Review of AME bioassay for ingredient matrix values of poultry

by Shubiao Wu
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Foreword

Dietary energy is often the first item for formulating animal feed as it is required for metabolism, physiological functions, maintenance, growth, tissue turn-over and production of heat in animal body. In poultry, metabolisable energy (ME) has been used for feed formulation since the 1950s. However, the accuracy of ingredient ME data is questionable, at least for some of the measurements reported in the literature due to various reasons. These include methodology flaws, human errors and nature of complexity in measuring ME. To ensure accurate formulation of poultry feed, it becomes necessary to identify the sources of ingredient ME inaccuracy. Thus, a thorough review of literature published in past 100 years reporting the measurement of poultry AME of feedstuff was proposed so as to clarify where the problems are present and to suggest more accurate bioassays for the estimate of feedstuff energy values in poultry.

Poultry industry in Australia and over the world has been grown dramatically due to the progress on the breeding of elite poultry breeds and better nutritional management. Energy is a major cost component of broiler feed, and the energy cost of ingredients has become increasingly variable as a result of increased demand and biofuel production. Therefore, accurate assessment of ingredient energy value becomes more important and the current research aimed to achieve this goal. Therefore, the poultry industry will benefit significantly from the research through refined formulations according to more accurate AME values of feed ingredients.

This literature review revisits the studies on ingredient ME assays performed in the past 100 years. It identified flaws present in the bioassays: a. the use of a nutritionally imbalanced diet in the bioassay as the result of replacing part of a balanced diet with test articles; b. variable AME values of supposedly standard ingredients; c. inability of in vitro assays to accurately mimic true metabolism occurring in live animals; and d. wrong computations or mathematical models used in the assays. Based on the identified sources of errors, the author suggested more accurate bioassay protocols: a. multiple linear regression method; and b. basal diet substitution with different levels of inclusion rates. These protocols will provide more accurate and consistent ME values for the formulation of feed in poultry.

As the available ME values of ingredients in the literature are diverse due to their chemical characteristics and methods used, a proper bioassay for determining ME values for poultry feedstuffs is the first step for accurate assessment of feedstuff ME values whether table values, prediction equations, in vitro assays, or NIR analysis are used in the feed mills. The available ME values so far seen in the literature have to be used with care due to possible flaws. Inaccurate bioassays, unnecessary adjustments, poor experimental designs, and flawed statistical analysis or mathematical calculations should be avoided. It is important to note that researchers should use simple designs with appropriate calculations in mind before embarking on an animal experiment.

This report for the chicken meat R&D program is an addition to AgriFutures Australia’s diverse range of over 2000 research publications and it forms part of our Growing Profitability arena, which aims to increase the productivity and efficiency of chicken meat production.

Most of AgriFutures Australia’s publications are available for viewing, free downloading or purchasing online at: www.agrifutures.com.au.

John Harvey
Managing Director
AgriFutures Australia
About the Author

Dr Shubiao Wu is an associate professor at University of New England. He has worked at the university for 12 years with interdisciplinary expertise in agro-biological areas. He took up a position in poultry including diseases and nutrition in year 2008. Prior to his current position, he worked as a research fellow at University of Otago, New Zealand in inherited retinal diseases. He received his PhD in molecular mapping and reproduction of olives from The University of Adelaide in South Australia in 2002. A/Prof published over 80 refereed journal papers, supervised/supervising more than 30 PhD and master students, and is serving as academic editor of PLOS ONE and referees for more than 30 scientific journals.

 Acknowledgments

Prof Mingan Choct at University of New England for critical review of the paper and Prof Gene Pesti at University of Georgie for important suggestions for improvement of the content.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>amino acids</td>
</tr>
<tr>
<td>AME</td>
<td>apparent metabolisable energy</td>
</tr>
<tr>
<td>AME&lt;sub&gt;bd&lt;/sub&gt;</td>
<td>AME of basal diet</td>
</tr>
<tr>
<td>AME&lt;sub&gt;fd&lt;/sub&gt;</td>
<td>apparent metabolisable energy of feed</td>
</tr>
<tr>
<td>AME&lt;sub&gt;n&lt;/sub&gt;</td>
<td>AME corrected to zero N retention</td>
</tr>
<tr>
<td>AME&lt;sub&gt;nfd&lt;/sub&gt;</td>
<td>AME&lt;sub&gt;n&lt;/sub&gt; of feed</td>
</tr>
<tr>
<td>AME&lt;sub&gt;s&lt;/sub&gt;</td>
<td>standard AME that is corrected for 50% N retention</td>
</tr>
<tr>
<td>AME&lt;sub&gt;td&lt;/sub&gt;</td>
<td>AME of test diet</td>
</tr>
<tr>
<td>AME&lt;sub&gt;ti&lt;/sub&gt;</td>
<td>test ingredient AME</td>
</tr>
<tr>
<td>AME&lt;sub&gt;ε&lt;/sub&gt;</td>
<td>AME error</td>
</tr>
<tr>
<td>b&lt;sub&gt;bd&lt;/sub&gt;</td>
<td>the estimate of the proportion of GE for basal diet that appears in the excreta</td>
</tr>
<tr>
<td>b&lt;sub&gt;ti&lt;/sub&gt;</td>
<td>the estimate of the proportion of GE for the test ingredient that appears in the excreta</td>
</tr>
<tr>
<td>C&lt;sub&gt;td&lt;/sub&gt;</td>
<td>energy digestibility coefficient of test diets</td>
</tr>
<tr>
<td>C&lt;sub&gt;ti&lt;/sub&gt;</td>
<td>energy digestibility coefficient of test ingredient</td>
</tr>
<tr>
<td>DE</td>
<td>digestible energy</td>
</tr>
<tr>
<td>EEL</td>
<td>endogenous energy loss</td>
</tr>
<tr>
<td>EM</td>
<td>energy metabolizability</td>
</tr>
<tr>
<td>FI</td>
<td>Feed intake</td>
</tr>
<tr>
<td>FI&lt;sub&gt;td&lt;/sub&gt;</td>
<td>test diet FI measured</td>
</tr>
<tr>
<td>FI&lt;sub&gt;ti&lt;/sub&gt;</td>
<td>test ingredient intake</td>
</tr>
<tr>
<td>GE</td>
<td>gross energy</td>
</tr>
<tr>
<td>GE&lt;sub&gt;bd&lt;/sub&gt;</td>
<td>GE of the basal diet</td>
</tr>
<tr>
<td>GE&lt;sub&gt;el&lt;/sub&gt;</td>
<td>GE of EEL</td>
</tr>
<tr>
<td>GE&lt;sub&gt;ex&lt;/sub&gt;</td>
<td>excreta gross energy</td>
</tr>
<tr>
<td>GE&lt;sub&gt;fc&lt;/sub&gt;</td>
<td>fecal energy from the feed taken</td>
</tr>
<tr>
<td>GE&lt;sub&gt;fd&lt;/sub&gt;</td>
<td>gross energy of feed taken</td>
</tr>
<tr>
<td>GE&lt;sub&gt;gs&lt;/sub&gt;</td>
<td>gaseous energy from the feed taken</td>
</tr>
<tr>
<td>GE&lt;sub&gt;lbd&lt;/sub&gt;</td>
<td>GE intake contributed by the basal diet proportion of the test diet (GE&lt;sub&gt;lbd&lt;/sub&gt; × P&lt;sub&gt;bd&lt;/sub&gt;)</td>
</tr>
<tr>
<td>GE&lt;sub&gt;ld&lt;/sub&gt;</td>
<td>test diet GE intake</td>
</tr>
<tr>
<td>GE&lt;sub&gt;li&lt;/sub&gt;</td>
<td>GE intake as the test ingredient in the test diet</td>
</tr>
<tr>
<td>GE&lt;sub&gt;ur&lt;/sub&gt;</td>
<td>urinary energy from the feed taken</td>
</tr>
<tr>
<td>HI</td>
<td>heat increment</td>
</tr>
<tr>
<td>II&lt;sub&gt;ti&lt;/sub&gt;</td>
<td>test ingredient intake</td>
</tr>
<tr>
<td>ME</td>
<td>metabolisable energy</td>
</tr>
<tr>
<td>ME&lt;sub&gt;bd&lt;/sub&gt;</td>
<td>ME of basal diet</td>
</tr>
<tr>
<td>ME&lt;sub&gt;d&lt;/sub&gt;</td>
<td>ME values of all diets including ME&lt;sub&gt;bd&lt;/sub&gt; and ME&lt;sub&gt;fd&lt;/sub&gt;</td>
</tr>
<tr>
<td>ME&lt;sub&gt;ε&lt;/sub&gt;</td>
<td>difference between diet ME values and ME contributed by the standard ingredients in that diet</td>
</tr>
<tr>
<td>ME&lt;sub&gt;fd&lt;/sub&gt;</td>
<td>ME value measured from the feed fed to birds</td>
</tr>
<tr>
<td>ME&lt;sub&gt;ld&lt;/sub&gt;</td>
<td>ME intake with the basal and test diets</td>
</tr>
<tr>
<td>ME&lt;sub&gt;li&lt;/sub&gt;</td>
<td>ME intake contributed by test ingredient</td>
</tr>
<tr>
<td>ME&lt;sub&gt;mi&lt;/sub&gt;</td>
<td>ME of standard ingredients</td>
</tr>
<tr>
<td>ME&lt;sub&gt;ti&lt;/sub&gt;</td>
<td>ME of test diet</td>
</tr>
<tr>
<td>ME&lt;sub&gt;ti&lt;/sub&gt;</td>
<td>ME value of test ingredient</td>
</tr>
<tr>
<td>N</td>
<td>nitrogen</td>
</tr>
<tr>
<td>NE</td>
<td>net energy</td>
</tr>
<tr>
<td>NIR</td>
<td>near infrared reflectance</td>
</tr>
<tr>
<td>N&lt;sub&gt;rt&lt;/sub&gt;</td>
<td>N retention by animal</td>
</tr>
<tr>
<td>P&lt;sub&gt;bd&lt;/sub&gt;</td>
<td>proportion of the basal diet (energy yielding ingredients) in the test diet</td>
</tr>
<tr>
<td>P&lt;sub&gt;mi&lt;/sub&gt;</td>
<td>minor ingredient inclusion rate</td>
</tr>
</tbody>
</table>
\( P_{si} \) proportion of standard ingredient in the test diet
\( P_{ss} \) proportion of standard ingredient substituted by test ingredient
\( P_{ti} \) proportion of test ingredient included in the diet
TME true metabolisable energy
TME\(_{bd}\) TME of the basal diet
TME\(_{fd}\) TME of feed
TME\(_{n}\) TME corrected to zero N retention
TME\(_{nf}\) TME\(_{n}\) of feed
TME\(_{ti}\) TME of the test ingredient
# Contents

Foreword .......................................................................................................................... iii
About the Author .............................................................................................................. iv
Acknowledgments ........................................................................................................... iv
Abbreviations .................................................................................................................. v
Executive Summary ........................................................................................................ ix
Introduction ..................................................................................................................... 1
Objectives ......................................................................................................................... 2
Energy partition of feed in poultry .................................................................................. 4
Metabolisable energy systems in poultry .......................................................................... 5
Metabolisable energy bioassays and factors affecting ME ................................................ 7
Techniques to determine ingredient ME .......................................................................... 10
  Direct feeding .................................................................................................................. 10
  Standard ingredient substitution ..................................................................................... 11
  Standard ingredient plus .................................................................................................. 11
  Basal substitution ............................................................................................................ 12
  Multiple linear regression ............................................................................................... 14
  Energy metabolizability ................................................................................................. 15
Pros and cons of ME systems .......................................................................................... 16
  AME vs TME .................................................................................................................. 16
  ME vs MEₐ ....................................................................................................................... 17
Flaws in ME bioassay ....................................................................................................... 18
  Wrong assumption of \( P_{bd} + P_{ti} = 1 \) in basal substitution method ......................... 18
  Non proportional basal energy yielding ingredients in test diet ................................... 20
  Miscalculations in simple linear regression .................................................................. 20
  Test diet ME value is assumed, not determined .............................................................. 21
  Forcing feeding to measure AME .................................................................................. 22
  Use diet metabolisability to predict test ingredient ME value ....................................... 23
  Ignored synthetic AA contribution as energy yielding ingredients ............................... 24
Proposed bioassay systems and diet designs for ingredient ME values ......................... 26
  Multiple linear regression method ............................................................................... 26
  Basal diet substitution ................................................................................................... 27
Implications ....................................................................................................................... 28
Recommendations ........................................................................................................... 29
References ......................................................................................................................... 30
Tables

Table 1. AME value difference resulted in mistaken calculation with constant minor ingredients between basal and test diets. ................................................................. 19

Table 2. Variables used in the Dozier et al (2008) for the comparison of accuracy with two different regressions............................................................. 21

Table 3. Calculation of test ingredient metabolisable energy coefficient .................... 25

Figures

Figure 1. Partition of feed energy in poultry showing the energy contents measurable under experimental conditions .................................................. 4

Figure 2. Regression of AMEn intake vs feed intake, glycerine intake and glycerine inclusion rate depicting calculation flaw present in the literature .................. 22
Executive Summary

What the report is about

This study critically reviewed the literature on poultry AME bioassay for ingredient matrix values for past 100 years to assess the accuracy of the reported ME values in the literature. By reviewing the design, methodology, the calculations and the values reported, the author revealed the historical flaws presented in the studies in assessing the ME values of poultry feed ingredients and suggested more accurate methods for such assays.

Who is the report targeted at?

The report targets poultry nutritionists who work in the industry and conduct research. The report probably is more important for new researchers and industrial nutritionists as they can start their research and formulation with the view that currently available energy values in the literature and database should be scrutinised for their applications.

Where are the relevant industries located in Australia?

- What is the location of the strongest industry representation in Australia?

Australia poultry industry is distributed among all the states including but not restricted to: outskirts of the Sydney metropolitan area, Mangrove Mountain / central coast, Newcastle, Tamworth and Griffith areas of New South Wales; Redland Bay south of Brisbane, and other areas to the south, south west and north of Brisbane in Queensland; Mornington Peninsula, east of Melbourne, and Geelong and Bendigo areas in Victoria; outskirts of Adelaide and the Two Wells area in South Australia; and outer metropolitan areas of Perth. Tasmania, Canberra and Northern Territory also have poultry farms.

- Describe the industry and indicate how many producers are involved and what the production levels and markets are.

There are currently more than 800 commercial meat chicken growers in Australia. Most grow chickens under contract to meat processing companies and are known as 'contract chicken growers'. These producers grow 80% of Australia’s meat chickens. The meat chicken industry is growing fast. The chicken meat consumption is steadily increasing and current annual consumption is 49 kg per capita. In 2017, the industry produce 1.2 million tonnes of chicken meat that is worth $2.8 billion. For egg industry, Australia produce greater than 500 million dozen eggs with a flock size of 22 million layers that is worth of $820 million in value in 2017. The consumption per capita in Australia is 245 eggs a year. Caged eggs has a share of 44%, free range eggs has overpassed caged egg to 45% and other productions see a 11% share. Australia turkey and duck industries are relatively small with a share of approximately $200 million and $100 million respectively.

- Who will benefit from this research and where are they located in Australia?

The poultry industry will benefit from this research and they are located as stated above.

Background

Dietary energy available to animals is key for formulating feed as it is required for all aspects of the animal’s life. In poultry, apparent (AME) and true (TME) metabolisable energy (ME) values have been used for feed formulation with or without correction for nitrogen balance. For the past 50 years, the accuracy of ME systems has been an ongoing debate and the
comparability of data produced using different bioassay systems developed at various institutes is often questionable. Overall, the ingredient matric values for ME used in feed formulation are not consistent, and to some extent, confusing.

**Aims/objectives**

The aim of the present review was to examine ME data produced for poultry feed ingredients published in the past century to elucidate the accuracy of different bioassay systems and examine whether the values for accuracy and useability. The poultry industry and researchers will benefit from this study.

**Methods used**

The literature of about 100 years was searched and reviewed thoroughly.

**Results/key findings**

A variety of flaws were identified in the literature that suggest a thorough re-thinking of feedstuff ME values currently used in practical feed formulation and in developing prediction equations. Two protocols, i.e., multiple linear regression and basal diet substitution methods, are proposed as more accurate bioassays for evaluating feedstuff ME values in this review. In addition, AME values without correction to zero nitrogen retention align more closely with the actual energy levels of feed ingredients likely available to growing birds, which should be used for poultry feed formulations instead of AME_n.

**Implications for relevant stakeholders**

- **Industry**
  Poultry industry in Australia and essentially over the world will benefit from the outcomes of this literature review on more accurate formulations of poultry feed so that costs to the feed can be reduced and the performance of birds improved.

- **Communities**
  Consumers will benefit indirectly from possible lowered costs of poultry products as the result of the savings made by the poultry industry.

- **policy makers**
  This study may have implications to policy makers on the importance of precision agriculture practice as the outcomes of this review will fine-tune the feeding practice of the industry.

**Recommendations**

The outcomes of the study correct the flaws in the literature and suggests bioassay methods for the measurement of the ingredient AME values. However, some researchers may still not be aware of the issues identified in the review, and thus new flaws may still be published following this review. Therefore, nutritionists are expected to apply any reported AME values carefully and only use the values in formulation practice after careful scrutinising. Furthermore, any *in vitro*, NIR or table values must be calibrated or computed based on the values produced from flawless bioassays so as to apply the derived values accurately. Although the flaws identified in this literature review can be avoided with care, whether the assumption that energy of individual ingredients is additive in a complete diet is still untrue at least under some circumstances. This may require efforts from industry and researchers to investigate relations among the main ingredients in a complete diet so that more accurate formulation can be performed based on the outcomes that may fine-tune the additivity assumption.
Introduction

Dietary energy is often the first item for formulating animal feed as it is required for metabolism, physiological functions, maintenance, growth, tissue turn-over and production of heat in animal body. In poultry, apparent metabolisable energy (AME), expressed as the gross energy of the feed minus the gross energy of the excreta, has been used for feed formulation since the 1950s (Hill and Anderson, 1958; Hill, et al., 1960). By accounting for the endogenous loss of energy in excreta, true metabolisable energy (TME) was developed later to measure available energy of feed to the animals (Sibbald, 1975). The general practice is to apply sourced (reported or measured) metabolisable energy (ME) values of ingredients as matrix values in poultry feed formulation so as to have an estimated ME value of a complete diet to satisfy the requirement of birds from feed. This practice requires a large database containing AME/TME values of grains, protein meals, fats and oils, and other minor ingredients and even additives, such as enzymes, of varying sources. All these values compiled in the database depend on data attained by individual measurements, reported in the literature, acquired from table values, predicted from values published in previous studies and, to a certain extent, guesstimated by experienced nutritionists. ME values of grains and other ingredient sources have been reported in the literature using different techniques: bioassays with live birds (Hill, et al., 1960; Tyagi, et al., 2008; Yegani, et al., 2013), table values according to prediction equations (Janssen, 1989; Sauvant, et al., 2004; CVB, 2009; Rostagno, 2017), in vitro analyses through artificial digestive systems (Smulikowska, 1992; Farrell, 1999; Gehring, et al., 2012; Zhao, et al., 2014), and near infrared reflectance (NIR) analysis (Valdes and Leeson, 1992; Garnsworthy, et al., 2000; Black, et al., 2009). However, the accuracy of ingredient ME data is questionable, at least for some of the measurements reported in the literature. This is due to: a. the use of a nutritionally imbalanced diet in the bioassay as the result of replacing part of a balanced diet with test articles; b. variable AME values of supposedly standard ingredients; c. inability of in vitro assays to accurately mimic true metabolism occurring in live animals; and d. wrong computations or mathematic models used in the assays. A recently published review on energy content estimation of diets and ingredients in poultry has thoroughly discussed the various reasons responsible for the discrepancies among AME values from different sources (Mateos, et al., 2018). The review focused on problems present in the table values, with prediction equations as well as the AME value discrepancies due to the environmental and physical factors affecting the energy content of diets and ingredients and, to an extent, the differences introduced by different wet chemistry methods used in different labs. Current review will focus on the methods used for measuring AME values of different feed ingredients, and revisit the ingredient values reported in the literature, so as to identify possible issues on accuracy of the measurements, and to suggest future directions for such measurements and/or prediction as has been suggested by Mateos, et al. (2018) that the standardisation of the procedures used in the in vivo trials should be a priority.
Objectives

A. To review all the protocols used so far since Fraps study (1939);
B. To identify issues involved in the measurements for each of the methods applied;
C. To determine the best protocol published in the literature;
D. To propose a more accurate bioassay for the measurement of the ingredient AME in poultry feed.
Methodology

This is a literature review project and thus following protocol was used:

a. Relevant literature was searched to have all the papers published available since the first publication on poultry AME study with available scientific publication database, i.e., Scopus, PubMed, and Google Scholar.
b. The bioassay methods were categorised.
c. The designs and diets used for the bioassays were examined to find their merits and shortcomings.
d. Reported data were re-analysed when possible to compare and assess the AME values obtained in the studies published.
e. The bioassay methods were evaluated based on the data reanalysis.
f. The best protocol in the literature was identified.
g. The possible corrections were suggested for the methods being inaccurate.
h. More accurate protocols were recommended according to the comprehensive.
Energy partition of feed in poultry

Feed energy can be partitioned to reflect the functionality and diversion of the energy content in the animal body (Figure 1). The gross energy (GE) of feed is stored as chemical energy which can be measured as energy released from the combustion of feed in a bomb calorimeter. This can be measured in a laboratory and predicted accurately based on the chemical composition of the feed (Ewan, 1989). However, it does not provide precise energy values that are available for animals to satisfy their requirement for maintenance and production. Therefore, the concept of ME was developed, and in poultry AME is widely used as default value for feed formulation, while TME was also used in the past albeit mainly in north America (NRC, 1994; Farrell, 1999). Metabolisable energy is defined as the feed energy available to the birds for anabolic and catabolic processes (Pesti and Edwards Jr, 1983), and measured as the GE of feed minus the GE of excreta (Armsby, 1903). As ME doesn’t differentiate between the energy required for production/maintenance and heat produced during digestion, metabolism and excretion, net energy (NE) was proposed to account for the loss of energy as heat for digestion, metabolism and excretion (defined as heat increment, or HI) (Noblet, et al., 2010). As NE is difficult to measure, and studies reported in the literature showed no or variable correlations of NE with the composition of diet in poultry due largely to the lack of accuracy in measurements, it has not been used for formulation of feed in poultry (Noblet, et al., 1994; Ferrell and Oltjen, 2008). However, efforts are being made in recent years to develop an NE system for practical feed formulation in poultry (Swick, et al., 2013; Carre, et al., 2014; Wu, et al., 2018). In the meantime, ME values are still used in poultry feed formulation. Accurate measurement of ME values for ingredient matrix is vital in the current and future formulation systems in poultry.

Figure 1  Partition of feed energy in poultry showing the energy contents measurable under experimental conditions. Excreted energy includes those from faeces and urine, and gaseous energy produced in poultry is negligible and thus not accounted for. *Digestible energy (DE) is not measurable under non-surgical experimental conditions as urine and faeces are excreted through a common cloaca to form excreta in poultry. Attempt can be made to measure DE through surgical approach but the digestive behaviour of modified birds may not be same as normal birds thus the measured DE value can be questionable.
Metabolisable energy systems in poultry

In poultry, several ME energy systems have been proposed and used in formulation of feed. As a consequence, energy evaluation for feed ingredients has produced ME values using various systems and hence most values are inconsistent. Among those, AME and TME with or without correction to zero-nitrogen (N) retention were used for decades (Farrell, 1999; Mateos, et al., 2018). While AME (or AME_n) has been used widely in the majority of the world, TME (or TME_n) was used in North America at least at some period of time and/or by some nutritionists (Farrell, 1999). Generally speaking, the nomenclatures of ME measured in different ways are confusing even though a more detailed naming system was proposed by Pesti and Edwards Jr (1983). The debate over the past century on which energy system should be used in poultry is yet to be settled. The breeding companies make recommendations for ME and amino acid levels but do not specify which system to use. Instead, a large volume of ME values for feed ingredients have been produced in the literature without an agreed standard system. The poultry industry worldwide on the other hand has been using those values in their feed formulations possibly without paying attention to the type of the values they are using, for example, AME or AME_n measured by which method. This may have resulted confusion about the differences with age and how additivity works with values measured at one age to formulate feed for different ages (Pesti, personal communications). Therefore, there is an urgent need for poultry nutritionists and researchers to standardise the type of ME system used to generate values for feed formulation. This will require a standardised approach to the bioassay protocol as has been the case in swine industry where digestible energy (DE) is used universally without confusion.

Metabolisable energy, defined primarily by Armsby (1903) for animals in general as: “potential energy of food minus potential energy of excreta, including under excreta, of course, all the wastes of the body, visible and invisible”. In poultry, Fraps and colleagues assayed metabolisable and productive energy extensively from 1920s to 1940s (Fraps, 1928; Fraps and Carlyle, 1939; Fraps, et al., 1940; Fraps, 1944), and ME was defined as “energy in the feed eaten less that excreted and is the maximum amount of energy that can be utilised by the animals”. Accordingly, ME of feed can then be expressed as:

$$ ME_{fd} = \frac{[GE_{fd} - (GE_{fc} + GE_{ur} + GE_{gs})]}{FI} \quad \text{Equation 1} $$

Where $ME_{fd}$ is metabolisable energy per unit feed; $GE_{fd}$ denotes gross energy of feed taken, $GE_{fc}$ indicates fecal energy from the feed taken, $GE_{ur}$ represents urinary energy from the feed taken, and $GE_{gs}$ equals to gaseous energy from the feed taken.

For the calculation of ME in poultry, $GE$ of feed taken ($GE_{fd}$) and $GE$ of excreta can be measured directly in the laboratory while the $GE_{gs}$ is usually ignored due to the negligible amount of gases produced by avian species. The above measurement of ME involves the excreta GE, where the energy is not only of feed origin but includes the energy from endogenous materials lost in the excreta defined as endogenous energy loss (EEL), which is the apparent values of ME thus called AME to distinguish it from true ME. To apply poultry situation, the above equation can be modified as:

$$ AME_{fd} = \frac{(GE_{fd} - GE_{ex})}{FI} \quad \text{Equation 2} $$

Where $AME_{fd}$ is apparent metabolisable energy of feed; and $GE_{ex}$ denotes excreta gross energy.

Harris (1966) proposed a system to measure ME with a correction for EEL so that the resulting ME is basically of feed origin, i.e., TME. Later, Sibbald (1976) developed a forcing feed system
using adult roosters to measure TME with correction for EEL through the measurement of the excreta voided by fasting birds. TME then can be expressed as:

\[
TME_{fd} = \frac{(GE_{fd} - GE_{ex} + GE_{el})}{FI}
\]

Equation 3

Where \(TME_{fd}\) equals TME of feed; and \(GE_{el}\) represents GE of EEL.

More than a century ago, Armsby (1903) proposed to correct the ME of protein gained by animals as the metabolism of such gained protein to produce kinetic form of energy would involve the energy cost of the production of urine to be excreted. Hill and Anderson (1958) later applied the correction of ME to N equilibrium with a value of 8.22 kcal per gram of N and stated it was for comparative purpose. Therefore, AME and TME can be corrected to N equilibrium as AME\(_n\) and TME\(_n\), and computed as below:

\[
AME_{n,fd} = \frac{(GE_{fd} - GE_{ex} - N_{rt} \times 8.22)}{FI}
\]

Equation 4

and

\[
TME_{n,fd} = \frac{(GE_{fd} - GE_{ex} - N_{rt} \times 8.22)}{FI}
\]

Equation 5

Where \(AME_{n,fd}\) is the AME\(_n\) of feed; \(TME_{n,fd}\) represents TME\(_n\) of feed; and \(N_{rt}\) equals to N retention by animal.

Other forms of energy, such as productive energy or net energy have been reported. However, these are beyond the scope of this review and thus will not be elaborated.
Metabolisable energy bioassays and factors affecting ME

ME can be measured using different bioassays according to how birds are fed and the excreta collected. Reported feeding techniques are:

- Ad libitum feeding applied by earlier researchers (Mitchell and Haines, 1927; Carpenter and Clegg, 1956), standardised by Hill and Anderson (1958), and used by different researchers for different feeding periods (1 to 7 days);
- Force-feeding with an intubation technique to deliver feed directly into the crop of birds at once (Sibbald, 1976);
- Rapid feeding by training birds to consume their feed allowance within a short span of time (1 hour) (Farrell, 1978);
- Controlled feeding to allow birds to take a proportion of feed during the experiment (Hari and Kriwusch, 1918; Fraps, 1928; Fraps, et al., 1940).

The ME bioassays with *ad libitum* feeding employed either total collection of the excreta or indigestible indicators such as chromic oxide, titanium dioxide, and acid insoluble ash to calculate the amount of energy excreted and the amount of energy retained relative to the marker – refer to the review of Pest and Edwards Jr (1983). In addition, for different feeding and excreta collection methods, there have been differences in the lengths of adaptation, the duration of excreta collection, age, sex and breed of the birds used, the number of birds per replicate, and the number of replicates. This review will not describe in detail regarding these methods and variations *per se*, rather it will focus on the accuracy and applicability of the methods.

Metabolisable energy values of feed are related to the characteristics of feed and the capability of animals to which it is fed. Therefore, the measurement of AME depends not only on the composition of energy yielding components of feed but also on health status, and possibly, age and physiological conditions, of the birds. For example, different chickens responded differently with the metabolism of lower AME wheat so that there is great variability in AME values measured with different individual chickens (Hughes, 1997 #2027). For the ease of text flow, the following sections will briefly touch on factors related to the characteristics of both the bird and feed that affect ME values due to methodological issues (Mateos, et al., 2018).

Feed intake (FI) affects AME values in roosters and broilers with controlled feeding regimes or variable FI due to aberrant nutrient balance in that EEL is not proportional to FI (Guillaume and Summers, 1970; Sibbald, 1975; Hätel, 1986; Zelenka, 1997; Yaghobfar and Boldaji, 2002). However, some other studies demonstrated that AME did not differ significantly when the chickens were fed the feed in certain ranges (30% to *ad libitum*) (Hill and Anderson, 1958; Bourdillon, et al., 1990a) presumably due to a low EEL. While earlier studies assumed that TME values are independent of FI due to a constant EEL produced by birds under standard conditions (Guillaume and Summers, 1970; Sibbald, 1975; Sibbald and Morse, 1983), this assumption has been shown to be untrue as EEL varies depending on the feed input and digestion of nutrients by the fasted birds may be abnormal (Hätel, 1986; McNab and Blair, 1988). However, in spite of the discrepancy on the influence of FI on ME, the values of AME, AMEn, TME or TMEn should be relatively stable at *ad libitum* feed consumption level provided the bioassay is conducted under standard conditions with the same type of birds and method. Therefore, given ME values are applied in feed formulation normally at *ad libitum* or at close to *ad libitum* FI, bioassays of any type of ME should be carried out with birds being fed *ad libitum*. Hence, caution should be exercised when using ME values of any type that have been corrected for FI.
Metabolisable energy values of feed have been measured in a wide range of birds, namely in different ages, sex, and breeds. It has been widely accepted that age affects the values of feed AME. For example, the AME value for a feedstuff is higher in adult chickens such as roosters than in growing broilers (Sibbald and Wolnyetz, 1985; Johnson, 1987; Bourdillon, et al., 1990b; Farrell, et al., 1998; Gonzalez-Esquerra and Leeson, 2000). Similarly in turkeys, old birds showed higher AME of pea diets than the younger counterparts (Palander, et al., 2006). However, the opposite has been reported: broilers showed higher diet AME values than roosters (Lopez and Leeson, 2007); AME, values, on the other hand, were higher in roosters due to little correction for N balance in roosters but greater correction in broilers. In growing chickens, although it has been recognised in general that the AME values are greater in older than in younger birds, except for some particular ingredients. For instance, a positive linear correlation between age and meat and bone meal AME was observed in broilers from days 0 to 21 (Adeola, et al., 2018), while the AME, values of biodiesel glycerine negatively correlated with the age of broilers up to day 30 (Lima, et al., 2013). Interestingly, fat AME values in broilers reached a plateau by the age of two weeks and no further increase was shown (Tancharoenrat, et al., 2013) and in some bioassay, age did not show effect in chickens (Sibbald, et al., 1960).

Sex is another variable of concern for bioassays of ME in poultry. Over the past century, ME has been measured mainly in male birds including roosters and male broilers, although in practice feed is formulated for both male and female birds. Comparisons for ME values obtained in male and female chickens have also been performed (Guirguis, 1976; ten Doeschate, et al., 1993; Zelenka, 1997; Ravindran, et al., 2004). It has been concluded that gender influences the digestive capacity of chickens through endogenous energy losses, gut structure and function, and metabolic activity of gut microflora (Hughes and Choct, 1999; Nalle, et al., 2011a). Thus, AME values measured in different gender can vary normally being lower in male than female. For most ingredients, ME values tend to be higher in female birds. However, like most studies related to ME values, information regarding the sex effect on ME values is not unequivocal. While the ME value of most feedstuff was not affected by sex (Zelenka, 1997), that of oats, fishmeal and tallow was significantly higher for female than for male chicks (Guirguis, 1975; Guirguis, 1976). It has also been shown that sex effect on AME, values of feed differs according to the age of the birds; while no sex effect was shown for 3 week old broilers, the AME, values for male broilers were higher than those for the females at 6-week old (Ravindran, et al., 2004).

The metabolisable energy assayed in different species or breeds of poultry can vary. The animals used include growing chicks (Hill and Anderson, 1958; Waititu, et al., 2018), layers (Mitchell and Haines, 1927; Zuber and Rodehutscord, 2017), roosters (Sibbald, 1975; Deng, et al., 2016), turkeys (Leeson, et al., 1974; Kozlowski, et al., 2018), ducks (King, et al., 1997; Kong and Adeola, 2010), geese (Wang, et al., 2017a), pigeons (Hullar, et al., 1999; Sales and Janssens, 2003) and quails (Mandal, et al., 2006; Pasquetti, et al., 2015). In industry, however, the database ME values invariably rely on broiler, to a lesser extent, adult rooster bioassays, with ME values of many ingredients unavailable for minor species. In fact, little has been done to systematically determine to what extent ME values of ingredients in different types of birds differ, and let alone setting any meaning correction factors to adjust the ME values obtained in one species for use in another species. However, sporadic comparisons among the poultry have been reported now and then (Lodhi, et al., 1969; Dale and Fuller, 1980; Cilliers, et al., 1994; Sell, et al., 2001; Collins, et al., 2003; Mandal, et al., 2006; Kianfar, et al., 2013). Higher AME and AME, values were observed in laying hens than in broilers for rapeseed meal (Lodhi, et al., 1969) and conjugated linoleic acid (Sell, et al., 2001). TME values of corn, soybean meal, corn gluten meal, fish meal and poultry by-product meal were similar among roosters, broilers and poults although broiler values tended to be lower (Dale and Fuller, 1980). Compared between the AME, values of roosters and ostriches, no difference was apparent for corn while ostriches had twice as much of lucerne AME as roosters (8.9 vs 4.5 MJ/kg) (Cilliers, et al., 1994) due to the ability of ostriches to digest fibre. The AME, values of barley
were higher in cockerels than in quails regardless of processing and enzyme supplementation (Kianfar, et al., 2013). However, no significant difference in the AME\textsubscript{n} values of different sorghum varieties was observed among cockerels, guinea fowls and quails (Mandal, et al., 2006).

The interactions among nutrients in feed also play a big role in the variation of AME values reported in the literature as all the assays or feed formulation have to assume that the energy provided by all the energy yielding ingredients or essentially the nutrients are additive. However, this may not be the case as dietary energy depends on the interaction between birds (Mateos, et al., 2018) and the nutrients can be interactive to each other. A typical example is the extracaloric effect of fat: added fat contributes more energy than predicted level of contribution to the diet. The interaction on ME between fat and possibly NSP in grains may be one of the reason (Mateos and Sell, 1980; Ward and Marquardt, 1983). (Ravindran, et al., 2016) made an excellent review on the factors such as the age, genetics, gender and health status of birds, characteristics of fat, fat inclusion level and other diet components that may be responsible for the measured values of energy content of fats. It has been reported that cereal type and fat sources interact in terms of fat digestion. For example, fat digestion can be suppressed by rye-based diets with tallow but less so with soybean oil (Antoniou, et al., 1980). Viscosity and microbial growth in the small intestine may be responsible for the depressed digestibility of fat (Ravindran, et al., 2016). Accessibility of lipid resulted from feed processing such as steaming cooking and pelleting may enhance fat digestibility in corn-based diet, while not in wheat or sorghum-based diets (Jiménez-Moreno, et al., 2009; Abdollahi, et al., 2014). In addition, dietary calcium and phosphorous levels and antinutrient factors such as tannins, trypsin inhibitors and various mycotoxins play significant roles in fat digestibility thus ME content of the feed (Ravindran, et al., 2016).

Other factors such as physico-chemical characters of diets and ingredients, heat processing, feed forms and particle size, dietary fiber and fat contents, antinutritional factors, and supplementation of additives such as enzymes, probiotics, prebiotics and organic acids have been extensively discussed, such as in a recent review (Mateos, et al., 2018). These are very important factors to be considered when the ME values are going to be used in feed formulation in feed mills.
Techniques to determine ingredient ME

Several protocols have been developed to determine ingredient ME values to be used for feed formulation by *in vivo* bioassays. The main consideration is how the test ingredients should be included in the test diet and fed to chickens so that the ME values can be derived. The techniques are summarised as follow:

a. Direct feeding of the test ingredient only (Direct feeding) (Mitchell and Haines, 1927; Fraps, et al., 1940; Sibbald, 1976);

b. The test ingredient to substitute an ingredient with known ME value in a basal diet to form a test diet. The ME values of basal and test diets are measured simultaneously (Standard ingredient substitution) (Hill and Anderson, 1958);

c. The test ingredient is mixed with one or more ingredients with known ME values to make a test diet. No basal diet is required in this case (Standard ingredient plus) (Carpenter and Clegg, 1956; Choct, et al., 1999);

d. The test ingredient mixed with a basal diet to make a test diet. The ME of basal and test diets are measured simultaneously (Basal substitution) (Sibbald, et al., 1960; Farrell, 1978);

e. Formulation of multiple test diets with multiple test ingredients at various independent levels (Multiple linear regression) (Young, et al., 1977; Noblet, et al., 1993; Applegate, 2005).

With different designs of experiment to perform bioassay, calculations of the test ingredient ME values can be very different. Even within the same category of the design, the calculation procedure can be different depending on how the basal and test diets are formulated. These can sometimes be very confusing even to the researchers themselves who designed the experiments, and not to mention the readers who wanted to understand it and possibly to emulate it. Therefore, it is necessary to detail all the steps of calculations according to how the bioassays are conducted to measure test ingredient ME values. It is worth to mention that terminologies regarding the techniques are very confusing in the literature. One term can cover many methods, while some other terms refer to the same method. Sometimes, terms for ingredient ME approach may be interchangeably used with diet ME bioassay methods. In addition, some methods reported in the literature was not given a term for the method – in fact some may even didn’t realise they were using a different method to measure ME of the ingredients. Therefore, we will use the terminologies in the present review according to our collection of the methods reported since the earlier 20th century and try to categorise while cover all of approaches to perform ingredient ME bioassay as complete and distinguishable as possible. The “commonly” used terms may not be used in the current review to minimize confusions due to incomplete definitions of the methods in the past.

**Direct feeding**

Direct feeding of the ingredient to birds can be implemented through force (Sibbald, 1976), controlled (Mitchell and Haines, 1927) or *ad libitum* (Fraps, et al., 1940) feeding of grains, protein meals, by-products and/or oil/fat (force-feeding only). The calculation of the test ingredient ME is straightforward as only the test ingredient is fed to birds.

\[
\text{ME}_{\text{ti}} = \text{ME}_{\text{fd}}
\]

Equation 6

Where \(\text{ME}_{\text{ti}}\) represents ME value of test ingredient; and \(\text{ME}_{\text{fd}}\) denotes the ME value measured from the feed fed to birds as shown in Equation 1 or varied to AME_{\text{fd}} (Equation 2) and TME_{\text{fd}} (Equation 3).
Standard ingredient substitution

A standard ingredient, of which the ME has been previously determined, is used to prepare a basal diet and a proportion or all of the standard ingredient is replaced by the test ingredient to make a test diet for the bioassay. The standard ingredients often include glucose/dextrose (AMEn, 3640 kcal/kg) (Hill, et al., 1960; Lodhi, et al., 1969; Daghir, et al., 2003; Applegate, et al., 2009; Kerr, et al., 2016), sucrose (AMEn, 3800 kcal/kg) (Lodhi, et al., 1969), corn starch (AMEn, 3989 kcal/kg) (Zuber and Rodehutscord, 2017), and barley (AMEn, 2980 kcal/kg) (Villamide, et al., 1997). The test ingredient ME value can be calculated according to the ME difference between test and basal diets and the substitution rate.

\[ \text{ME}_{\text{si}} = \frac{\text{ME}_{\text{si}} - (\text{ME}_{\text{td}} - \text{ME}_{\text{bd}}) / P_{\text{ss}}}{P_{\text{ss}}} \]  

Where ME_{si} denotes the ME of standard ingredients; ME_{td} represents ME of test diet; ME_{bd} is ME of basal diet; and P_{ss} indicates the proportion of standard ingredients substituted by test ingredient.

To determine the dose response, the standard ingredients was substituted at multiple levels by the test ingredient to form several test diets (Sell, et al., 2001; Lammers, et al., 2008). The ME can be determined by regressing the diet ME values less ME contributed by the standard ingredients (e.g., glucose) in the diets against the level of substitution (Lammers, et al., 2008). The model can be expressed as:

\[ \text{ME}_{\delta} = \text{ME}_{d} + \text{ME}_{\text{si}} \times P_{\text{sb}} \]  

ME_{d} is calculated by equation

\[ \text{ME}_{\delta} = \text{ME}_{d} - \text{ME}_{\text{si}} \times (1 - P_{\text{sb}}) \]  

Where ME_{d} is the ME values of all diets including ME_{bd} and ME_{si}; and ME_{\delta} represents the difference between diet ME values and ME contributed by the standard ingredients in that diet.

The same result can be obtained using different regression models (Sell, et al., 2001).

Standard ingredient plus

One or more standard ingredients with known ME values is used to prepare a test diet that includes a set of the test ingredient. The standard ingredients traditionally used in this bioassay are different to that described for the standard ingredient substitution method discussed earlier. However, it appears there is no reason why the standard ingredients used in these two methods cannot be used interchangeably.

This method has the advantage of using a reasonably balanced diet in determination of a test ingredient ME value. However, there are a number of issues that diminish the reliability of the data generated using this method. First, the various “standard ingredients” used and their lack of consistency. For instance, there have been vast differences in the casein AME values used in different assays ranging from 3000 to 4800 kcal/kg (Carpenter and Clegg, 1956; Annison, 1991; Annison, et al., 1994; Ravindran, et al., 1999). Although it is understandable that batches of casein produced at different location or with different methods are expected to differ in their AME values, the problem is researchers often don’t detail the origin, type, residual fat level, salt level and protein content. Other “standard ingredients are soybean oil with an AME value of 8795 kcal/kg (Hew, et al., 1998); cod liver oil with and AME_n value of 8600 kcal/kg (Carpenter and Clegg, 1956); poultry fat with an AME value of 8126 kcal/kg (McCracken, et al., 2008). Secondly, the assay includes a fixed ingredient mixture with AME/AME_n values...
determined based on the NRC values (Scott, et al., 1998; Seyedi, et al., 2013; Yegani, et al., 2013) of which the accuracy of standard AME values are questionable. The test ingredient ME value can be calculated according to the measured ME value of the test diet and proportions of standard and test ingredients in the diet.

\[
\text{ME}_i = \frac{(\text{ME}_{td} - \text{ME}_{si} \times P_{si})}{P_{ti}} \quad \text{Equation 10}
\]

Where \( P_{si} \) is the proportion of standard ingredient in the test diet; and \( P_{ti} \) represents the proportion of test ingredient included in the diet.

The accuracy of values used for the standard ingredients is critical to the calculation, indeed the accuracy of the test ingredient ME value. There is no reason as to why ME values from tables other than the NRC cannot be used for the standard ingredients, but the use of NRC values for basal diet is highly questionable as doubts have been expressed about the usefulness of the NRC values. The lack of standardisation of the so-called "known standard ingredients" is a major drawback of this bioassay.

## Basal substitution

A basal diet is formulated to be adequate in nutrients and energy, and a proportion of the basal diet is substituted by the test ingredient to produce a test diet (Sibbald, et al., 1960). The ME values of the basal and test diets are measured in birds so that the ME value of test ingredient can be derived. The test ingredient ME value can be calculated according to the measured ME values of basal and test diets and the proportion of test ingredient substitution and the proportion of the basal diet (energy yielding ingredients) in the test diet.

\[
\text{ME}_i = \frac{(\text{ME}_{td} - \text{ME}_{bd} \times P_{bd})}{P_{ti}} \quad \text{Equation 11}
\]

Where \( P_{bd} \) is the proportion of the basal diet (energy yielding ingredients) in the test diet. If \( P_{bd} + P_{ti} = 1 \), the equation can be simplified to:

\[
\text{ME}_i = \text{ME}_{bd} - \frac{\text{ME}_{bd} - \text{ME}_{td}}{P_{ti}} \quad \text{Equation 12}
\]

This is the most common technique to measure ingredient ME values for poultry, and it is also a technique that has many variants accompanied by many different mathematic equations/models to calculate \( \text{ME}_i \). Not surprisingly, the calculations are replete with errors. The mistakes include the use of calculation Equation 12 assuming \( P_{si} + P_{bd} = 1 \) in the test diet while the minor ingredients were kept constant between the reference and the test diets (Newkirk, et al., 1997), formulation of test diets with different ratios between energy yielding ingredients to the basal diet (Rodriguez, et al., 1998), and missing energy yielding ingredients in the test diet compared to the basal diet, except for the test ingredient (Barekatain, et al., 2015).

A list of variations to this approach is summarised as below.

**Practical basal substitution** Considering that a simple replacement of the basal diet with a test ingredient may lead to nutrient imbalance, such as in vitamins and minerals, for the test diet, a variation of the test diet formulation has been frequently used, i.e., to formulate a test diet with the same levels of minor ingredients including vitamins, minerals and possibly amino acids (AA) so that the test diet is more balanced at least for those micro nutrients. Since a small proportion of the basal diet or test ingredient is replaced by minor ingredients, as compared with the standard substitution technique, \( P_{bd} + P_{ti} \) no longer equals one. Therefore, Equation 12 does not apply to this modified method. More details on such flaws related to the use of Equation 12 will be elaborated later.
Multiple level basal substitutions Another variation to this technique is the use of graded levels of the ingredient (i.e., $P_i$). The inclusion levels vary depending on the nature of the test ingredient, which can be at 30%, 40%, 50%, and 60% for barley (Villamide, et al., 1997), 25%, 50% or 75% for corn, and 0%, 10%, 20%, and 30% for soybean meal (Lopez and Leeson, 2008). The ME value for the test ingredient ($ME_i$) is determined by regressing the diet ME value on the test ingredient inclusion levels, and extrapolating the regression line to 100% test ingredient inclusion level (Potter, et al., 1960; Mateos and Sell, 1980; Villamide, et al., 1997; Gonzalez-Esquerra and Leeson, 2000; Applegate, 2005; Borsatti, et al., 2018). This calculation is the most widely used method and does not leading to errors in calculation even when $P_{bd} + P_i ≠ 1$ (Gonzalez-Esquerra and Leeson, 2000; Applegate, 2005; Lopez and Leeson, 2008).

\[
ME_d = M_{bd} + (M_{bd} - ME_{ti}) \times P_{ti}
\]

Equation 13

Variations of design and calculations of the ingredient ME in this method have been also carried out in different ways.

Test ingredient associated ME intake regression A model was proposed by Adeola and Ileleji (2009) to regress ME intake associated with the test ingredient ($ME_{ti}$) against the test ingredient FI ($FI_{ti}$) (Adeola and Ileleji, 2009; Pekel, et al., 2015) with Equation 14.

\[
ME_{ti} = ME_{ti} \times FI_{ti}
\]

Equation 14

ME intake contributed by the test ingredient ($ME_{ti}$) can be calculated by:

\[
ME_{ti} = (ME_{td} - ME_{bd} \times P_{bd}) \times FI_{td}
\]

Equation 15

And, test ingredient intake $FI_{ti}$ by:

\[
FI_{ti} = FI_{td} \times P_{ti}
\]

Equation 16

In equations (15), (16) and (17), $FI_{ti}$ is test ingredient intake; $FI_{td}$ denotes test diet FI measured; and $ME_{ti}$ represents ME intake contributed by test ingredient.

Excreta energy regression Cilliers, et al. (1994) developed a different regression method to calculate TME$_{ti}$ by regressing GE intake against GE excretion to produce an estimate of the true proportion of GE intake that is metabolisable. The model is:

\[
GE_{ex} = EEL + GE_{bdt} \times b_{bd} + GE_{li} \times b_{li}
\]

Equation 17

$GE_{bdt}$ and $GE_{li}$ can be calculated using equations:

\[
GE_{bdt} = GE_{bd} \times FI_{td} \times P_{bd}
\]

Equation 18

\[
GE_{li} = GE_{ld} - GE_{bdt}
\]

Equation 19

where, $GE_{bdt}$ is GE intake contributed by the basal diet proportion of the test diet; and $GE_{bd}$ represents GE of the basal diet; $GE_{li}$ indicates GE intake as the test ingredient in the test diet; $GE_{ld}$ denotes test diet GE intake; $b_{bd}$ equals to the estimate of the proportion of GE for basal diet that appears in the excreta; $b_{li}$ equals to the estimate of the proportion of GE for the test ingredient that appears in the excreta.

TME$_{bd}$ and TME$_{ti}$ for the basal diet can be calculated using the following equations:

\[
TME_{bd} = GE_{bd} \times (1 - b_{bd})
\]

Equation 20
and

\[ \text{TME}_{ii} = \text{GE}_{ii} \times (1 - b_{ii}) \]  

Equation 21

Where \( \text{TME}_{bd} \) is TME for the basal diet; and \( \text{TME}_{ti} \) is TME for the test ingredient, and \( 1 - b_{bd} \) and \( 1 - b_{ti} \) equal, respectively, the energy utilisation coefficients of the basal diet and the test ingredient.

The advantage of this is that EEL can be calculated through regression rather than through a total excreta collection in fasted birds. Unfortunately, the calculation of \( \text{GEI}_{bdt} \) was wrong in the study of Cilliers, et al. (1994) as they suggested to calculate GE intake contributed by the basal diet proportion in the test diet with an equation:

\[ \text{GEI}_{bdt} = \text{GEI}_{td} \times P_{bd} \]  

Equation 22

\( \text{GEI}_{bdt} \) was \( X_{i} \) in Cilliers, et al. (1994), which equals GE intake (i.e., \( \text{GEI}_{td} \) here) \( \times \) proportion of diet as basal (\( P_{bd} \) here). They did not realise \( P_{bd} \) is the weight proportion of basal diet in test diet not the proportion of its energy contribution to the test diet energy. This equation stands only when \( \text{GE}_{bd} = \text{GE}_{ii} \) where proportions of energy contributed by basal diet and test ingredient are the same as their weight proportions in the test diet. Therefore, this most likely results in mistaken values in the calculations when \( \text{GE}_{bd} \neq \text{GE}_{ii} \).

**Proportional ad libitum feeding** Further variation is to adjust feeding levels to a proportion of ad libitum FI so that the level of basal diet intake in all test diets are identical, and the levels of the test ingredient included in the test diets are 0%, 10%, 20% and 30% of ad libitum FI, respectively. In this case, it is assumed that the change in the ME values of the diets is due purely to the test ingredient. Simple linear regression of ME intake against test ingredient intake (however it was mistakenly stated in the original paper that ME intake was regressed against FI instead!) is used to determine ME_{ii} (Adeola, 2001; Dozier, et al., 2008).

\[ \text{MEI}_{d} = \text{ME}_{bd} + \text{ME}_{ii} \times \text{II}_{ii} \]  

Equation 23

Where \( \text{MEI}_{d} \) is the ME intake with the basal and test diets, and \( \text{II}_{ii} \) represents the test ingredient intake. It appears that controlling feeding is somewhat redundant as ad libitum feeding of the test diets with different levels of the test ingredient does not pose a problem for regression analysis. Such an approach may be more feasible in swine (Adeola, 2001) than in poultry (Dozier, et al., 2008).

**Multiple linear regression**

Multiple linear Regression has been used widely in pigs (Young, et al., 1977; Noblet, et al., 1993) but rarely in poultry (Applegate, 2005). This approach measures the ME (or DE in pigs) values of various ingredients by using multiple diets with several test ingredients at different levels. The number of diets must be more than the number of energy-yielding ingredients. The ME values of test ingredients can be calculated using multiple linear regression of the measured ME value of diets against the inclusion levels of each ingredient. In the resulting regression equation, the coefficient obtained for an ingredient corresponds to its ME value, namely, the ME of a diet is the sum of ME contributed by each of the ingredients included in the diet. The energy values of the feedstuffs can be estimated from the multiple regression model:

\[ Y = b_1x_1 + b_2x_2 + b_3x_3 + \ldots + b_ix_i \]  

Equation 24
Where Y is the predicted ME value of the diet; \( x_1 \) through to \( x_i \) represent the percentages of individual feedstuffs included in the diet; and \( b_1 \), through to \( b_i \) denote the estimated metabolisable energy values of the feedstuffs as coefficients in the equation.

**Energy metabolizability**

Energy metabolizability (EM) together with their gross energy of nutrients in feedstuffs are quite often used to calculate ME values presented in the nutrient tables (Titus, 1955; Janssen, 1989; Rostagno, 2017). In the calculations, GE values of nutrients used are: fat, 9280 kcal/kg; crude protein, 4310 kcal/kg; and NFE, 4140 kcal/kg (Janssen, 1989). The calculation of test ingredient AME (AME\(_i\)) can be achieved through the summation of the calculated nutrients AME values that equal to their respective EM in the ingredient and their gross energy values described above. Please note that EM of the same nutrients in different feedstuff may differ thus EM of the nutrients must be measured individually and accurately so that the AME of feedstuffs can be produced accordingly. However, this may not always be the case as it is unlikely to measure all the EM of all the nutrients in all the feedstuff so as to acquire AME values. Rather, same EM of a nutrient may be used for the same species of feedstuff in the calculations of AME in the tables or feed formulation database. Therefore, the AME values produced in such way are more or less a guesstimate compared to the measured values through bioassay.
Pros and cons of ME systems

Debates have been documented concerning which energy system should be used for poultry, how the ME values should be measured, and what strategy should be taken to analyse ingredient ME values (Pesti and Edwards Jr, 1983; Sibbald and Wolynetz, 1987; Farrell, 1999). The pros and cons have been discussed regarding ME energy systems (AME vs TME, and/or ME vs ME\textsubscript{n}), bioassay protocols (i.e., total collection of excreta or using indigestible markers, force, restricted or \textit{ad libitum} feeding, fasting birds prior to excreta collection or not), and to less extent, the techniques to measure ingredient ME values. Some of these have been discussed extensively while others have escaped attention.

The key point of emphasis is that the first item set in feed formulation is the energy value of the feed ingredients. Indeed, in feed formulation, all nutrients are set as ratios to energy. The economic implication of setting a wrong energy value in feed formulation is immense. But there is a lack of clarity about origin of the ME values in most databases today. The issues related to the fact that the ME values have been generated in various laboratories using different ME systems, bioassays and techniques. Therefore, despite numerous reviews, it is still necessary to have another look at the pros and cons of the ME systems so that justifications of a standard ME system for poultry can be made.

**AME vs TME**

The earlier measurements of ME used the AME system as EEL was not considered and accounted for in the assays (Mitchell and Haines, 1927; Fraps, 1944; Hill and Anderson, 1958). Harris (1966) stated that EEL should be considered and hence a TME system was proposed. Apparent ME is based on the study performed by Hill and Anderson (1958) with growing chickens fed \textit{ad libitum} whereas the TME system was championed by Sibbald (1976). Since the development of these two methods, comparative studies have performed to assess their accuracy, applicability and usefulness for estimating feedstuff energy content.

AME is currently the default system for energy evaluation in poultry (Farrell, 1999; McNab, 1999; Mateos, et al., 2018). Thus, the AME data generated from various sources have been the foundation of many databases used for practical feed formulation. AME can be measured in different types of poultry regardless of species and age. However, AME values of feedstuffs can be variable due to age, species and sex of birds, different bioassays, and techniques with associated erroneous calculations (to be discussed in later sections). Nevertheless, the AME system is still preferred for the estimate of feedstuff energy content before other systems, such as net energy (NE), are implemented in poultry.

Pesti and Edwards Jr (1983) and Farrell (1999) were critical of the TME system. Their view is that the ME values generated using the TME assay may not be applicable in practice because it employs force-feeding of birds, often adult cockerels, with a set amount of feed as well as a long period of fasting; force-feeding birds a low amount of feed may have exaggerate the role of EEL in ME calculation compared with that in birds fed \textit{ad libitum}. In addition, force-feeding doesn’t meet welfare standards.

Furthermore, it has been shown that EEL for TME is overestimated by fasting (Sibbald, 1975) or through regression of energy void as excreta on the weight of feed consumed under force feeding (Hätel, 1986; Farrell, et al., 1991). Undoubtedly, fasting for an extended period of time alters the physiology of birds compared with \textit{ad libitum} feeding. Notwithstanding, the TME system overestimates ME values by correcting for EEL because at the end all energy gained or lost by the bird comes from the feed it has ingested. Therefore, the loss of such energy should not be added to the ME value as it is not available to the animal for utilisation.
However, from an operational rather than an accuracy point of view, the TME assay has some merits; it is rapid, requires a small amount of feed, does not need feed mixing, and uses the birds repeatedly (Sibbald, 1976). Despite these advantages and, at time good agreements between AME and TME values of some ingredients, such as corn (Baidoo, et al., 1991), the TME system has largely been phased out and TME values are rarely used.

**ME vs MEₙ**

AMEₙ, or usually interchangeable with MEₙ, values are commonly used as the available energy poultry feed (Hill and Anderson, 1958), and as such, they are the default table values for feedstuffs (Janssen, 1989; NRC, 1994; Rostagno, 2017). AMEₙ is corrected from AME and the correction is made based on the energy content of N-containing excretory materials that is actually retained in the body. The correction factors of 8.22 kcal (Hill and Anderson, 1958) or 8.73 kcal (Titus, et al., 1959) have been used for 1 g of N retained. Hill and Anderson (1958) assumed that uric acid was the sole N excretory product in the chicken, while Titus, et al. (1959) suggested less uric acids in the excretory products of protein metabolism thus different values.

Correction for N retention is based on data obtained in non-growing adult roosters, which was then applied to growing chickens as a comparison (Hill and Anderson, 1958; Farrell, 1999). As growing chickens retain more than half of the N consumed, the concept of standard AME (or AMEs) has been proposed where it is suggested that 50% N retention to be applied to AME values (Cozannet, et al., 2010). However, such a correction has not been accepted in poultry ME measurement and formulation to date.

Whether the correction to zero N retention should be used for describing available energy for poultry has been an ongoing debate since Hill and Anderson (1958). First of all, researchers realised that such a correction doesn’t change the relative values of ingredients (Baldini, 1961; Proudman, et al., 1970) and the correction, in most cases, makes little difference in the ME values in older birds (Shannon and Brown, 1970; Farrell, et al., 1991; Lopez and Leeson, 2007). Secondly, N correction under experimental conditions and in certain test diets may introduce errors as N-retention in birds fed an amino acid imbalanced test diet can be different from those fed a balanced diet. Thus, MEₙ values obtained under such circumstances may be erroneous, for example after fasting and birds fed test diets with a high protein level (Farrell, et al., 1991). Thirdly, the ME values may be penalised for protein meals and overestimated for the energy grains and fat (Farrell, 1999). Lastly, correcting ME values to zero N balance does not reflect the true requirement of energy in productive birds, such as broilers and layers, where retained energy as protein will never be excreted as uric acid and other N compounds. The argument that MEₙ should be used to make energy values among different poultry classes more consistent doesn’t stand. It is because this ignores the physiological differences that exist among different classes of birds. Thus it is recommended that ME instead of MEₙ be used and ME values of ingredients be measured in the same type of birds for which they are intended.
Flaws in ME bioassay

While a plethora of studies have been performed to estimate the ME values of poultry feedstuffs, the accuracy of the assays is questionable. Therefore, it is necessary to thoroughly review all the studies, carefully assess their design and calculations, and scrutinise the data critically. A summary of identified flaws in many studies is reported here focusing on the protocols and calculations that affected the accuracy of the data.

Wrong assumption of $P_{bd} + P_{ti} = 1$ in basal substitution method

In the Basal Substitution method, formulation of the test diet rather than direct substitution of the basal diet with the test ingredient is frequently used to allow for the inclusion of a set level of minor ingredients for both the basal and test diets. Such an exercise is designed to make the test diet balanced in micro nutrients, such as trace minerals, vitamins and amino acids. In this case, the proportion of energy contributed by the basal diet to the test diet plus the inclusion rate of the test ingredient does not equal to 100% as already stated in previous section, i.e., $P_{bd} + P_{ti} \neq 1$.

Herein, a simply example is presented to demonstrate a significant error introduced by this wrong assumption, where $P_{bd} + P_{ti} < 1$. As shown in Table 1, the basal diet is formulated to contain energy yielding ingredients, including wheat (60%), soybean meal (33%) and canola oil (2%) that constitute 95% of the diet, and the minor ingredients makes up the rest, i.e., 5% of the diet. When a test diet is formulated to include 30% test ingredient (i.e., sorghum), the level of minor ingredients (5%) remains the same, leaving 65% as the energy yielding ingredients in the basal diet, i.e., wheat, 41.05%, soybean meal 22.58%, and canola oil 1.37%. Accordingly, the $P_{bd} = 65%/95% = 68.42%$ rather than 70% as if 30% basal diet is replaced by test ingredient.

By giving the measured basal diet AME value of 3200 kcal/kg ($AME_{bd}$), and test diet AME value of 3280 kcal/kg, the sorghum AME value $AME_{ti} = (AME_{td} – AME_{bd} \times P_{bd})/P_{ti} = (3280 – 3200 \times 68.42%)/30% = 3635$ kcal/kg according to Equation 11. However, if $P_{bd} + P_{ti} = 1$ is assumed, i.e., $P_{bd}$ would be $1 – P_{ti} = 1 – 30% = 70%$, and thus $AME_{ti} = AME_{bd} - (AME_{bd} – AME_{td})/P_{ti} = 3200 – (3200 -3280)/30% = 3467$ kca/kg according to Equation 12. A significant underestimation of (3635 – 3467) = 168 kcal/kg is made by the mistaken assumption of $P_{bd} + P_{ti} = 1$ that neglects the 1.58% lower inclusion rate of basal energy yielding ingredient in test diet.

Overall, such errors occurred in the bioassays to measure ME values of wheat (Saki, et al., 2009), barley (Saki, et al., 2009), full fat sunflower seed (Salari, et al., 2009), sunflower meal (Moghaddam, et al., 2012), Brassica meals (Newkirk, et al., 1997), and canola meal (Woyengo, et al., 2010; Toghyani, et al., 2014). In contrast, some authors realised that basal diet inclusion rate ($P_{bd}$) does not equal to 1 - $P_{ti}$ and they used appropriate equations with accurate $P_{bd}$ applied in the calculations as stated (Bartov, 1996; Rodriguez, et al., 2005; Mandal, et al., 2006; Adeola and Ileleji, 2009; Cozannet, et al., 2010).

The underestimation of the AME by this erroneous calculation can be corrected by adding the value calculated by the following equation:

$$AME_{e} = AME_{bd} \times P_{mi}/(1-P_{mi})$$

Equation 25

Where $AME_{e}$ represents the error, $P_{mi}$ is the minor ingredient inclusion rate which is the same in both basal and test diets.
Table 1. AME value difference resulted in mistaken calculation with constant minor ingredients between basal and test diets.

<table>
<thead>
<tr>
<th>Ingredients/energy</th>
<th>Basal diet</th>
<th>Test diet</th>
<th>Test/basal</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat (%)</td>
<td>60.00</td>
<td>41.05</td>
<td>68.42</td>
<td>(AME\text{td} – AME_{bd}^*P_{bd})/P_{ti}</td>
</tr>
<tr>
<td>Soybean meal (%)</td>
<td>33.00</td>
<td>22.58</td>
<td>68.42</td>
<td>AME_{bd} - (AME_{bd} – AME_{td})/P_{ti}</td>
</tr>
<tr>
<td>canola oil (%)</td>
<td>2.00</td>
<td>1.37</td>
<td>68.42</td>
<td>(AME_{td} – AME_{eAA} - AME_{bd}^*P_{bd})/P_{ti}</td>
</tr>
<tr>
<td>Mineral and vitamin</td>
<td>5.00</td>
<td>5.00</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>premix (%)</td>
<td>30.00</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sorghum (%)</td>
<td>100.00</td>
<td>100.00</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>3200</td>
<td>3280</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Measured AME (kcal/kg)</td>
<td>3635</td>
<td>3467</td>
<td>3591</td>
<td></td>
</tr>
<tr>
<td>Sorghum AME (kcal/kg)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Error (kcal/kg)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>44</td>
</tr>
</tbody>
</table>
Non proportional basal energy yielding ingredients in test diet

The rationale to have a test diet that has a set proportion of the basal diet replaced by a test ingredient is to be able to deduce the amount of ME attributable to the unknown proportion, i.e., the test ingredient. In this case, the test diet needs to be formulated in such a way that the ratios of inclusion of all the basal energy yielding ingredients should be identical between the test diet and the basal diet. In the example outlined above the ratios of corn, soybean meal and canola oil inclusions in the test diet to basal diet are 68.42%. However, this is not always the case in the ME bioassays reported in the literature. For example, Rodriguez, et al. (1998) formulated a test diet containing yellow maize, soybean meal and sunflower oil at 82.3%, 75.1% and 0%, respectively, to those in the basal diet in order to measure the ME value of hulled full-fat sunflower seed. In the study, the calculation of the test ingredient ME would have been either impossible or grossly inaccurate, depending on the extent of differences in the ratios. Such inconsistence occurred in the measurement of canola seed AME by Barekatain, et al. (2015). In the study, the test diet accounted for 88.0% of corn and soybean meal in the basal diet while no canola oil was added. Taking into account the calculation of the basal diet inclusion rate of 85% in the study, the basal contribution to the test diet ME was overestimated by 160 kcal and consequently the canola seed contribution to the test diet ME by approximately 160 kcal. This erroneous formulation meant that the canola seed ME value was possibly underestimated by 1067kcal/kg (160 kcal/0.15 = 1067).

Other studies have also made similar errors. For instance, enormously inconsistent proportions of energy-yielding ingredients were formulated in the test diet relative to the basal diets to measure the AME values of pea and lupin varieties (Nalle, et al., 2011a; Nalle, et al., 2011b; Nalle, et al., 2012). A pea test diet is described here to show the erroneous formulation of the diets. Pea substitution rate was 20% while the basal diet energy-yielding ingredients inclusion rates were 72.7%, 81.4%, 100.0%, 100.0% and 325.0% for maize, soybean meal, meat and bone meal, tallow and soybean oil, respectively, in the test diet as opposed to the supposed inclusion rate of 80% for all the ingredients in the test diet relative to the basal diet. Such a wide range of inconsistent ingredient ratios rendered the calculation of the pea AME value invalid. There are numerous other examples to quote (Sahraei, et al., 2012; Mirghelenj, et al., 2013).

Miscalculations in simple linear regression

By simple linear regression of diet ME values against the inclusion rates of the test ingredient in the test diets, the test ingredient ME can be obtained by extrapolating the inclusion of the test ingredient to 100% (Potter, et al., 1960). Over the years, a number of variants of this method appeared in the literature. For example, Adeola (2001) proposed to compute DE of test ingredient by regressing energy intake against test ingredient consumed in pigs and this method was applied in poultry (Dozier, et al., 2008; Adeola and Ileleji, 2009; Borsatti, et al., 2018). On the other hand, Cilliers, et al. (1994) regressed excreted GE against GE intakes independently as GE intake of basal diet portion and GE intake of test ingredient portion in test diet to calculate the basal diet TME and test ingredient TME. While the rationale of the above regression calculations are logical purely in calculating ME of ingredient, the statistical outcomes on the accuracy of the calculations can be misleading by including, for example, FI other than ingredient inclusion levels - as the $r^2$ and possibly P values can look much better statistically than when inclusion levels of ingredient is used. Here is an example for the demonstration of a “flawed” simple linear regression that showed a high coefficient of determination by the regression of AMEn intake against FI (Dozier, et al., 2008). In the study, the birds were fed 100%, 97%, 94%, and 91% of ad libitum intake so that differences in AMEn consumption were only attributable to the glycerine inclusions at 0%, 3%, 6% and 9%. The variables for the regression are shown in Table 2. The flaws are identified as follow. Firstly, the slope of the equation produced by regressing the AMEn intake against FI does not equate.
to the AME value of glycerine. This is because such regression implies that diet AME\textsubscript{n} value is a function of AME\textsubscript{n} intake divided by FI (Figure 2a), which gives the AME\textsubscript{n} of feed rather than test ingredient. The correct approach should have been the AME\textsubscript{n} intake regressed against the glycerine intake with the basal AME\textsubscript{n} as the intercept and glycerin AME\textsubscript{n} as the slope (Figure 2b). From the calculation point of view, this gives correct value. Secondly, the significance (P ≤ 0.0001) and regression coefficient (r\textsuperscript{2} = 0.98) were only the reflection of the model that fits the values of test ingredient intake against AME\textsubscript{n} intake (Figure 2b). The already expected linear relationship between test ingredient intake and AME\textsubscript{n} intake would have masked the poor fitness of the model that only takes account the linear relationship between diet AME\textsubscript{n} and glycerine inclusion rate, upon which glycerine AME\textsubscript{n} value was determined. Figure 2c on the other hand suggests a correct regression model that reflects the true relationship between AME\textsubscript{n} of the diet and the glycerine inclusion rate. The glycerine AME\textsubscript{n} can then be derived from the regression equation, where the intercept is the basal AME\textsubscript{n} value and extrapolation of glycerine inclusion rate to 100% results in glycerine AME\textsubscript{n} value. As is shown in the Figure 2c, the r\textsuperscript{2} of the model is actually very low (0.27), indicating the lack of robustness in estimation of glycerine AME\textsubscript{n} value. Table 2 further explains this problem. For instance, the test diet AME\textsubscript{n} values vary depending on the inclusion level of the test ingredient in the test diet, leading to vastly different AME\textsubscript{n} values at 3% and 6% and 9% inclusions of glycerine. In fact, at 3% inclusion level, the calculated glycerin AME\textsubscript{n} value was much lower (1717 kcal/kg) than the at 6% and 9% glycerine inclusions (2967 kcal/kg and 3206 kcal/kg, respectively) indicating poor liner relationship between inclusion rate and AME\textsubscript{n} change of test diets or in another word strong interaction between glycerin and other ingredients in the diet (Table 2). Consequently, it can be concluded that a linear model does not fit the data measured by Dozier, et al. (2008). A similar regression method was reported in Lima, et al. (2013), however the results reported are not sufficient to allow readers to examine the fitness of the data. In summary, a direct regression of diet AME values against the test ingredient inclusion levels should be employed so that the statistical power of the analysis can be shown and accuracy of the experimental data can be directly visualised.

### Table 2

<table>
<thead>
<tr>
<th>Glycerin level (%)</th>
<th>Glycerin intake* (kg)</th>
<th>Feed intake (kg)</th>
<th>AMEn intake (kcal)</th>
<th>Diet AMEn (kcal/kg)</th>
<th>Glycerin AMEn (kcal/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.0000</td>
<td>0.197</td>
<td>588</td>
<td>2984</td>
<td>-</td>
</tr>
<tr>
<td>3.00</td>
<td>0.0061</td>
<td>0.203</td>
<td>598</td>
<td>2946</td>
<td>1717</td>
</tr>
<tr>
<td>6.00</td>
<td>0.0126</td>
<td>0.210</td>
<td>626</td>
<td>2983</td>
<td>2967</td>
</tr>
<tr>
<td>9.00</td>
<td>0.0194</td>
<td>0.216</td>
<td>649</td>
<td>3004</td>
<td>3206</td>
</tr>
</tbody>
</table>

**Test diet ME value is assumed, not determined**

Quite often, researchers use literature values for the ingredients included in the test diet, so that the ME value of a test ingredient can be calculated (Scott, et al., 1998; Austin, et al., 1999; Wiseman, 2000). While certain energy or protein sources, such as glucose or casein, are used (Armsby, 1903; Anderson, et al., 1958; Annison, et al., 1994), one would expect the ME values are basically accurate. However, this is not always the case, e.g., four different casein ME values ranging from 3000 to 4804 kcal/kg have been used in the literature as described previously (Carpenter and Clegg, 1956; Annison, et al., 1994; Ravindran, et al., 1999). The use of casein values differing by 1804 kcal/kg obviously leads to massive differences in obtaining ME values of test ingredients. Furthermore, by assuming ME values for grains and protein meals using table values, prediction equations, or previously measured values, there
is no doubt the resultant ingredient ME values are questionable. A case in point is the wheat AME values for broilers (Scott, et al., 1998; Austin, et al., 1999; Wiseman, 2000; Seyed, et al., 2013), rye AME values for broilers (Boros and Bedford, 1999), and meat and bone meal AME values for ducks (Adedokun and Adeola, 2005) reported over the past two decades. Interestingly, Adedokun and Adeola (2005) stated “the corn and soybean meal used in this study, although from the same batch, were different from those used in the report by Adeola (2003)”, but “the AME values of corn and SBM at 3,100 and 2,600 kcal/kg (Adeola, 2003)” were still used as AME values used to calculate the test diet AME value. Therefore, it stresses the importance of full characterisation of all the ingredients used in ME bioassays.

![Figure 2](image)

**Figure 2** Regression of AME\textsubscript{n} intake vs feed intake, glycerine intake and glycerine inclusion rate depicting calculation flaw present in the literature. When AME\textsubscript{n} intake is regressed against feed or glycerin intake, coefficients of determination are very high ($R^2 = 0.98$). However, when AME\textsubscript{n} is regressed against glycerin inclusion rate, the coefficients of determination is very poor ($R^2 = 0.27$) which shows the true the effect of glycerine inclusion level on measured glycerine AME\textsubscript{n} value. (a) AME\textsubscript{n} intake vs feed intake; (b) AME\textsubscript{n} intake vs glycerin intake; and (c) AME\textsubscript{n} vs glycerin inclusion rate.

**Forcing feeding to measure AME**

Forcing feeding was a frequently used assay to determine the TME value of feedstuffs, although its use is no longer widespread today (Sibbald, 1976; Sibbald and Wolynetz, 1987; Sell, et al., 2001; Wang, et al., 2017a). In such assays, many researchers report the AME or AME\textsubscript{n} values by subtracting EEL from TME (Lin, et al., 2003; Tshovhote, et al., 2003; Pirgozliev, et al., 2006; Latshaw and Freeland, 2008; Zhao, et al., 2008; Farran, et al., 2010; Jie, et al., 2013; Jahanian and Rasouli, 2014). This calculation does not take into the account that force-feeding assay applies a longer period of excreta collection (36 – 72 h) for a limited FI. Therefore, the force-feeding assay with such a long period of excretion overestimates EEL compared to *ad libitum*
feeding for the same amount of feed. This leads to a significant underestimation of AME or AMEn values. Henceforth, such “AME” or “AMEn” values are not comparable to those measured in ad libitum bioassays such as the studies of Sibbald (1975) and Sibbald (1976) vs those of Hill, et al. (1960) already indicated by Pesti and Edwards Jr (1983). Unfortunately, the point raised three decades ago was disregarded by many researchers. On the other hand, some studies measured TME and AME with more appropriate bioassay systems (Dei, et al., 2008; MacLeod, et al., 2008).

Use diet metabolisability to predict test ingredient ME value

There is no doubt that AME values of the basal and test diets can be obtained by using metabolisability coefficients of basal (Cbd) and test (Ctd) diets together with their GE. However, calculation of AMEi through energy metabolisability coefficients of the test ingredient (Ci) is problematic as it is impossible to derive Ci without knowing AMEi. It is completely logic to assume that AME in a test diet (AMEtd) is the sum of energy contributions from basal diet (AMEbd × Pbd) and the test ingredient (AMEti × Pti). Such additivity assumption has been used widely in the measurement of feedstuff ME in poultry as described previously. However, similar assumption of additivity does not apply to energy metabolisability coefficient as has been used by the Adeola group (Olukosi and Adeola, 2009; Adeola, et al., 2010; Adeola, et al., 2018) and others (Olukosi, et al., 2017) i.e., Ci = (Ctd × Ptd) + (Cti × Pti) and thus Cti = Cbd + (Ctd - Cbd)/Pti. These studies employed derived Cti through Cbd and Ctd to calculate test ingredient AME by the basal substitution method.

We take an example to show why the coefficients are not additive and how such additive assumption leads to mistaken outcome for test ingredient coefficient and thus AME. Table 3 shows an example that the calculations of Cti are conducted in three different ways in literature by:

1. dividing ingredient AME by ingredient GE;
   \[ C_{ti} = \frac{\text{AME}_{ti}}{\text{GE}_{ti}} \]  
   Equation 26

2. deriving from Cbd and Cti with Pbd and Pti as the proportions of the basal diet (in weight) and the test ingredient in the test diet;
   \[ C_{ti} = \frac{(\text{Ctd} - \text{Cbd} \times P_{bd})}{P_{ti}} \]  
   Equation 27

3. deriving from Ctd and Cti with Peti and (1 - Peti) as the energy contribution proportions of basal diet and test ingredient respectively in test diet.
   \[ C_{ti} = \text{Cbd} + \frac{(\text{Ctd} - \text{Cbd})}{P_{eti}} \]  
   Equation 28

By definition, Cti calculated with Equation 26 is the direct answer. Equation 27 has two flaws. Firstly, it assumes C is additive which is untrue and secondly the weight inclusion rates cannot be used to calculate energy metabolisability coefficient. Equation 28 is flawed with the assumption that energy metabolisability coefficients are additive. Based on these equations, Cti of 0.635, 0.674 and 0.624 are produced respectively by the Equation 26, Equation 27 and Equation 28. Apparently, the value of 0.635 produced by Equation 26 is correct by definition of energy metabolisability coefficient, while Equation 27 overestimated and Equation 28 underestimated the coefficient. Thus, resulted AME from mistaken Cti is undoubtedly wrong. Henceforth, we suggest that the AME values reported in many studies need to be recalculated (Adeola, et al., 2010; Bolarinwa and Adeola, 2012; Adebiyi and Olukosi, 2015; Pekel, et al., 2015; Olukosi, et al., 2017; Adeola, et al., 2018). Furthermore, Wang, et al. (2017a) and Wang, et al. (2017b) calculated AME of dry citrus pulp and mulberry leaf meal by using energy metabolisability coefficient of test diets (Ctd, calculated in their equation as [GEdiet
−GEexcreta/GEdiet) rather than that of test ingredients (Ct). Apparently, dry citrus pulp and mulberry leaf meal have lower metabolisability than the test diets thus their energy content can be overestimated significantly by the authors. Similar faulty calculations are also present in the calculations of ileal digestibility and digestible energy (Adeola, et al., 2010; Toghyani, et al., 2017; Adeola, et al., 2018).

**Ignored synthetic AA contribution as energy yielding ingredients**

In a practical diet, AA such as lysine, methionine and threonine is usually added to make the diet balanced in essential AA. In the ME bioassay, if a proportion of basal diet is substituted directly with the test ingredient to form a test diet, whether AA are regarded as energy yielding ingredient is not an issue as they are included as part of the basal diet proportionally. However, when the minor ingredients are formulated to a constant inclusion level in both the basal and test diets and AA are considered as minor ingredients together with minerals and vitamins, ignoring AA as energy yielding ingredients will produce a calculation error despite that the error can be relatively small (Salari, et al., 2009; Woyengo, et al., 2010; Moghaddam, et al., 2012; Toghyani, et al., 2014).

Here is an example. A basal diet and a test diet are formulated as shown in Table 1 that include 5% minor ingredients with 0.3% lysine, 0.4% methionine and 0.2% threonine supplemented in addition to the minerals and vitamins. When AA are not considered, the AME value of sorghum (at an inclusion rate of 30%) is calculated to be 3635 kcal/kg as shown in Table 1 by using Equation 11. When AA are considered as energy yielding ingredients, the calculation should be adjusted as the AA inclusion in test diet is not proportional to other energy yielding ingredients in the basal diet, i.e., instead of 68.42% of the basal AA, it is 100%. Therefore, the extra 100% - 68.42% = 31.58% AA contribution is not from the basal diet. The energy supplied by the extra AA should be deducted from ME of the test diet in the calculation of the test ingredient ME, i.e., for sorghum AME. The AME of extra AA (AMEEAA) can be calculated as:

\[
AME_{EAA} = (3748 \text{ kcal} \times 0.3\% + 5528 \text{ kcal} \times 0.4\% + 4029 \text{ kcal} \times 0.2\%) \times 31.58\% = 13.08 \text{ kcal}
\]

Where, 3748 kcal is the GE of lysine-HCl (78.4%), 5528 kcal the GE of methionine (98%), and 4029 kcal the GE of threonine (98%). It is assumed that AA are 100% digestible (Karakas, et al., 2001).

The calculation of sorghum AME then becomes to:

\[
AME_{li} = (AME_{td} - AME_{EAA} - AME_{bd} \times P_{bd})/P_{li} = (3280 - 13.08 - 3200 \times 68.42\%)/30% = 3592 \text{ kcal/kg}.
\]

Thus, an overestimation of 3635 – 3592 = 43 kcal/kg sorghum AME was produced by ignoring the AA as energy yielding ingredients without adjustment.
Table 3. Calculation of test ingredient metabolisable energy coefficient

<table>
<thead>
<tr>
<th>Items</th>
<th>Basal diet</th>
<th>Test diet</th>
<th>Ratio in test to basal</th>
<th>Test ingredient (calculated C&lt;sub&gt;ti&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy yielding, %</td>
<td>95.0</td>
<td>65.0</td>
<td>68.4</td>
<td>-</td>
</tr>
<tr>
<td>Minor, %</td>
<td>5.0</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Test ingredient, %</td>
<td>0.0</td>
<td>30.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GE, kcal/kg</td>
<td>4200</td>
<td>4100</td>
<td>-</td>
<td>4088</td>
</tr>
<tr>
<td>Excreta GE from 1 kg feed</td>
<td>1100</td>
<td>1200</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AME, kcal/kg</td>
<td>3100</td>
<td>2900</td>
<td>-</td>
<td>2596</td>
</tr>
<tr>
<td>P (energy contribution to test diet)</td>
<td>73.1</td>
<td>-</td>
<td>-</td>
<td>26.9</td>
</tr>
<tr>
<td>C&lt;sub&gt;1&lt;/sub&gt;*</td>
<td>0.738</td>
<td>0.707</td>
<td>-</td>
<td>0.635</td>
</tr>
<tr>
<td>C&lt;sub&gt;2&lt;/sub&gt;*</td>
<td>0.738</td>
<td>0.707</td>
<td>-</td>
<td>0.674</td>
</tr>
<tr>
<td>C&lt;sub&gt;3&lt;/sub&gt;*</td>
<td>0.738</td>
<td>0.707</td>
<td>-</td>
<td>0.624</td>
</tr>
</tbody>
</table>

* C<sub>0</sub> is calculated by Equation 26 to produce C<sub>1</sub>, Equation 27 to produce C<sub>2</sub> and Equation 28 to produce C<sub>3</sub>.

Abbreviations: C, energy metabolizability coefficient; GE, gross energy; P, proportion of basal diet or test ingredient in test diet; ti, test ingredient;
Proposed bioassay systems and diet designs for ingredient ME values

The bioassay systems, diet designs and corresponding data analyses for measuring feed ingredient ME values are complex. The aspiration is to use a nutritionally adequate diet with a statistically robust design that is practical and user-friendly to determine feed ingredient ME values used in poultry feed formulation. Data thus obtained will form the basis for all other systems, including in vitro assays and NIR predication.

The following criteria are proposed for ME bioassays.

a. The age, sex and breed of animals are representative of industrial applications.
b. Animals are not interrogated during the assay so that the physiological status is similar to the animals under normal growth conditions.
c. Birds are ad libitum.
d. All the feed ingredients used in the assay should be characterised using wet laboratory analysis.
e. Diets should be nutritionally balanced and palatable.
f. The protocol for feed withdrawal periods before and after total collection should be uniform.
g. The method of excreta collection, drying and grinding should follow a standard practice.
h. Purified or semi-purified diets not recommended.
i. Long periods of fasting should be avoided.
j. Calculations and data analysis should be standardised.

According to the above criteria, the total collection bioassay with multiple levels of a balanced diet is recommended to measure AME values of multiple ingredients (multiple repression or basal diet substitution at multiple levels of single test ingredient). The detailed protocols are described below.

Multiple linear regression method

Formulate six or more nutritional balanced diets to include 4 or more test ingredients in the diets as energy yielding ingredients that include grains, oils or fats and protein meals. The principle is that the number of test diets is more than the number of ingredients to be tested.

a. The inclusion levels of different ingredients should not correlate significant with each other in the diets allowing independent measure of AME contributions from the individual energy yielding ingredients.
b. Perform six or more replications for each diet depending on the inherent variation of the assay system.
c. Broiler aged 25-28 days is recommended as this period is estimated for birds to consume the average amount of feed during the total period of the bird growth (35 d). Thus, ME measured is representative of the average value. The age can change depending on the bird performance with the progress of breeding, management and marketing practices.
d. Use 4 to 6 birds in each replication in cases of broiler and layer chickens. The number of birds per replicate for other poultry may vary.
e. Employ the total collection method with 4 days of adaptation and 3 days of excreta collection. Note, the exact time accurate to minute should be recorded for the start and end of the balance experiment to reduce errors.
f. Feed consumed, excreta collected, ingredients included in the diets, gross energy of diets and ingredients should be measured as dry matter (DM) basis. Therefore, the DM of the feed should always be determined when the feed is weighed out for feeding.

g. Perform multiple linear regression with the inclusion levels of ingredients (DM basis) and diet AME or AME<sub>n</sub> values to calculate the AME or AME<sub>n</sub> of the ingredients under question according to Equation 24 as the regression model.

In circumstances that the above design is not suitable, the basal diet substitution method should be used.

**Basal diet substitution**

a. Formulate a basal nutritionally balanced diet.

b. Formulate 2 to 4 test diets supplemented with the same levels of minerals, vitamins and supplemental AA and different levels of the test ingredient.

c. Perform six or more replications for each diet depending on the inherent variation of the assay system.

d. Broiler aged 25-28 days for broiler chickens is recommended as this period is estimated for birds to consume the average daily intake of the total period of the bird growth (35 d). Thus, ME measured is representative of the average value. The age can change depending on the bird performance with the progress of breeding, management and marketing practices.

e. Use 4 to 6 birds in each replication in cases of broiler and layer chickens. The number of birds per replicate for other poultry may vary.

f. Employ the total collection method with 4 days of adaptation and 3 days of excreta collection. Note, the exact time accurate to minute should be recorded for the start and end of the balance experiment to reduce error.

g. Feed consumed, excreta collected, ingredients included in the diets, gross energy of diets and ingredients should be measured in dry matter (DM) basis. Therefore, the DM of the feed should always be determined when the feed is weighed for feeding.

h. Calculate the AME or AME<sub>n</sub> of the ingredient under question using Equation 11 with correct P<sub>bd</sub> and P<sub>i</sub> in DM basis.

i. When AME<sub>n</sub> is calculated, the N correction factor should be standardised as 8.22 kcal/g.
Implications

Poultry industry in Australia and essentially over the world will benefit from the outcomes of this literature review on more accurate formulations of poultry feed so that costs to the feed can be reduced and the performance of birds improved.

Consumers will benefit indirectly from possible lowered costs of poultry products as the result of the savings made by the poultry industry.

This study may have implications to policy makers on the importance of precision agriculture practice as the outcomes of this review will fine-tune the feeding practice of the industry.
Recommendations

The available ME values of ingredients in the literature are diverse not only due to their chemical characteristics because of genetics and production conditions, but also owing to methods used, corrections applied, and calculations erroneously conducted. Therefore, a proper bioassay for determining ME values for poultry feedstuffs is the first step for accurate assessment of feedstuff ME values whether table values, prediction equations, in vitro assays, or NIR analysis are used in the feed mills. The currently available ME values reported in the literature have to be used with care as numerous flaws are present in the data which are difficult to identify. This review has highlighted these flaws as much as possible and any values in the studies where flaws are present should be used with great caution. Most importantly, the aim is to avoid repeating similar mistakes. Inaccurate bioassays, unnecessary adjustments, poor experimental designs, and flawed statistical or mathematical calculations can be avoided. It is important to note that researchers should use simple designs with appropriate calculations in mind before embarking on an animal experiment. Although the flaws identified in this literature review can be avoided with care, whether the assumption that energy of individual ingredients is additive in a complete diet is still untrue under some circumstances at least. This may require efforts from industry and researchers to investigate relations among the main ingredients in a complete diet so that more accurate formulation can be performed based on the outcomes that may fine-tune the additivity assumption.
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Review of AME bioassay for ingredient matrix values of poultry

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