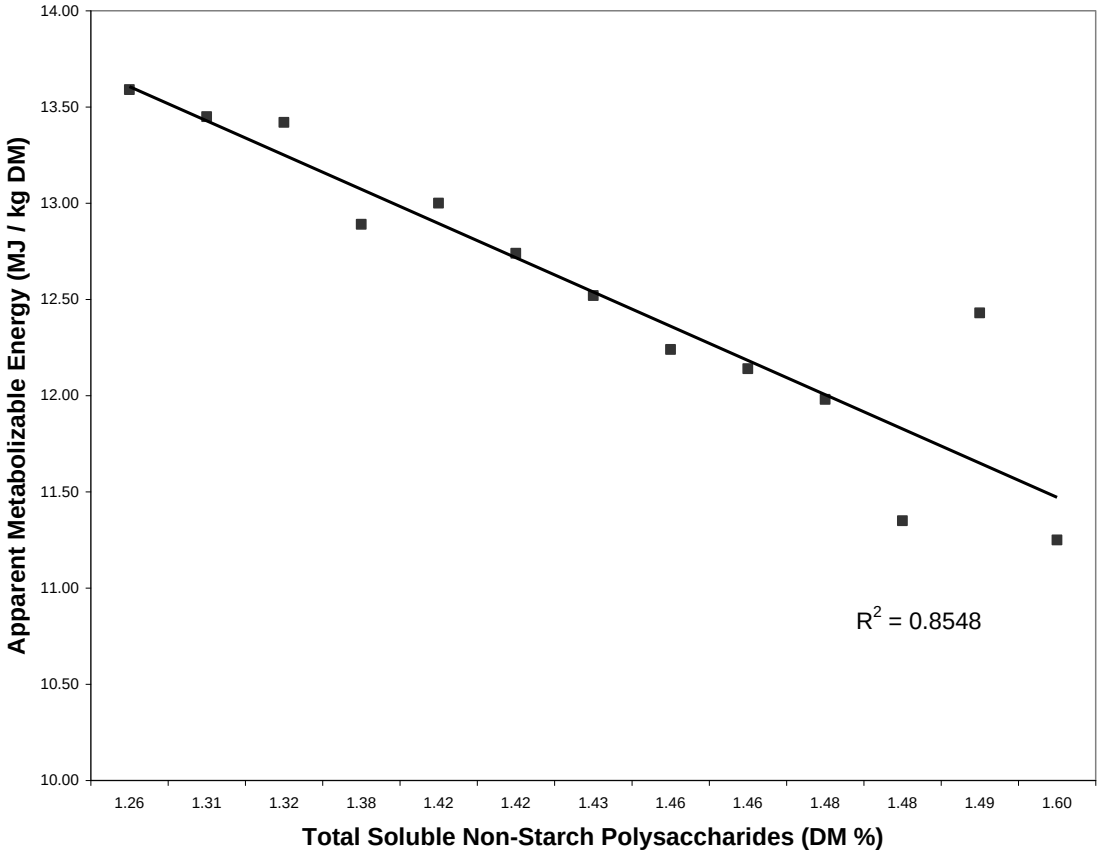
In a subsequent study, Annison (1991) determined AME values of 13 wheat varieties with reference to soluble, insoluble and total NSPs. AME was significantly correlated with the total soluble NSP content as shown in Figure 1.2. The results suggested that variation in the soluble NSPs was responsible for the reduced energy availability for broilers fed wheat-based diets. This supported earlier findings that soluble pentosans in rye (Fengler and Marquardt, 1988a, b) and β - glucans in barley (Campbell *et al*., 1989) cause anti-nutritive effects in broiler diets. Fengler and Marquardt (1988b), Classen and Bedford (1991) and Choct *et al*. (1996) suggested that the viscous nature of the NSPs is the primary cause for their anti-

nutritive effects. The increased bulk and viscosity of the intestinal contents are believed to decrease the rate of diffusion of substrates and digestive enzymes and to hinder their effective interaction at the mucosal surface (Ikegami *et al*., 1990; Angkanaporn *et al*., 1994; Iji *et al*., 1996a, b). Ikeda and Kusano (1983) and Graham *et al*. (1993) further suggested that viscous polysaccharides might also directly complex with digestive enzymes and reduce their activity.

Other substances like amylase and trypsin inhibitors, lectins, tannins (Liener, 1989; Classen and Campbell, 1990; Scott and Bedford, 1994); alkaloids (Ruiz, 1976); phytates (Reddy *et al*., 1982; Fordyce *et al*., 1987), hydrocyanic acid (Rajaguru and Ravindran, 1985) and saponins (Cheeke, 1979) have also been shown to depress nutrient digestibility and utilisation in birds fed some types of grain legumes. With reference to values of ME_n of toxic and detoxified cassava root meal, 13.38 ± 0.41 MJ/kg and 15.92 ± 0.16 MJ/kg, respectively, Rajaguru and Ravindran (1985) explained that the difference was due to HCN interfering with energy utilisation in poultry. Kopinski *et al*. (1995) therefore suggested that it is important for nutritionists to be aware of anti-nutritive factors, particularly as pentosans along with β - glucans can influence the energy digestibility of diets for broiler birds with deleterious effects on performance.

It has been demonstrated that the addition of suitable enzymes say, glucanase, to wheat diets for broilers can reduce the viscosity of the digesta caused primarily by arabinoxylans, thereby improving chick production (Rogel *et al*., 1987; Pettersson and Amman, 1988, 1989; Annison, 1992; Graham *et al*. 1993; Bedford 1994; Choct *et al*., 1994). The same workers explained that this was due to partial depolymerisation of the arabinoxylan polysaccharides by enzymes, which reduced molecular chains containing more than 5,000 sugars to those consisting of just above 1,000 sugars. Subsequent studies similarly indicated that supplementation with an enzyme product remarkably ($P<0.01$) increased the AME of low ME-wheats from 12.02 to 14.92MJ/kg DM (Choct *et al*., 1995). Increased solubilisation of the NSPs within the gastrointestinal tract of birds demonstrated that NSP - degrading enzymes markedly increase the nutritive value of low ME-wheat in broiler diets. This implied that increased levels of viscous NSPs may cause the low-AME content found in some wheats (Figure 1.2). Bedford and Morgan (1996) suggested that the improvement of feed efficiency with enzyme addition was often greater in the finishing period than in the growing period. The effect of enzymes has been found to vary considerably depending on the composition of different diets. In a subsequent study, Geraert *et al*. (1997) indicated that addition of xylanase to a wheat-based diet allowed young birds as early as 8 days of age to derive more nutrients from their feed, resulting in enhanced profitability in broiler production.

Figure 1.2: Relationship between the content of non-starch polysaccharides and the AME of wheat in diets of broiler chickens



Source: Annison (1991)

In the case of lupins, one way to counteract possible anti-nutritive effects caused by both NSPs and the oligosaccharides is to supplement diets with enzymes which degrade these carbohydrates (Brenes *et al*. 1993; Bryden *et al*., 1994; Wiryawan *et al*., 1995; Annison *et al*., 1996). Annison *et al*. (1996) demonstrated that supplementation of a lupin-based diet with an enzyme supplement containing xylanase, pentosanase and hemicellulase activities increased the AME of the lupins from 10.01 MJ/kg DM to 11.65 MJ/kg DM. A further investigation by Wiryawan *et al*. (1997) showed that the protein quality of lupins increased remarkably ($P<0.01$) after supplementation with a multi enzyme product containing xylanase, α-amylase and protease. On the other hand, another enzyme supplement containing β-glucanase, hemicellulase and pectinase activities failed to produce an improvement in the lupin's nutritive value, but caused an increase in the concentrations of soluble NSPs in the ileal digesta of chickens which was accompanied by an increase in the viscosity of the ileal digesta.

These results demonstrate that the NSPs in legumes are extremely complex and that it is difficult to find enzymes that precisely target these substrates. However, the cereal NSPs have been responsive to this approach since their structure is well understood and abundant information on their anti-nutritive activity is available.

Processing of feeds. The most common treatments applied to broiler feeds are crushing, grinding, cooking and pelleting. Sibbald and Slinger (1963) recommended that satisfactory ME assays require diet ingredients to be ground in such a manner that the birds are not able to sort the feed. Cave *et al*. (1965) and Bayley *et al*. (1968) reported that the ME values of some types of diet are markedly increased by steam pelleting. The latter workers reported that the performance of growing chicks on a diet short of inorganic phosphate was markedly increased when the diet was steam pelleted. In agreement with Bayley *et al*. (1968), Reddy *et al*. (1961) had earlier observed that pelleting slightly increased the ME content of a diet. In addition, Williams *et al*. (1997) showed that pelleting tended to improve live weight gain and significantly improved feed conversion efficiency of broiler chickens over the period of 1-50 days of age.

Although the advantages of pelleting poultry feeds are well documented, those of more advanced forms of mechanical feed processing, such as expansion before pelleting, are still poorly documented. Nevertheless, Williams *et al*. (1997) discovered that feed conversion efficiency was further improved by expansion of the diets prior to crumbling or pelleting. An additional advantage from expansion compared with pelleting alone indicated a significant processing × sex interaction ($P<0.01$). Female broilers responded with significant increases in live weight gain whereas the males' response was not consistent. Feed processing has also been found to contribute to the improvement of the microbiological status of the feed (Table 1.1) as well as the bulk density and quality of the pellets.

Table 1.1 Microbiological status of grower feed in different forms

Diet	Total Aerobic/g	Yeast and Fungi/g
Mash	28000	1590
Pelleted	12000	110
Expanded and pelleted	9000	180
Expanded and crumbled	1000	320

Source: Williams *et al*. (1997)

Some studies have shown that the physical form of the diet that is fed can have profound effects on the development of the gizzard. Godwin (1995) found that diets that have been

finely ground and pelleted produce birds with poorly developed gizzards and dilated proventriculi. However, McDonald *et al*. (1995) reported that grinding of cereals for poultry has no consistent effect on ME values. Therefore, the manner in which the feed is prepared may in some cases affect its ME value.

Level of feeding. An increase in the quantity of feed eaten by an animal generally causes a faster rate of passage of digesta (Preston and Leng, 1994). McDonald *et al*. (1995) suggested that there may be a reduction in digestibility and therefore the ME value when feed is exposed to the action of digestive enzymes for a shorter period. On the other hand, when feeding levels are raised to 2.3 - 3.0 times of maintenance in poultry, there is still little effect of feeding level on the digestibility of conventional (low-fibre) diets.

Effect of additives. March *et al*. (1972) recorded that antibiotic supplementation of broiler diets enhanced ME values by 0.13 - 0.38 MJ/kg. The investigators found that with practical broiler diets, the beneficial effects of dietary antibiotics were immediate but did not persist following withdrawal of the antibiotic from the diet. It was then recommended that in order to achieve the maximum effect at the earliest age from the feeding of antibiotics to broilers, it was necessary to fortify the diet continuously with antibiotics. Alternatively, supplementation during the last four weeks of the normal eight-week growing period was found to be effective. Moran and McGinnis (1968), Misir and Marquardt (1978) and Classen *et al*. (1985) observed that the positive effect of antibiotics such as, penicillin, appeared to be related to the elimination of fermentative micro-organisms mainly butyric acid producers, from the small intestine. However, the finding of Choct *et al*. (1996) was contradictory in that the addition of the antibiotic Amoxil to the birds' drinking water failed to counteract the anti-nutritive effect of NSPs in broiler diets. It was hypothesised that in this case, the antibiotic selectively suppressed glycanases but not fermentative microbes, elevating gut viscosity and VFA production throughout the gut. Another possibility was that the antibiotic had a wide antibacterial spectrum but was destroyed by penicillinase produced by *Staphylococcus aureus.*

Age. The ME value of a diet for young chicks may vary by 1.13 MJ/kg during the first few weeks of life. Zelenka (1968) found that a few days after hatching the ME value fell, after which it increased from the 7th - 9th day to attain a maximum value at 50 d of age. The same author explained that the initial fall was due to the reducing nutrient contribution of the residual yolk at that time and the subsequent rise was due to the development of the chick's ability to digest feed. Carew *et al*. (1963) had previously noted that the ME of a diet

containing 20% corn oil was higher for 3-week-old than for 2-week-old birds, and attributed the whole of this increase to better absorption of fat by the older birds.

The utilisation of fat by birds is unique in that there is a well-defined dependence, noted above, on bird age. The digestibility and/or ME of fats have been shown to increase with age (Whitehead and Fisher, 1975; Katongole and March, 1980; Kussaibati *et al*., 1982; Krogdahl, 1985). The age effect was most pronounced with saturated fats, tallow in particular. The addition of bile salts, lipase or phospholipids to chick diets improved the digestibility of animal fats, demonstrating that lipid digestive processes are not fully functional in the very young (Leeson, 1993). In addition, the development of enterocytes with fatty acid binding-protein (FABP) activity seems to parallel the development in lipase activity and bile salt secretion in poultry (Kussaibati *et al*. 1982; Krogdahl, 1985). Sell *et al*. (1986) and Katongole and March (1980) documented up to a 5-fold increase in FABP in chicks from hatch to 8 weeks of age. This provides evidence that FABP is not produced in adequate quantities by young birds. Bile salt secretion seems to be the limiting function for lipid digestion during the first few weeks after poultry hatch (Krogdahl, 1985). Whether lipase secretion, FABP synthesis, or another physiological factor is the next limiting function cannot be ascertained from the information available.

Strains and species. Differences in the ME values of various diets for different strains and breeds of chicken have been observed (Begin, 1969; Proudman *et al*., 1970; Farrell, 1975; Pym and Farrell, 1977), but several comparisons have produced conflicting information. Proudman *et al*. (1970) observed differences among AME values measured with lines of chickens selected from the same base population. However, Washburn *et al*. (1975) found no AME difference when breeds and strains of meat-type birds were compared. Although Hew *et al*. (1996) indicated that strain and diet type interactions were not significant for the AME of the three strains, Strains A and C tended to have higher AME values (13.49 and 13.57 MJ/kg, respectively, $P<0.09$) than Strain B (13.10 MJ/kg).

Davidson *et al*. (1957, 1964) observed differences among strains in the utilisation of AME for tissue synthesis and suggested that they might result from differences in feed intake. Farrell (1975) reported between-strain differences in heat production and AME utilisation for tissue synthesis. Furthermore, Pym and Farrell (1977) found differences in daily maintenance energy requirements among broiler lines. Although there were no significant differences among the breeds of chicken and their ability to metabolise energy, there was some indication

that the egg-laying breeds studied metabolised less energy per unit of feed than did the heavier, faster growing breeds (Begin, 1969).

Nutrient balance. Dietary energy levels are closely related to those of other components and will therefore affect their nutritive value (Feltwell and Fox, 1978). Reid *et al*. (1980) proposed that extremes in nutrient balance, such as very low concentrations of vitamin A or low or high concentrations of dietary protein or fat, might affect the digestibility and metabolisability of energy.

An example of such a relationship is the energy-protein balance denoted by the number of energy units percent of crude protein in the mixture. This refers to the amount of energy in the ration that is required to metabolise the protein supplied to body tissues. Protein utilisation has been shown to be higher at low protein levels than at high levels. However, the biological value (BV) which denotes the proportion of feed protein that can be utilised by the bird for synthesising body tissues was generally found to decrease when protein level was increased (McDonald *et al*., 1995).

Environmental temperatures. It is well established that optimum environmental temperatures are necessary for maximum growth and productivity in chickens (Osbaldiston, 1966; Lei and Slinger, 1970; Olson *et al*., 1972; Miller, 1974; Farrell and Swain, 1977). When the environmental temperature was reduced from 32^{o}C to 7^{o}C, Osbaldiston (1966) observed that the classical ME values of a commercial pelleted ration declined from 10.8 MJ/kg to 9.84 MJ/kg. This difference in the ME values was attributed to differences in N-retention that varied significantly with environmental temperature. In addition, the latter author noted that differences among older birds were less marked. In support of the above findings, Olson *et al*. (1972) recognised the effect of elevated (40.5^{o}C) or depressed (13.0^{o}C) ambient temperatures on chick growth and on diet consumption. Olson *et al*. (1972) estimated that a one-degree drop in temperature from either 40.5^{o}C or 13.0^{o}C decreased ME efficiency by approximately 1%. To maintain carcass energy gain, it was concluded that an additional 0.25 - 0.33 MJ/kg diet was required for each 1^{o}C decrease in temperature. From this, the need of energy for body temperature maintenance is clearly evident in the higher requirement for the birds in the cooler condition. Although Lei and Singer (1970), Olson *et al*. (1972), and Miller (1974) agreed that elevated and depressed environment temperatures lowered weight gain and feed efficiency, the severity of the stresses did not affect the ME content of the feed.

On the other hand, increasing the ME content 14% (from 13.48 to 15.45 MJ/kg) increased the energy consumption (ME of diet x g consumed) 5% in the warmer regime and 2% in the

cooler regime. From this evidence, Olson *et al*. (1972) concluded that chicks do not control consumption entirely on the energy content of the diet, but that the environmental factor plays a great role as well. Miller (1974) ascertained that the ME value of a particular feed sample is not constant, but is subject to variation due to the factors affecting the digestion and assimilation of nutrients. Furthermore, Miller (1974) noted that while the effects of these factors may be small relative to the diet's mean ME value, they may be large relative to the differences in ME values between diets that might normally be regarded as of economic importance. From the above it is clear that ME is a biological measurement dependent on the interaction between the animal, its feed and its environment.

1.2.3 *In vivo* techniques for the assessment of the ME values of feedstuffs.

Introduction

This section is concerned with *in vivo* methodologies. Several assays are described and their advantages and disadvantages are assessed in terms of logistics. It is important to note that the major feeding techniques used to derive the ME content of feedstuffs include the *ad libitum* method developed first by Hill and Anderson (1958), the rapid *ad libitum* methods proposed by Farrell (1978) and the force-feeding technique developed by Sibbald (1976). As earlier mentioned, four types of ME value exist: the AME, AME_n, TME and the TME_n (McNab, 1990; Sibbald, 1989; Farrell *et al*., 1991). The assays used to measure ME of feedstuffs range from the conventional (Hill and Anderson, 1958; Sibbald and Slinger, 1963; Miller, 1974) to rapid (Sibbald, 1976; Farrell, 1978, 1981; Farrell *et al*., 1991) and semi-rapid methods (du Preez *et al*., 1986).

Conventional methods

Under this category of methods, there are total collection and indicator procedures. The ME content of feedstuffs by conventional methods is evaluated from measurements of the heat of combustion of representative feed samples and excreta output relative to feed intake (Miller, 1974; Cullison, 1982). Apart from this procedure, an indigestible substance in the feed can be used as a marker. In such a case, quantitative measurement of feed intake and excreta output are unnecessary and ME can be assessed from laboratory measurements of marker concentration in samples of feed and excreta alone (Miller, 1974; Sibbald, 1982; Sibbald, 1989; Farrell *et al*., 1991).

Total Collection. Miller (1974) reasoned that the excretion of urine and faeces together makes this direct method of assessment technically very easy. The classical total-collection

procedure allows birds free access to a diet and the AME is calculated on the assumption that excreta voided over a specific period of time corresponds to feed ingested during the same time (SCA, 1987; Sibbald, 1982). The energy lost as by-products of digestion is considered insignificant in this approach and is therefore ignored (Sibbald, 1989). Thus, AME is represented by the equation:

$$\text{AME / g of feed} = \frac{\left(F_i \times GE_f\right) - \left(E \times GE_e\right)}{F_i}$$

Where F_i is the feed intake (g); E is the excreta output (g); GE_f represents the GE/g of feed; and GE_e is the GE/g of excreta, on a DM basis. A 3-day pre-test is followed by a 4-day collection period. This balance, together with several hundred grams of feed intake over the period, is thought by some to permit discrepancies in gut-fill at the beginning and end of the experiment to cancel each other out (Miller, 1974). However, this assumption had been disputed because rates of intake and excretion vary (Sibbald, 1982) and it had been suggested that a period of starvation be imposed at the beginning and end of the collection period in order to identify the start and finish of the bioassay period (March and Bailey, 1973). However, the latter assay tended to give low estimates of AME (Sibbald, 1975b). This was probably because the metabolic faecal energy plus the endogenous urinary energy (F_mE + U_eE) voided over a period of 4 days was charged against the feed consumed in 3 days (Sibbald, 1982). Alternatively, an inert marker could be included in the feed to colour the excreta for the purpose of identifying the bioassay period (Miller, 1974; Sibbald, 1982). The classical collection procedure is advantageous because it approximates closely to the real situation and enables bioassays to be conducted with all types and ages of birds. The procedure is also readily understood and capable of duplication among laboratories.

On the other hand, the major difficulty with the classical approach is that feed consumption and quantitative collection of excreta are difficult to measure. Feed spillage is a problem, especially in chick experiments, which may explain why in North America, assays which have been developed employ indicators (Sibbald, 1982). Sibbald (1989) emphasised the fact that accurate measurements are extremely difficult to perform since chicks have a tendency to bill feed out of the trough and since some excreta tends to be retained on the wire-mesh floors of the cages. Spilled feed, feathers, down and scales contaminate excreta and make accurate estimation of output and composition difficult, and lead to incorrect results (Miller, 1974; Sibbald, 1982; 1989). The method is slow and has a high labour requirement and it involves

the handling and storage of relatively large quantities of excreta, which is an unnecessary procedure when an indicator is employed.

Another difficulty associated with the total collection procedures is variation in moisture content of feed from the time of preparation, throughout the assay and during analysis. Feedstuffs gain or lose moisture because of changes in the environment and through physical processes such as grinding. Sibbald (1982) reviewed the available evidence and concluded that, during grinding, up to 15% of the moisture evaporated when the initial dry matter (DM) content was roughly 85%. Variations in moisture content also affect the excreta and lead to variable losses of energy especially during drying (Shannon and Brown, 1969; Sibbald, 1982). In support of such difficulties, Sibbald (1979b) and Wallis and Balnave (1983) suggested thorough oven drying at 60^{o}C for 18 to 24h.

Furthermore, difficulty may arise when diets high in fibre are fed. Such diets lead to low feed intake and metabolic and endogenous energy contribute substantially to the energy excreted, hence producing an underestimate of ME (Miller 1974; Classen and Bedford, 1991). Miller (1974) and Sibbald (1982) noted that the precision of measurement of the ME value of a diet by this method depends on the variation in measurements made on different birds or on the same bird on different occasions.

The indicator method. If an indigestible substance in the food is used as a marker, the quantitative measurement of food intake and excreta output is unnecessary (Miller, 1974; Sibbald, 1982; Fisher and McNab, 1989). Those same workers confirmed that ME can be assessed from relatively straightforward laboratory measurements on samples of food and excreta alone. The use of indicators to provide quantitative measure of the ratio of faecal output to food intake depends on the principle that the total amount of the inert indicator substance excreted equals the amount ingested during a given time period. This may be represented by the equation:

$$C_iI = C_fF \quad \text{and} \quad C_i/C_f = F/I$$

Where I and F are total feed intake and faecal output during the collection period and C_i and C_f are the concentrations of the indicator substance, for example Chromic oxide (Cr_2O_3) and acid insoluble ash (AIA) in the food and faeces, respectively.

The indicator procedure has both merits and demerits. The merits include obviation of the need to measure feed intake and for total collection of excreta, and a substantial reduction in the amount of excreta that has to be stored for analysis. Miller (1974) and Sibbald (1982)

agreed that this is an important consideration when measurements are made on groups as opposed to individual chickens. In favour of the method is the fact that excreta samples free from contamination with feed are readily obtained (Fisher and McNab, 1989). Indicators are also considered attractive because they permit the derivation of acceptable ME values even when feed is spilt and some excreta is not recovered (Sibbald, 1982). Several authors have considered the indicator procedure to be more precise than total collection since the standard errors associated with the mean estimates are lower than for the total collection procedure (Coates *et al*., 1977; Halloran and Sibbald, 1979). Furthermore, indicators are assumed to be dispersed uniformly throughout the feed and excreta, flow through the alimentary canal at the same rate as other feed residues and are unabsorbed. Such characteristics make them suitable in determination of ME values.

However, it has been suggested that the variability associated with Cr_2O_3 analysis is a major cause of error in ME determination when this marker is used. This fact has contributed to the difficulty of obtaining reproducible assay data among laboratories. Vohra (1972) established that due to its electrostatic properties, Cr_2O_3 also fails to be evenly distributed in the diet. This led to the use of alternative markers such as AIA. AIA has advantages over Cr_2O_3 in that it is non-electrostatic and does not require any special equipment for its determination (Vogtmann *et al*., 1975). Its limitations include requirement of a relatively large sample (1-3g) for the determination of the marker itself. This poses problems when nutrient digestibility is needed in the case of ileal samples from individual birds (some have only 1.5-2g of DM per sample). Another disadvantage of AIA marker is the length of its determination (2 days) due to repeated ashing and drying. To overcome these drawbacks, Choct and Hughes (1996) introduced a new marker: the long chain alkanes, such as hexatriacontane ($C_{36}H_{74}$). The alkane marker appears to be simple and rapid and it offers distinct advantages over the other markers. Although the indicator procedure has a number of problems and involves additional analyses, it yields more precise results, particularly when feed spillage and excreta contamination are problems (Sibbald, 1982).

Rapid methods

The measurement and application of ME has changed over time, from the conventional ME assays that are based on young chickens to rapid assays which use adult cockerels. Hence, values obtained for adult birds are now used to formulate diets for young chickens (Johnson, 1987). There are two types of rapid bioassay, namely: the AME assay (Farrell, 1978; 1981; Farrell *et al*., 1991) and the TME assay (Sibbald, 1976; 1986). The ME determined by these

procedures is often adjusted to nitrogen equilibrium to give values designated as AME_n and TME_n (Wolynetz and Sibbald, 1984; Sibbald, 1986, 1989; Lessire, 1990; Farrell *et al*., 1991).

The rapid AME bioassay. A comparatively recent rapid bioassay using cockerels was developed by Farrell (1978, 1980) and has been found simpler, faster and less expensive than the conventional methods. The bioassay involves training individually caged cockerels to consume their daily pelleted feed allowance in 1h. Studies with adult cockerels demonstrate that a minimum daily feed intake is necessary for valid ME measurements. At least 70g (Farrell, 1978; Sibbald, 1989) during the feeding hour is now considered necessary rather than the 45g suggested by Guillaume and Summers (1970) to ensure that endogenous excreta does not significantly depress ME values. Pelleting is required to permit the rapid intake of large amounts of feed and to facilitate the measurement of feed intake, although it may not be practical in case of small quantities of test materials (Sibbald, 1985). Total collection of excreta voided per bird during the next 24h represents all the feed consumed and thus gives an accurate assessment of the ME of the diet (Farrell, 1978). Control birds receive a similar diet, but *ad libitum*. The rapid AME bioassay requires a comparison between chicks and cockerels offered the same diet to eliminate possible differences in ME values of the same diet, due to age and to the need to adjust ME values on the basis of nitrogen retention.

The Farrell assay is generally considered to have several advantages. Birds are easily trained and may be available for assays to be started at short notice and completed rapidly. A major merit lies in the fact that there is no need for cockerels to adjust to the test diet before measurements are made. Sibbald (1989) and McNab (1990) referred to the technique as valuable because of the reproducible ME data obtained without correction for nitrogen retention and for the same feed with cockerels and chicks.

While assays of the type proposed by Farrell (1978) have obviously proved to be successful, there are nevertheless several problems associated with them (Sibbald, 1982; Fisher and McNab, 1989). The fact that the feed must be mixed and pelleted prior to an assay requires access to a pelleting device and this may cause delay. Schang and Hamilton (1982) found that many birds could not be trained to eat their feed requirements in one hour and, once trained, they had to be maintained in that state; this is a time consuming and laborious exercise. Similar to earlier experiments by Guillaume and Summers (1970), Fisher and McNab (1989) showed that AME values for diets fed to adult cockerels were profoundly affected by the amount of feed eaten during the assay; the lower the feed consumption, the lower the AME estimate. McNab (1990) explained that this effect was attributed to the contribution made to

the excreted energy by the EEL. Another problem associated with this assay is its use of adult birds that have different metabolic rates. Compared to conventional assays, the Farrell assay is considered faster and less expensive.

Determination of AME_n. AME_n is an estimate of ME (Sibbald, 1989; Bourdillon *et al*., 1990a, b; Farrell *et al*., 1991) which differs from AME in that a correction is made for NR, which may be either positive or negative (Shannon, 1982; Sibbald, 1989).

$$AME_n = \frac{\left[\left(F_i \times GE_f\right) - \left(E \times GE_e\right)\right] - \left(NR \times K\right)}{F_i}$$

Where $NR = (F_i \times N_f) - (E \times N_e)$; N_f is the N/g of feed (g); N_e is the N/g of excreta (g); and K is a constant, usually either 34.4 or 36.5 kJ/g on a DM basis. Although AME_n data are used widely by poultry nutritionists, the need for the N correction remains debatable as discussed under section 2.2. The controversy over N correction will continue, however, its effect in most situations is small and the additional work involved in its determinations is difficult to justify.

Determination of TME. Sibbald (1975a, 1985 and 1986) argued that the anomalies that had earlier been associated with assays designed to derive AME values were a direct consequence of EEL effects. Similarly, SCA (1987), Dale and Fuller (1982) and McDonald *et al*. (1995) concluded that TME is distinguishable from AME in that a correction to the latter for FE_m and UE_e gives TME. Combined, $FE_m + UE_e$ represents a maintenance cost that should not be charged against the feed (Sibbald, 1989). In view of the abnormal condition (fasted birds) in the TME assay, Muztar and Slinger (1981) mentioned that it was doubtful that birds would retain enough NR from a single feeding to revert to a positive body N equilibrium.

$$TME = \frac{\left[\left(F_i \times GE_f\right) - \left(E \times GE_e\right)\right] + \left(FE_m + UE_e\right)}{F_i}$$

A simple, rapid TME assay was developed on the basis of GE assays of the test feed and of excreta voided over 24h by roosters which were force-fed (Sibbald, 1976; SCA, 1987). Prior to each such TME assay, birds were starved for 21h to empty their digestive tracts and individual empty body weights were recorded (Sibbald, 1976). Force-feeding was accomplished by inserting a 5.5mm (internal diameter) glass tube into the crop via the oesophagus. SCA (1987) noted that force-feeding of adult cockerels allows an optimum

intake of 40g of a pelleted or 30g of mash feed. Sibbald (1976) used 4.76mm (diameter) cold pressed pellets. The birds were weighed again after exactly 24h, and the voided excreta were collected quantitatively for GE analysis. Birds of equal weight were starved for 21h and the FE_m and the UE_e voided during the next 24h was used as the correction factor to obtain TME (SCA, 1987).

The correction for FE_m and UE_e has several important effects. The data obtained confirm that because of the correction for EEL, TME values are invariably larger than the corresponding AME values, as indicated in Table 2.2. Sibbald (1982, 1989) suggested that determination of TME would be the only alternative if meaningful energy levels were to result under the optimal intake of 40g of a pelleted feed by adult cockerels. The presentation of the feed by tube permits the most accurate measurement of energy intake, and its use helps avoid feed spillage and changes in dry matter content (McNab, 1990). TME values were also proved to be independent of variations in feed intake and this contributes to their reliability and reproducibility.

Although TME investigation has not been extensive, it appears that values obtained with adult cockerels can also be used successfully in the formulation of diets for young birds (Sibbald, 1976, 1978). The assay is simple and can be initiated at short notice if a flock of birds is maintained for the purpose and it was also observed that the overall cost of the assay is less than that of conventional AME assays (Sibbald, 1989).

Table 1.2 Apparent and true metabolisable energy values of feedstuffs at low feed intakes

Feedstuff	AME (MJ/kg)	TME (MJ/kg)
Barley	11.5	14.0
Maize	13.7	16.5
Oats	8.3	11.2
Soybean meal	9.4	12.7
Wheat	12.4	14.7

Source: SCA (1987)

Much of the criticism of the TME assay has focussed on the procedure of estimating the EEL of fed birds from the excreta energy outputs of fasted birds (Sibbald, 1985; Härtel, 1986; Pesti *et al*., 1988a, Farrell *et al*., 1991). The mentioned authors argued that starved birds are always in negative N balance and fed birds are not depending on the feed ingredients being tested. From his review, McNab (1990) concluded that in the starved state, individual birds void

variable amounts of energy; ranging from 33 to 82 kJ/24h (Farrell, 1978) and from 25 to 69 kJ/24h (Sibbald and Price, 1978).

More complications have arisen due to the marked difference between the TME calculated for the same diet when fed to birds at different temperatures (Farrell and Swain, 1977; Dale and Fuller, 1981; Yamazaki and Zhang, 1982; McNab, 1990). At a lower (5^{o}C) and at a hotter environmental temperature (30^{o}C), the TME values derived were, for example, 70 kJ/24h and 38 kJ/24h, respectively (Dale and Fuller, 1981). Yamazaki and Zhang (1982) reported another interaction between temperature, EEL and TME. The named workers demonstrated that starved cockerels excreted 63 kJ/24h at temperatures ranging between 5-15^{o}C and 32 kJ/24h at hotter temperatures ranging between 25-35^{o}C. This point is controversial because in subsequent studies with birds fed glucose solutions during the pre-feeding period and tube fed 50g glucose, McNab (1990) observed that although the EEL differed slightly with variations in environmental temperature, TME values of the feedstuffs tested were unaffected by temperature (41.5 kJ/24h at 5^{o}C and 42.4 kJ/24h at 35^{o}C). The measurement of EEL and TME at various temperatures therefore requires further investigation.

Other disadvantages of the TME technique are the limit on dose size and welfare attitudes towards the acceptability of a procedure frequently referred to as "force-feeding". At the purely practical level, this feeding may cause stress to the bird that may interfere with normal digestive processes. However, proponents of the system argue that the feeding technique is extremely rapid (15-30s/bird with most feeds) and that there is little visual evidence of more stress beyond that involved in handling. If force-feeding is impractical the assay of Parsons *et al*. (1984), in which the test materials are available *ad libitum* for 6h and excreta is collected for 54h, may be a useful alternative.

SCA (1987) and Sibbald and Morse (1983b) commented that the small amount of feed consumed results in further potential problems in that only a small amount of excreta is voided and collection and weighing this plus the small quantity of EEL is not easy. Another complex and unresolved issue is the time required to ensure complete clearance of a feedstuff. The original proposal of 24h (Sibbald, 1976; Farrell, 1978) produced unreliable data and is now considered to have been too short. SCA (1987) recommended that a fasting period longer than 24h would lead to more consistent results. Subsequently, various modifications have been suggested and adopted since Sibbald (1976) described a bioassay for TME. For example, periods of fast to clear feed residues have been extended from 24h to 32h (Farrell, 1981;

Sibbald, 1982) to 48h (Sibbald, 1986, McNab and Blair, 1988) and Sibbald and Morse (1983a) extended it to 72h.

McNab (1990) has more recently noted that extending the collection period to 72h would provide a practical solution but at a cost of some stress to the birds. Sibbald (1986) suggested a further extension to "a sufficient time to allow all feed residues to be voided". McNab and Blair (1988) improved the accuracy of TME values by delivering an aqueous solution of glucose to starved birds and a comparison with the Sibbald (1976) procedure indicated that this modification reduced the coefficient of variation of TME from 5.5% to 1.5%. Stress on the birds would presumably also have been reduced.

In Table 2.3 TME values derived from 48h and 72h collections in birds fed 50g feed are compared. This data suggests that a period of 48h is insufficient to allow all the undigested residues to be voided in the case of ingredients such as blood meal. Fisher and McNab (1989) suggested that lower intakes may alleviate this problem, but at the cost of both reduced accuracy and increased influence of endogenous effects. McNab (1990) added that incomplete clearance is a problem with high animal protein products that are finely ground. Sibbald and Morse (1983a) concluded that because such materials have low density, they fully pack the crop, causing problems in wetting of the feedstuff. McNab (1990) also observed that palatability might be another influential factor because when birds fed blood meal are given water, distaste seems to be experienced and regurgitation may occur. In support of this, Sibbald (1989) had earlier observed that an extremely low TME value might be due to regurgitated feed being mixed with the excreta.

The importance of water intake in ME assays is another area where firm conclusions have not been reached and yet it may be a significant source of variation. McNab and Blair (1988) observed that despite the presence of water, tube-fed birds were rarely seen drinking. It is only a speculation that low and variable water intakes may have been responsible for erratic feed passage rates and consequently erratic residue clearances. McNab (1990) reported that 90% of water consumption by birds is associated with voluntary feed intake. Thus, it may be the lack of access to feed that reduces the stimulus to drink.

Although the TME procedure considerably reduces the time required for the completion of a bioassay, and requires only a small amount of feed, it is not applicable to field conditions and is restricted to use with adult cockerels. The slow recovery of body weight following the assay, due to the small amounts of feed given and the relatively long period of starvation, combined with force-feeding, mean that the technique is not practical with young birds.

Farrell (1977) affirmed that widespread conversion to an evaluation bound on the TME of feeds would necessitate changing the existing standards for the measurement of energy requirements of poultry. Subsequently, research led to modifications and the system of TME bioassay values corrected to zero nitrogen balance (TME_n) was developed (Sibbald, 1985; McNab and Blair, 1988; Farrell *et al*., 1991).

Table 1.3 Comparison of TME_n values derived after 48h and 72h excreta collection

Ingredient	Samples	A TME_n (48h)	B TME_n (72h)	B/A Ref
Full-fat soy meal	4	14.44	14.42	1.00
	12	12.77	12.75	1.00
	12	13.29	13.06	0.98
	5	13.37	12.09	0.90
	7	8.59	10.46	0.97
	12	8.59	8.56	1.00

Source: McNab (1990)

Estimating TME_n in poultry feedstuffs. Similar procedures to those used to determine TME by Sibbald (1976) were followed, but the assay was modified by extending the preliminary fast period to 24h and the excreta collection period to 48h (Sibbald, 1978). A subsequent experiment, Sibbald (1979a) and Sibbald (1980) confirmed that problems associated with the delayed passage of some feedstuffs require a longer excreta collection period than 24h. Two important features, namely the mode of feeding and the inclusion of an estimate of EEL, differentiate the TME_n bioassay from the commonly used AME and AME_n assays (Parsons *et al*. 1982; Sibbald, 1985; Muztar and Slinger 1981; Wolynetz and Sibbald, 1984). Wolynetz and Sibbald (1984) suggested that EEL controls much of the variation associated with the type of bird, feed intake and the environment. Hence, TME_n values are said to be less variable among assays than are AME_n values, which are more dependent upon assay conditions.

$$TME_n = \frac{\left[\left(F_i \times GE_f\right) - \left(E \times GE_e\right) - \left(NR \times K\right)\right] + \left[\left(FE_m + UE_e\right) + \left(NR_o \times K\right)\right]}{F_i}$$

Where NR and NR_o are estimates of nitrogen retention for fed and fasted birds, respectively. TME_n bears the same relationship to TME as does AME_n to AME. The correction to nitrogen equilibrium is similarly made to that in AME_n and its value is thus subject to the same debate as previously discussed.

1.3 A critique of ME

The major recent point of concern is that diets with the same ME are not necessarily used with equal efficiency when fed to birds (Hughes and Choct, 1997; Petersen and Farrell, 1997). Petersen and Farrell (1997) explained that this might be due to considerable fermentation of the DM component, the extent of which depends on the chemical composition of the feed and the age of the birds. In support of this, Hughes and Choct (1997) provided evidence that highly variable responses by broiler chickens on the same low-ME wheat diet were exhibited not only because of the physio-chemical nature of the diet but also because of significant individuality in the digestive physiology of birds. Another point of concern is that the AME or TME value for a feedstuff or diet must be determined using birds of an age at which the value will be applied (Johnson, 1987). Johnson pointed out that all conventional bioassays were based on young chickens, and this had earlier caused concern (Lodhi *et al*., 1970; Peterson *et al*., 1976) because the ME values derived were applied to both young and adult birds. However, because of the widespread use of more recently developed rapid bioassays, which use adult cockerels, the situation has now been reversed. Hence, ME values determined using adult birds are now used to formulate diets for young chickens. Emmans (1994) and MacLeod (1994) indicated that there are improved systems, for example, the productive, effective and NE, that may overcome some of these shortcomings, but they have yet to be used in commercial practice.

1.4 Determination of productive energy (PE)

Productive energy is a measure that was employed for the estimation of NE values of many feedstuffs (Fraps, 1946; cited by Farrell, 1974b). It was based on a carcass analysis or comparative slaughter technique using growing chicks (Hill and Anderson, 1958; Farrell, 1974b; McDonald *et al*., 1988, 1995). McDonald *et al*. (1988, 1995) stated that the energy values obtained were called "productive" to emphasise that they were NE values for growth, and not for maintenance. The procedure was based on the difference in energy gain after feeding one group of chickens the experimental diet *ad libitum* and restricting a second group to about 50% of *ad libitum*. Maintenance energy requirement and thus the PE values of the diet were derived (Hill and Anderson, 1958; Farrell, 1974b) by the use of simultaneous equations of the form:

$$WM+G = FX$$

for each input level and solved for X, the PE/unit weight of feed. W represents the average chick weight (W1 + W2 + W3)/4 where W1, W2, and W3 are initial, mid time, and final body weights during the assay; M is the maintenance energy requirement per unit of body weight; G is the carcass energy gain; and F is the feed intake. There was an assumption that M was a constant for the range of body weights of interest. As a result, the measurement of PE by Fraps' method was criticised on the grounds that the maintenance energy requirement of growing chickens was not proportional to body weight (Hill and Anderson, 1958; Poczopko and Kowalczyk, 1965; Sibbald, 1982). This might have contributed to the fact that Fraps' PE values have never been widely employed (McDonald *et al*. 1988; 1995). Hill and Anderson (1958) also disagreed with Fraps' PE values because of their high variation for a single diet. Farrell (1974b) and the National Research Council (NRC, 1994) noted that the inability to obtain a single precise value for PE should not preclude its use as an important feed parameter.

1.5. Determination of effective energy (EE)

Emmans (1994) introduced a new energy system known as effective energy (EE). As described by Farrell (1996), it is in effect a NE system that takes into account the energy costs required to process a diet. The system considers the heat increment of feeding to be linearly related to five measurable parameters. These were, with their HI in parentheses; urinary N (w_u: MJ/kg), faecal organic matter (w_d: MJ/kg), positive protein retention (w_p:), positive lipid retention from feed lipid (w_n: MJ/kg), and positive lipid retention not from feed lipid (w_l: MJ/kg). The values of w_u, w_d, and (w_l - w_n) allow an energy scale called EE to be defined. With reference to this scale, the values of w_p and w_l together with the heats of combustion of protein and lipid of 2.38 and 3.95 MJ/kg, respectively, allow the energy requirement to be expressed as (MH + 50PR +56LR). PR and LR represent the rates of positive protein and lipid retention w_p (kg/d) respectively, and MH is the maintenance heat production (MJ/d) which can be estimated as 0.96 of the fasting heat production. The EE yielded can be estimated as EE (kJ/g or MJ/kg) = 1.71 ME – 4.2 CP – 2.44, where ME is measured at, or corrected to, zero N-retention (AME_n) and CP (g/g) is the crude protein (N x 6.25) content of the feed ingredient.

The system is criticised for the enormous time and effort involved in generating EE values for feed ingredients (Hancock *et al*., 1995). Other shortcomings include the difficulty in

determining the digestibility of CP that should be measured at the bird's terminal ileum and the need to know how much carcass lipid is synthesised directly from dietary lipid. Despite the shortcomings, Emmans (1994) reported that the system is accurate and its tabulated values can be used to formulate diets and to predict growth rate using programs such as the Gompertz growth function (Hancock *et al*., 1995).

1.6. Determination of net energy (NE)

It is recognised that diets of similar ME do not necessarily promote the same biological responses when fed to various classes of poultry, indicating that the availability of ME is not constant (SCA, 1987). It is also true that ME is a reliable predictor of the amount of energy which is potentially available for maintenance and for production. However, there is evidence that it is not a predictor of how efficiently the bird then uses what is available to it (SCA, 1987; Close, 1990; MacLeod, 1994). MacLeod (1994) argued that the simplicity and reproducibility of the ME system is achieved at the cost of ignoring the metabolic fate of the absorbed nutrients. The NE of a feed represents the true energy available for productive purposes (NE_p), and takes account of the losses in the metabolism of absorbed nutrients (NE_m), as illustrated in Figure 1.1.

In case of broiler birds, the NE_p is used for growth and is stored in the body and the quantity so used is referred to as the bird's energy retention (ER). NE_m is mainly used to perform work within the body and will leave the animal as heat. The heat produced is a function not only of the bird *per se* but also of the feed it consumes and the rate at which tissues or products are formed within the body. Of the total heat loss of the bird only that associated with the feed, that is the heat increment (HI) of feeding, is truly wasteful and can be regarded as a direct tax on the feed. The deduction of this component from the ME intake gives the NE value of the feed (Sibbald, 1982; Close, 1990; McDonald *et al*. 1988, 1995).

It is therefore necessary to measure either the bird's heat production (HP) or its ER to study the extent to which the ME of the feed it eats is utilised. Examination of Figure 1.1 makes it clear that, if one of the quantities is known, the other can be determined by subtracting the known one from the ME value, thus, ME = HP + ER (Close, 1990). One of the basic procedures for estimating the HP is the use of closed-circuit calorimeters involving measurement of the bird's respiratory exchange.

1.7. Conclusion

For the last three and a half decades the energy concentrations of feeds and the energy requirements of poultry have been described in terms of ME. It is presently still the energy system of choice, and many authors believe it will remain so in the future (Miller, 1974; Farrell, 1978; SCA, 1987). Several methods have been employed to determine ME. The conventional method, involving excreta collection over several days rather than its estimation using Cr_2O_3, has been recommended. SCA (1987) stated that none of the rapid methods available had then been tested thoroughly enough to allow them to replace the conventional method and hence, more work is needed to prove the reliability of these methods. On the contrary, Sibbald (1975b) advanced reasons for the conventional methods being unsuitable. Sibbald noted that high labour input, large sample size, slow determination time and high costs require that an alternative method be employed.

Farrell *et al*. (1991) recognised that there has been considerable debate on the relative merits of methods used to measure the ME of poultry foodstuffs. The controversies that exist originated partly from the validity of measuring in a TME, the EEL of starved birds, and using this mean value to estimate the EEL of fed birds. Much of the subsequent debate concerning the effectiveness of the rapid methods revolves around which is the better system to use, AME or TME (McNab and Blair, 1988). The latter authors argued that because AME and TME are mathematically related, the assays should be judged on how well feed intake and EEL can be measured and that whether AME or TME values are finally derived from the data is irrelevant. SCA (1987) expressed uncertainty as to the likely survival of TME; they anticipated that new animal welfare regulations might preclude its use in the future. In support of this du Preez *et al*. (1986) strongly argued that the means of determining of EEL in the TME procedure is physiologically undesirable since birds would be in an energy and protein-deficient state. Added to this, Parsons *et al*. (1982) had previously reported that administration of feed by intubation caused birds to suffer post-feeding stress. Arguing to the contrary, Sibbald (1985) explained that the apparent conflict may be due to misunderstanding of the time required to "force-feed" a bird; usually it requires much less than one minute, and that the procedure should thus cause little stress to the birds if done correctly.

Although the rapid methods are open to criticism, Farrell (1981) recognised that there is a close relationship between them and the conventional ones. Furthermore, Farrell (1981) concluded that due to the deficiencies associated with the TME method, the inability to confirm the values that are provided and the fact that current poultry feed requirements are

expressed in terms of “apparent” and not “true” ME, there is no justification for changing to a TME system. However, recent research findings have advanced two further criticisms of ME values of feedstuffs or diets, and suggest that there may now be a real need to reconsider the position.

Diets of similar ME do not necessarily promote the same biological responses in birds under the same test and this has led to a major criticism. The second criticism is concerned with the age of birds at which bioassays are conducted and having to apply the derived values to formulate diets for birds of different ages. DeGroote (1974), Emmans (1994), MacLeod (1994) and Farrell (1996) have shown that such shortcomings may be overcome by employing the NE system, which has been proposed as a positive step towards the more accurate prediction of bird performance and feed composition than the existing AME system. DeGroote’s (1974) study illustrated the superiority of the NE over the ME system in broiler diet formulation because it took into account differences in the metabolic utilisation efficiency of the ME of different feedstuffs and it allowed a more accurate energetic evaluation of dietary ingredients.

The experiments reported in this thesis examine in broiler chickens the effects of type of cereal grains, used in a diet, on HP during metabolism and VFA production in the hindgut. The effect of a diet incorporated with NSPs with or without enzyme supplementation on gut microflora and energy availability to a bird is also examined.

Chapter Two

Materials & Methods

2.1 Ethical considerations

The Animal Care and Ethics Committee of the University of New England (Armidale, NSW) approved this study. Health and husbandry practices complied with the *Code of Practice for the Welfare of the Domestic Fowl* issued by the Australian Bureau of Animal Health (1997).

2.2 Experimental birds

Broiler Experiment: Day-old male broiler birds (Cobb strain) were obtained from the Baiada Hatchery, Tamworth, and were raised on commercial broiler starter crumbles containing 12.5 ME MJ/kg and a minimum 20% CP (Ridley AgriProducts, Tamworth, NSW), in standard chick brooders for 18 days. Four groups, each consisting of two randomly selected birds, approximately of equal weight, were selected for the experiment. The experiment began when each group of birds was 18 days old. The birds were transferred into the chambers two by two, where they were allowed to acclimatisation period of 7d, followed by a 4-d collection period. Each batch contains two replicates of each diets as well as a control diet. For each diet, there had to be two identical runs to enable a total of 4 replicates per diet.

Layer Experiment: ISA Brown layers at 56 weeks of age were transferred to single layer cages in the same animal house where the NE work to be conducted. They were given the experimental diets for 3 days before being transferred to the respiratory chambers. Four laying hens who were used to consuming the experimental diets were placed into the 8 chambers individually and were allowed to acclimatise themselves to the chambers for 7 days, followed by two a 4-day excreta collection periods to enable a total number of replicates to be 4 for each diet. The same birds were used repeatedly until all the diets were assayed.

2.3 Feed formulation and mixing

The Agri-Data package (Agri-Data Systems, Inc., Maryland, USA) was used to formulate the experimental diets, using the recommended levels of nutrients (NRC, 1997) for optimum performance. Diets for both experiments were cold-pelleted. The basal diets are shown in Tables 2.1 and 2.2.

Table 2.1. The basal diet for the Broiler NE Experiment

Diet	Lupin Canola	SBM MBM	Grain
Wheat 14%	56.3	58.8	82
Casein 90%	4.5		
Protein source	35	36.5	13.4
L-Lysine	0.4	0.1	
DL-Methionine		0.3	
Dicalcium P	2.1	2.6	2.6
Lime 38% Ca	1	1	1.14
Premix	0.5	0.5	0.5
Salt	0.2	0.2	0.36
TOTAL	100	100	100

Table 2.2. The basal diet for the Layer NE Experiment (%)

	Wheat	Barley	Millrun	Sorghum	Oats	Lupins	SBM	M&B	Canola
Wheat	80.04					60.21	69.65	71.54	64.46
Barley		80.04							
Millrun			80.04						
Sorghum				80.04					
Oats					80.04				
Protein Meal						28.33	18.89	17.00	24.08
Limestone	8.86	8.86	8.86	8.86	8.86	8.86	8.86	8.86	8.86
Casein	8.5	8.5	8.5	8.5	8.5				
Dicalcium Phosphate	2	2	2	2	2	2.00	2.00	2.00	2.00
Salt	0.3	0.3	0.3	0.3	0.3	0.30	0.30	0.30	0.30
DL-Methionine	0.2	0.2	0.2	0.2	0.2	0.20	0.20	0.20	0.20
Premix*	0.1	0.1	0.1	0.1	0.1	0.10	0.10	0.10	0.10
TOTAL	100	100	100	100	100	100	100	100	100

* Commercial vitamin and mineral premix for layers supplied by Ridley AgriProducts, Tamworth.

2.4 Respiration Chambers

The trials were conducted using 8 sets of the "closed-circuit calorimetry system" described by Farrell (1972). The system was effectively an ME cage capable of measuring oxygen intake and carbon dioxide output. Swain (1980), Pesti *et al*. (1988b) and Pesti *et al*. (1990) recorded some modifications that led to an improved oxygen flow and pressure control as illustrated in Figure 2.1. The system used water instead of mercury (Farrell, 1972) to seal the chamber (A). Pressure sensitive solenoid valves controlled the chamber pressure. The dimensions of each chamber (A) and wire mesh cage (B) were:

Chamber size: 600mm high x 380mm wide x 830mm long.

Cage size: 470mm high x 270mm wide x 480mm long.

Each chamber was constructed of poly-carbonate material and sat on a sheet-metal base containing a water trough. The cage rested on a tray (C) with sides about 10cm deep and there was sufficient room for a feeder (D) and drinker (E). Wet (G) and dry bulb (F) thermometers were located at one side of each cage. A 200 L cylinder (H), fitted with a regulator and a reducing valve (I), provided oxygen. Chamber air was circulated by a diaphragm pump (J) fitted with an electronic speed control (K), and was passed successively through a 2 L flask containing 1.5 L of potassium hydroxide (KOH) (L) and a moisture absorption train (M) containing 2 kg of calcium chloride before returning to the chamber. The KOH concentration used during Experiments 1 and 2 was adjusted according to the live-weight of the test birds (40% KOH at 500 g up to 50% KOH at 850 g), as required to provide a safety margin as the birds grew older, i.e. the KOH concentration was maintained in excess of that required to absorb the carbon dioxide (CO2) that was expected to be produced.

2.5 Principle of Operation

Two chicks at 14 days of age were placed in each of the 4 cages for Experiments 1 and 2 and the chambers were sealed. Initial readings of atmospheric pressure, temperature and humidity for each chamber were recorded, and an aliquot of chamber air was withdrawn through an outlet into a 0.5 L, gas-tight syringe. The air sample was subsequently analysed for O_2 and CO_2 using a Servomex digital oxygen analyser, model 570A and a Haldane gas analyser, respectively. Room temperature was controlled by an air conditioning unit at $25 \pm 2^{o}C$ to maintain a constant temperature within the chamber. The pump continuously circulated chamber air and as the air bubbled through the KOH solution, carbon dioxide expired by the birds was absorbed. This reduced the pressure in the

system, which in turn caused a solution of sodium bicarbonate ($NaHCO_3$) in the glass manometer (N) to rise in the closed arm. When the $NaHCO_3$ solution reached the stainless steel contact (O), the electrical circuit was completed and a relay system (P) opened a solenoid valve (Q) to permit oxygen to enter the chamber so as to restore chamber pressure. As the chamber pressure rose, the solution rose in the open arm until it reached the stainless steel contact (R) and the relay system shut off the solenoid valve. At the end of each 20-22 h experimental period, pressure, temperature and humidity in the chamber were recorded and a final sample of air was withdrawn for analysis.

Figure 2.1: The respiration chamber and ancillary equipment.

A - Chamber	**J** – Pump
B - Wire mesh cage	**K** - Motor speed control
C - Collection tray	**L** - Flask containing 1.5 L of KOH
D - Feeder	**M** - Calcium chloride train
E - Drinker	**N** – Manometer
F - Dry-bulb Thermometer	**O** - Steel contact in closed arm
G - Wet bulb thermometer	**P** - Relay system
H - Oxygen cylinder	**Q** - Solenoid valves
I - Oxygen regulator and reducing valve	**R** - Steel contact in open arm

The KOH solution into which the CO_2 was absorbed during each run was washed into a 2 L volumetric flask and made up to volume. An aliquot (10 mL) was analysed gravimetrically to determine carbonate content and thus CO_2 production was determined. Corrections were made to CO_2 and O_2 values on the basis of the initial and final composition of chamber air at STP.

2.6 Analytical methods and related procedures

GE determination

GE of the excreta and of the diets was determined in each experiment. Live weight, daily feed intake and water consumption were recorded. Excreta were collected separately from each cage for 4 days. Spilled feed and feathers were discarded and the excreta were dried at 80^{o}C for 24 h. The GE content of the diets and excreta were determined using an adiabatic bomb calorimeter (DDS CP 500, DIGITAL DATA SYSTEMS PTY, LTD.). Benzoic acid was used to standardise the bomb calorimeter. The dry excreta weight was adjusted to include the calculated dry weight of a sub sample that was used for VFA analysis.

AME determination

The GE values were then used to calculate those of the energy metabolised from the GE intake of the birds in a given period on both an as-fed and DM basis, thus:

$$\mathrm{AME\,(as\,fed)} = \frac{\mathrm{GE_{in}} - \mathrm{GE_{out}}}{\mathrm{FI_{as\,fed}}}$$

Where FI = feed intake and GE = gross energy

Dry Matter (DM) content of feeds (%)

The dry matter content (%) of the diets was measured after drying sub-samples (range 4-5 g) at 105°C for 24 h and calculated as follows:

$$\text{DM \%} = \left[100 - \left(\frac{\text{Weight of wet feed sample (g)} - \text{Weight of dry feed sample (g)}}{\text{Weight of wet feed sample (g)}}\right)\right] \text{x } 100$$

Dry Matter (DM) content of excreta (%)

Fresh excreta were dried in a force-draught oven at 80°C for 24h and the DM content was calculated as:

$$\text{DM \%} = \left[100 - \left(\frac{\text{Weight of wet excreta (g)} - \text{Weight of dry excreta (g)}}{\text{Weight of wet excreta (g)}}\right)\right] \text{x } 100$$

Oxygen consumption

The difference between the weight of the oxygen cylinder at the beginning and end of each run (20-22 h) gave the O_2 consumption value by weight.

Carbon dioxide recoveries

The recovery of CO_2 from KOH solution was accomplished by a modified barium chloride ($BaCl_2$) precipitation technique described by Swain (1980) as a variation of the earlier method of Annison and White (1961). Modifications involved the concentrations of ammonium chloride (NH_4Cl) (200 g/l) and $BaCl_2$ (300 g/l) used in the precipitation of $BaCO_3$ from the KOH solution, as described below. The gravimetric determination of CO_2 absorbed by the KOH solution was based on the following chemical reactions:

$$CO_2 + 2KOH \rightarrow K_2CO_3 + H_2O$$

$$K_2CO_3 + BaCl_2 \rightarrow BaCO_3 + 2KCl$$

The KOH solutions (40 to 50% w/v) for both Experiments were made by dissolving 400, 425, 450 or 500 g of KOH chips in 1.5 L of distilled water in a conical flask. The solution from each 20-22 h calorimeter run was made up to the 2 L volume and the dissolved CO_2 was then precipitated as follows:

1. 10 mL of the KOH solution was pipetted into a dried and weighed centrifuge tube followed by the addition of 6 mL of NH_4Cl and then 20 mL of $BaCl_2$ solution that was gently swirled and mixed thoroughly.

2. The resulting suspension was then centrifuged for 15 min at 7500 *g*.

3. The supernatant was carefully decanted and the carbonate pellet re-suspended in 20 mL of distilled water and centrifuged again for 40 min at 8000 *g*.

4. The supernatant was then decanted and the tube dried for 12 h at 105°C in a laboratory oven.

5. Finally, the centrifuge tube was cooled in a desiccator and re-weighed. The weight difference was recorded as the $BaCO_3$ recovered from the 10 mL aliquot of KOH solution. Recoveries were carried out in duplicates with an acceptable maximum difference of < 1% between duplicates. The dry weight of $BaCO_3$ was then used to calculate total CO_2 recovery as follows:

1.

$$CO_2 \text{ recovered in sample (g)} = \frac{\text{M. Wt. } CO_2}{\text{M. Wt.} BaCO_3} \times BaCO_3 \text{ Wt.} = 0.229 \times BaCO_3 \text{ Wt.}$$

2.

$$\text{Total } CO_2 \text{ recovered (g)} = \frac{\text{Volumetric flask mixture (2L)}}{\text{Aliquot volume (10ml)}} \times CO_2 \text{ (g)} = CO_2 \text{ (g)} \times 100$$

3.

$$\text{Volume } CO_2 \text{ recovered at STP (litres)} = \frac{CO_2(g)}{\text{M. Wt. } CO_2 \text{ (g)}} \times K = CO_2 \times 0.509$$

Where K is a constant (22.414) that represents the volume of 1 mole of gas at STP.

4.

$$\text{Combine factors: } \times \frac{\text{M. Wt. } CO_2}{\text{M. Wt.} BaCO_3} \times \frac{\text{Volume 1 mole gas at STP}}{\text{M. Wt.} CO_2} \times \frac{2000 \text{ mL}}{10 \text{mL}} = 11.35$$

5. Total CO_2 recovered (litres, STP) = $BaCO_3$ from 10mL aliquot x 11.35.

Heat production (HP)

The respiratory quotient (RQ) during each run refers to the ratio between the volume of CO_2 produced by the birds to the volume of O_2 used (RQ = CO_2 produced / O_2 used). The value obtained indicates the degree of oxidation of the diet on trial. By reference to the thermal equivalent of O_2 (kJ/L) for such a mixture, heat production from a known O_2 consumption was estimated, using the Brouwer equation (Johnson, 1981) incorporated into the Closed Circuit Respiratory Calorimetry (CCRC) computer program (Pesti *et al*., 1988b). Observations of HP were made over 4 days but were suspended for about 2 h each day while the feed and water containers were replenished, excreta were collected and the system was readjusted for the next run, including increasing the KOH

concentration in the CO_2 absorbed. The HP was calculated on an hourly basis and then converted to a 24 h basis.

Net energy (NE)

From the principle of the conservation of energy, the ME provided to a bird by its diet is either retained (ER) in the body or lost as heat (Kleiber, 1975; Sibbald, 1982; McDonald *et al*., 1995). Thus, ME = HI + ER. The deduction of the HI component from the ME intake gave the NE value of the feed.

Basal metabolism

The measurement of basal metabolism as estimated by fasting heat, involved the removal of the complicating effect of the heat increment of feeding by starving the birds. The period of fasting required for the digestion and metabolism of previous meals to be completed was 2 days as recommended for poultry (Farrell, 1972; 1974c; Pym and Farrell, 1977; McDonald *et al*., 1995). The RQ and HP from the known O_2 consumption and CO_2 produced by each pair of birds, during the starvation period were estimated using the Brouwer equation incorporated into the CCRC computer programme. By reference to the above programme, the link between basal metabolism and body weight was assumed (kJ/kg $^{0.75}$/day).

Statistical Analysis

All data were analysed using Repeated Measures Analysis. Statgraphics (Manugistica Inc., Maryland, USA) was used to perform the analyses. The conventions below have been used to indicate statistical significance throughout the text and tables:

NS	non significant; P>0.05
*	P<0.05
**	P<0.01
***	P<0.001

Chapter Three

The Net Energy Value of Poultry Feedstuffs Commonly Used in Australia

3.1 Introduction

It is argued that the net energy system is a better measure of the true availability of energy to animals because it takes into account of heat energy loss during metabolism. However, the system is a lot more tedious than the AME bioassay and it requires a higher degree of technical level to run it routinely. In the current study, the most common ingredients including wheat, barley, sorghum, millrun, canola, lupins, meat and bone meal, and soybean meal were assayed for their net energy value in both layers and broilers.

3.2 Results

Respiratory quotient (RQ), the ratio of oxygen intake and carbon dioxide output, but the birds were all around 1, which indicates a reasonable functioning of the calorimeters and a good health of the birds. Broilers fed canola meal and lupin meal produced significantly ($P<0.05$) more heat than those fed the other diets. This was reflected in the very low NE values for these ingredients. As expected, the NE value of sorghum was higher ($P<0.01$) than that of other ingredients tested in this study. The standard deviations of the NE values were generally high due to the low number of replicates available for the experiment. All the broiler data are shown in Table 3.1.

The results from the layer experiment were very different to that fro the broiler work. The RQ was generally lower and tended to be more variable than in broilers. Heat production on a metabolic body weight was half of that in broilers. Mature laying hens could extract a great deal of energy out of a range of ingredients, except millrun and canola. Some birds were able to utilise a diet containing a high level of canola well, whereas other were totally unable to handle it. Thus the variation in the NE value of canola was extreme. All the data are shown in Table 3.2.

Table 3.1. The NE value and associated parameters for commonly used ingredients in broiler chickens.

Diet	Respiratory Quotient	SD	Heat Prod. kJ/kg$^{0.75}$/day	SD	Diet ME MJ/kg	SD	Ingredient NE MJ/kg	SD
Barley	1.026	0.0241	1001a	65.01	11.91b	0.311	10.64b	0.700
Canola	1.043	0.0077	1046a	13.14	10.32c	0.782	5.28c	0.551
Sweet Lupin	1.019	0.0118	1107a	26.68	8.77c	0.619	3.87d	0.413
Meat Meal	1.038	0.0248	898b	10.91	12.84b	1.022	7.44c	0.534
Millrun	1.024	0.0224	882b	57.40	9.01c	0.341	8.75c	0.353
Soybean meal	1.015	0.0055	942b	46.69	12.69b	1.124	6.74c	0.876
Sorghum	1.095	0.0082	1073a	32.47	15.12a	0.603	13.18a	0.781
Wheat	1.055	0.0291	997a	49.90	13.14b	0.970	11.89b	0.618
Significance	NS		0.01		0.01		0.01	

[abc] Values with the same superscript within a column are not significantly different (P>0.05).

Table 3.2. The NE value and associated parameters for commonly used ingredients in laying hens.

Diet	Respiratory Quotient	SD	Heat Prod. kJ/kg$^{0.75}$/day	SD	Diet ME MJ/kg	SD	Ingredient NE MJ/kg	SD
Barley	0.980	0.0383	479	59.41	14.11a	0.401	10.26b	1.125
Canola Meal	0.970	0.0419	484	37.42	3.31c	1.844	9.27b	3.723
Sweet lupin	0.909	0.1490	532	81.69	7.95c	0.782	12.90b	1.328
Meat Meal	0.994	0.0435	513	39.35	9.14b	3.029	15.75a	1.532
Millrun	0.880	0.0301	493	31.57	9.43b	0.964	6.73c	1.220
Oats	0.971	0.0428	483	26.96	14.84a	0.224	11.44b	0.423
Soybean Meal	0.969	0.0558	514	73.59	5.04c	3.192	11.71b	1.610
Sorghum	0.973	0.0999	547	93.01	16.03a	0.845	12.25b	0.884
Wheat	0.929	0.0789	517	43.58	15.13a	0.389	10.31b	0.519
Significance	NS		NS		0.01		0.01	

[abc] Values with the same superscript within a column are not significantly different (P>0.05).

3.3 Conclusion

NE values cereal grains obtained in broilers comparable to those obtained in laying hens, but broilers appear to be unable to obtain as much NE energy from protein sources as laying hens.

Determination of NE is extremely tedious and it will require large amounts of investment to establish a commercially meaningful NE database for practical feed formulation.

Chapter Four

The Performance of Broiler Chickens Fed Diets formulated on ME or NE

4.1 INTRODUCTION

The default system of energy for poultry is the apparent metabolisable energy (AME) assay, determined either using the total collection method or the marker technique. It relies on a simple import-output balance of based on the total amount of food energy consumed vs. the total amount of energy excreted in the excreta. The AME value of an ingredient does not take into account energy losses due to heat production during digestion and absorption. Thus, an "efficiency of utilisation" of the food energy can be estimated. If a raw material takes more digestive and metabolic "effort" for the animal to utilise it, then this material will be less efficient in providing energy for maintenance and production. This is the basis of the argument that NE, rather than ME, should be used for practical diet formulation for animals. However, the measurement of NE is extremely complicated because it requires not only the quantification of O_2 consumption and CO_2 output, but also the energy expenditure in the form of other gases, such as methane and hydrogen. The animal's heat production at varying levels of food intake including at zero intake.

In the current study, a limited number of commonly used Australian raw materials were tested for their NE values and the efficacy of diets formulated either on AME or on NE values for bird performance was compared.

4.2 MATERALS AND METHODS

Two broiler diets were formulated either using the net energy (NE) values or the AME values (Table 4.1). A commercial diet was obtained from Ridley AgriProducts, Tamworth, NSW and was used as a control. One hundred and fifty (150) day-old Cobb male broiler chicks were used in the experiment. Birds were randomly distributed to 30 cages with 5 birds each, i.e, allocated to 10 cages per treatment. The birds were fed starter diets for the first three weeks and then changed to finisher diets for last two weeks. All the birds were fed *ad libitum* throughout the experiment. Clean water was available all times. At the beginning of fifth week, feed intake was recorded daily and excreta were collected quantitatively for AME determination. Body weight and feed intake were recorded weekly on a cage basis and FCR calculated correspondingly.

Table 4.1. The composition of the experimental diets.

Starter diet (0-21 days)				**Grower diet (21-35 days)**			
Formulated on NE		**Formulated on ME**		**Formulated on NE**		**Formulated on ME**	
Ingredient	**%**	**Ingredient**	**%**	**Ingredient**	**%**	**Ingredient**	**%**
Sorghum 10% CP	46.09	Sorghum 10% CP	36.59	Sorghum 10% CP	45.78	Sorghum 10% CP	**38.39**
Wheat 14% CP	27.44	Soy 47.9% CP	17.00	Wheat 14% CP	22.41	Wheat 14% CP	**3.99**
Soy 47.9% CP	15.00	Canola 35% CP	13.00	Barley 14% CP	0.19	Barley 14% CP	**20.00**
Meat ML 50% CP	10.38	Millrun 16%	12.89	Soy 47.9% CP	25.00	Soy 47.9% CP	**25.00**
Lysine-HCl	0.41	Lupin	9.00	Meat ML 50% CP	2.00	Meat ML 50% CP	**2.00**
Dl-Methionine	0.30	Dicalcium phosphate	5.23	Lupin	1.26	Millrun 16% CP	**5.00**
Premix	0.20	Sunflower oil	4.18	Dicalcium phosphate	1.00	Dicalcium phosphate	**2.57**
Salt	0.18	Lime 36% Ca	1.00	Lime 36% Ca	1.00	Lime 36% Ca	**1.43**
		DL-Methionine	0.40	Sunflower oil	0.47	Sunflower oil	**0.66**
		Lysine-HCl	0.30	Lysine-HCl	0.10	Lysine-HCl	**0.10**
		Salt	0.20	Dl-Methionine	0.35	Dl-Methionine	**0.35**
				Premix	0.20	Premix	**0.20**
				Salt	**0.25**	Salt	**0.30**
Analysis							
ME (kcal/kg)	3000		3000		3000		3000
Protein (%)	21.50		21.54		21.36		21.39
Digestible lysine (%)	1.08		1.12		0.94		0.94
Calcium (%)	1.35		1.23		0.93		1.07
Available P (%)	0.77		0.79		0.44		0.47

4.4 RESULTS

The weekly body weights of the birds fed the three diets (commercial, NE and ME) are shown in Table 4.2. The birds given the ME diet grew better up to week 2, but by week 3 this advantage disappeared. By week 4, the birds fed the commercial diet gained markedly less weight compared to those given the ME and NE diets.

Table 4.2. The body weight of broilers fed diets formulated either on ME or on NE in comparison to a commercial diet

Diet	0d	7d	14d	21d	28d	35d
Commercial	42.2	142.8[a]	402.5	743.3	1172.4[a]	1722.3[a]
Formulated on ME	39.7	154.5[b]	405.0	780.3	1328.3[b]	2008.4[b]
Formulated on NE	41.3	148.8[ab]	393.2	757.0	1302.4[b]	2030.0[b]
Pooled SE	1.5	3.3	13.0	18.4	19.3	24.7

[ab] Values with the same superscript within a column are not significantly different (P>0.05).

Feed conversion efficiency of the birds fed the three diets followed a similar pattern to the weight gain data. FCR was the lowest with the commercial diet during the first week, but this advantage disappeared by the time the birds were 2weeks of age. Clearly, the NE based diet gave the best FCR during the grower period (Table 4.3).

Table 4.3. The FCR of diets formulated either on ME or on NE in broiler chickens comparison to a commercial diet

Diet	0-7d	7-14d	14-21d	21-28d	28-35d	0-21d	21-35d	0-35d
Commercial	1.22[ab]	1.30	1.51	1.93[a]	2.03[a]	1.36	1.98	1.68
Formulated on ME	1.21[a]	1.31	1.51	1.80[b]	1.99[a]	1.39	1.90	1.68
Formulated on NE	1.23[b]	1.33	1.50	1.70[b]	1.80[b]	1.39	1.75	1.60
Pooled SE	0.02	0.04	0.06	0.04	0.02	NS	NS	NS

[ab] Values with the same superscript within a column are not significantly different (P>0.05).

The apparent metabolisable energy value of the three diets were determined during week 4 in order to ensure that the diet formulations were accurate and meaningful. The ME value of the diets was within 0.5MJ of each other, but the diet formulated on ME had significantly (P<0.05) lower ME value than the other two diets (Table 4.3).

Table 3 The AME value of the grower diets in broilers.

Diet	AME(MJ/kg)
Commercial	12.15[ab]
Formulated on ME	11.88[b]
Formulated on NE	12.25[a]
Pooled SE	0.11

[ab] Values with the same superscript within a column are not significantly different (P>0.05).

4.5 CONCLUSION

It is reasonable to conclude that diets formulated on net energy can give a significant advantage over those formulated on ME. This is probably due to the true additivity of NE values in feed formulation.

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