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Executive Summary

Apparent metabolisable energy corrected to zero nitrogen retention (AMEn) values derived from growing broilers and adult cockerels are used to formulate most of Australia's 800,000 tonne annual production of layer feed. The use of net energy (NE) values to formulate layer feed is not currently being practised but may offer cost savings as this method accounts for heat increment (HI) or heat wasted during digestion. Cost savings in the pig and cattle industry using NE formulation have been demonstrated. The project described herein examined AMEn and NE of diets for layers using commercial style cages and respiration chambers. NE was calculated by subtracting heat increment from measured AMEn in different test diets. Regression was used to generate an equation to predict the NE content of ingredients based on AMEn and chemical components in the feed. The equation was validated in respiration chambers and also in a cage experiment.

In Experiment I, the AMEn of corn, soybean meal (SBM) and wheat supplemented with or without xylanase was determined. The values obtained were applied to formulations of test feeds for the first validation experiment. Determined AME and AMEn values were: corn, 14.47 MJ/kg and 14.19 MJ/kg; SBM, 10.10 MJ/kg and 9.60 MJ/kg; wheat, 13.70 MJ/kg and 13.35 MJ/kg; and wheat with xylanase, 14.89 MJ/kg and 14.40 MJ/kg respectively. Wheat had lower AMEn than corn. Xylanase increased wheat AMEn to that of corn.

In Experiment II, 16 diets with different levels of nutrients, but meeting minimum nutrient requirements, were fed to layers in respiratory chambers. AMEn, nitrogen retention, heat production and gaseous exchange were determined. Previous fasting heat production values for laying hens were used to calculate HI and NE. An equation was generated by linear regression to predict NE and HI of feed ingredients based on nutrient composition. Energy partitioning analysis showed diets had different AMEn, NE, heat increment, and NE:AMEn ratios. The prediction equation generated by regression for NE was: NE (MJ/kg) = 0.786 × AMEn (MJ/kg) + 0.0844 × ether extract (%) – 0.0295 × crude protein (%).

In Experiment III, the NE of ingredients was predicted and used to formulate two diets - one low and the other high in NE:AMEn ratio. Layers were fed these diets in respirometer chambers. Measured and predicted NE and NE:AMEn were examined and found to be close to one another thereby confirming precision of the equation.

In Experiment IV, a 62 hen validation trial in commercial cages compared diets formulated to be low and high NE:AMEn ratio. The low NE:AMEn diet had 0.44% added refined canola oil and 5.0% wheat mill run while the high NE:AMEn diet 4.06% added canola oil and 24.0% millrun. Final 0 to 11 week results (44 week old hens) showed an advantage for the high NE:AMEn diet in terms of egg weight, FCR (feed/eggs), Haugh units, yolk colour when compared to low NE:AMEn diet. The cost per kg egg depended on price of oil. At $885/t there was a clear advantage for the high oil diet.

This study demonstrates that increasing fat/oil and decreasing crude protein levels in layer diets increases NE:AMEn. Depending on ingredient prices, formulating layer feed on an NE basis may reduce feed cost or lower feed cost per kg egg with higher feed cost.

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Introduction

According to the Stockfeed Manufacturers Council of Australia, annual feed for table egg layers is 6.2% of the total 13.0 million tonnes of feed produced or about 800,000 tonnes (Anon, 2017). Most of the layer feed formulated today is based on the apparent metabolisable energy (AME) system and most AME values used for ingredients are derived from measurements in growing broilers or adult cockerels and adjusted to zero N retention. There have been several major initiatives over the years to improve AME methods and develop poultry net energy (NE) values. However, formulation of feed using NE in laying hens has been lacking.

Globally, the poultry industry is a major consumer of energy in the form of grains, protein meals and feed fats. Nutrients consumed by chickens yield energy when oxidised during metabolism. Energy is required for growth, egg production, maintenance and locomotion. Energy consumed beyond requirement is retained for short periods as glycogen and longer term deposited as fat. Nutritionists aim to formulate diets to meet energy requirements for growth or egg production without compromising body composition. Genetic changes and management improvements make this an ongoing task. Understanding energy measurement and use in chickens is important for formulating nutritionists and vital for further progression of the industry.

During the 1940's, Fraps measured the NE of series of poultry ingredients based on dietary energy balance and energy gain in bird carcasses. In their study, simultaneous equations were used to determine energy for maintenance and energy for production, and productive energy or NE of production was calculated. However, the procedure was time consuming and expensive. Following the applications of Titus's energy value table for AME (Titus, 1955; Hill and Anderson, 1958; Sibbald and Slinger, 1962) the true metabolisable energy (TME) assay was developed. This refined and simplified the dietary energy assay system with corrections made for endogenous energy losses (Sibbald, 1976; Farrell, 1980). A further development was the correction of ME values for carcass nitrogen (N) retention. This decreased variation in both the AME and TME assays. This was deemed necessary as it is impossible to ensure that all birds grow at the same rate, or at all in the case of adult roosters (Muztar and Slinger 1981, Sibbald and Morse, 1983; Dale and Fuller, 1984; Lopez and Leeson, 2007). However, Lopez and Leeson (2007) found no difference in performance of birds fed corn–soy based diets formulated using AME values either corrected or not corrected for N retention. The AME assay, corrected for N retention, using growing broilers is the most widely accepted system today used for feed formulation in most world areas. Although AME has been reported to be both an accurate and repeatable (across laboratories) method of determining available energy of raw materials (Bourdillion et al., 1990a; Bourdillion et al., 1990b), some questions and idiosyncrasies remain. Published values often do not indicate if they were obtained using roosters, laying hens or growing chicks, and may not indicate if there has been correction for N retention. Of greater importance is the fact that AME does not give a complete picture of the amount of energy actually available for maintenance, growth or production. Formulation on an NE basis may thus be a more accurate and cost effective system than the current AMEn system. However, this system has not been successfully assessed and is not ready for use in the formulation of poultry diets.

The heat increment (HI) of feeding is defined as the heat produced by an animal in excess of that associated with basal or fasting metabolism. Different ingredients and combinations of ingredients generate more or less heat as their nutrients are digested and metabolised. In growing chicks, the relative efficiency of energy utilisation for carbohydrate, fat and protein has been determined to be 100%, 113% and 78%, respectively (De Groote, 1974). Intuitively, this means that the ME system overvalues the energy value of high protein feeds and undervalues fat or ingredients with a high fat content. While this seems to be relatively straightforward, adjusting ME for metabolic heat loss requires measurement of total heat production in birds fed complete diets and fasting heat production. This can be accomplished in two ways: 1) determining carcass energy in serially slaughtered birds as Fraps reported in the 1940s; or 2) using indirect calorimetry involving the measurements of respiratory gas exchanges. Calorimetry measures gaseous exchange with heat production determined by applying the Brouwer equation to the data: HP (kJ) = (O_2 consumption, L × 3.866 + CO_2 generation, L × 1.2) / 0.239 (McLean, 1972). Prediction equations of HI and thus NE can be generated based on chemical composition of the diets and /or digestibility of various feed components. Such equations are in commercial use in the pig (Noblet et al., 1994) and ruminant (Ferrell and Oltjen, 2008) industries. In the pig industry, cost savings of €4.00 to 4.50 per tonne of feed have been demonstrated using NE formulation as compared to ME formulation with no negative impact on production (van der Klis et al., 2010). Published equations (De Groote, 1974; Hoffman and Schiemann, 1980; Emmans, 1994) for estimating NE values of feed ingredients based on nutrient digestibility for poultry were examined by Pirgozliev and Rose (1999) and applied to 40 feedstuffs ranging in ME content between 8.0 and 18.0 MJ/kg. The NE content of these feeds were previously estimated using prediction equations based on crude protein, ether extract and nitrogen free extract derived from the serial slaughter methodology (Fraps, 1946). Their results indicated that ME accounted for 78% of the variation in NE. It was revealed that ME overestimated energy of high protein feeds of animal origin when compared to cereals, cereal by-products and vegetable protein feeds. This report suggested economic merit in developing a system to predict poultry NE from chemical composition of feeds and feed ingredients.

The measurement of NE has been proposed for poultry but implementation has been lacking. There are several reasons for this. The ME system has been entrenched for decades and is easy to use. Obtaining data for HI by serial slaughter is laborious and costly. In addition, several reports examining HI in broilers using open circuit calorimetry have suggested higher variability than those obtained with pigs. Reported differences in HI between diets varying in ether extract and crude protein content in these studies were also not significant (Carré et al., 2002; Noblet et al., 2003; Noblet et al., 2007; Noblet et al., 2009; Warpechowski, et al., 2004). At the University of New England, closed circuit chambers have been used to develop equations for predicting NE of raw materials for broiler chickens. The results have indicated potential savings in formulation of feed using equations generated in the NE system.

If net energy formulation were be shown to improve energy efficiency in layers, significant feed cost savings may be realised. However, some questions on the use of AME should also be answered first as AME is a component of the NE calculation: 1) are the AME values derived from adult cockerels valid for hens in production? 2) what is the effect of production level and age on these values? 3) are AME values derived from growing

broilers suitable for hens in production? 4) as the hen in production retains approximately 50 to 60% of nitrogen consumed, mainly to produce egg albumin, is it valid to correct AME values to zero N retention ie. AMEn?

Attempts have been made to assess the NE content of layer feed ingredients (Burlacu et al., 1974; Sakomura et al., 2005; Waring and Brown, 1965). However, further studies and application of the data have been scarce due to variation in the measurements. The measurements of the energy partitions have been performed in laying hens to determine energy responses to environmental variables, restriction of dietary energy, sulphur amino acids and/or protein levels in the feed by several groups. It was observed that higher temperature tended to increase availability of metabolisable energy in laying hens (O'Neill and Jackson, 1974a; O'Neill and Jackson, 1974b,c). Higher protein level in the feed demonstrated a higher energetic efficiency. For example, 72.4% NE:AME was achieved with an 18% protein diet, whereas only 60.9% was observed with a 12% protein diet at the ambient temperature of 21^{o}C (Valencia et al., 1980). Reid and Maiorino in 1984 demonstrated total sulphur amino acid (TSAA) deficiency and methionine toxicity was found to alter the energetic efficiency (NE:AME) significantly. It was shown that the NE:AME was improved from 52.1% to 63.8% by an increase of TSAA from 0.47% to 0.51% of feed. On the other hand, feeding of toxic levels of TSAA (3.4%) increased about maintenance ME requirement by 20 kcal/day thus lowering efficiency. Overall, the energetic efficiency in layers was reported to range from 64% - 86% with the growing conditions close to industry standards. Such a large range provide the opportunity for the nutritionist to formulate diets in a more efficient way to maximise feed efficiency and achieve the best possible output for the layer farmers.

Objectives

The objectives of the current study were:

- Generate a basic database of NE values using a range of well-defined diets for developing a prediction equation for NE;
- Determine measured and predicted NE values of diets to validate the equation;
- Perform semi-commercial and commercial scale experiments to examine whether diets formulated on a NE basis will give a commercial advantage.

Methodology

Experiment I AME bioassay

Birds

For the bioassay of feed ingredient AME, 60 42-week old Hy-Line Brown layer hens were sourced from The Glenwarrie Partnership, Tamworth, NSW. They were fed a standard commercial diet on arrival until the start of the trial. The birds were housed in a layer shed at the University of New England, Armidale, NSW. Two birds were kept in each layer cage measured at 55 cm × 50 cm × 50 cm with each cage separated by an empty cage on either side. Each cage was equipped with individual feeder designed to minimise spillage. Two nipple drinkers were provided per cage. Oversized removable dropping pans hanging from the wire mesh cage floor were used to collect excreta. The study was approved by the Animal Ethics Committee of the University of New England and designed to follow the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2013).

Diets

Energy yielding ingredients, corn, soybean meal and wheat were sourced from the local market. Nutrient content was measured using wet chemistry for proximate (ether extract, Dumas N), Ca and total P and NIRS (Evonik) for total and standardised ileal digestible amino acids. AME and AMEn were determined in cages and compared to results obtained using EU prediction equations (Janssen, 1989). A reference diet and four test diets were formulated for the bioassay of corn, soybean meal and wheat with or without supplementation of xylanase as shown in Table 1 and Table 2. The formulas in Table 2 were used to produce the four diets. All diets were in mash form with ingredient particle size suitable for hens in lay.

Table 1 Measured nutrients and chemical components in corn wheat and soybean meal used in experimental diets (percent as is basis unless indicated).

Ingredient	Corn	SBM	Wheat	Wheat plus xylanase
Dry Matter (%)	88.0	90.2	89.6	89.6
AMEn calc EU, MJ/kg	13.78	9.59	13.24	13.66
Crude protein (%)	8.97	47.1	10.73	10.73
Crude fibre (%)	1.65	3.30	2.30	2.30
Ether extract (%)	3.03	1.80	2.15	2.15
Ash (%)	1.53	6.50	1.52	1.52
ADF (%)	3.40	5.10	2.57	2.57
NDF (%)	8.06	8.40	9.83	9.83
Starch (%)	48.9	0.00	63.9	63.9
Free sugars, g/kg	21.9	108.0	22.5	22.5
NSP total, g/kg	57.6	120	81.1	81.1
NSP soluble, g/kg	3.30	8.00	13.3	13.3
NSP insoluble, g/kg	54.3	112	67.8	67.8
Lys (%)	0.31	2.93	0.36	0.36
Met (%)	0.16	0.64	0.16	0.16
Thr (%)	0.31	1.85	0.33	0.33
Arg (%)	0.39	3.44	0.51	0.51
Val (%)	0.41	2.23	0.50	0.50
Ile (%)	0.32	2.11	0.40	0.40

Chemical components were measured by wet chemistry using AOAC methods (petroleum ether for ether extract and Leco Dumas method for N, crude protein = N x 6.25) with the exception of amino acids measured using the Evonik Amino NIR system.

The diets consisted of a corn-soybean meal reference diet and four test diets. The reference diet provided a baseline AME value allowing the AME of each test diet to be calculated. The test diets consisted of 30% of each test ingredient added to slightly less than 70% of the reference diet. The balance consisted of vitamins, minerals (including limestone) and amino acids added to ensure sufficient and similar nutrient levels in all diets. In the wheat plus xylanase diet, Econase XT 25 (AB Vista, Marlborough, UK) was included at the expense of a small portion of the energy yielding ingredients of the reference diet. The entire mix of reference diet, test ingredient and added limestone, amino acids, vitamin and mineral premixes are shown in Table 2.

Diets were fed for a 7-day adaptation period followed by a 3-day collection period with measurements of feed intake and excreta. Feathers, scales, and spilled feed were removed from the excreta daily. Feed spillage was minimised. Body weight of birds was measured before the initiation of the excreta collection period and also at the end of experiment to ensure that dietary treatments have not limited growth.

Table 2 Ingredient composition of reference and test diets for AME experiment

Ingredients	Ref Diet	Corn Diet	Soymeal Diet	Wheat Diet	Wheat+Xyl Diet
Corn (%)	60.5	69.8	39.8	39.8	39.8
Wheat (%)	0.00	0.00	0.00	30.0	30.0
SBM, Arg (%)	25.8	16.9	46.9	16.9	16.9
Canola oil (%)	1.28	0.84	0.84	0.84	0.84
Limestone (%)	9.69	9.69	9.69	9.69	9.69
Dical Phos (%)	1.56	1.56	1.56	1.56	1.56
Xylanase (%)	0.00	0.00	0.00	0.00	0.005
Salt (%)	0.27	0.27	0.27	0.27	0.27
Na bicarb (%)	0.20	0.20	0.20	0.20	0.20
UNE pmx (%)	0.20	0.20	0.20	0.20	0.20
Choline Cl 60% (%)	0.078	0.078	0.078	0.078	0.078
L-lysine HCl (%)	0.079	0.079	0.079	0.079	0.079
D,L-methionine (%)	0.271	0.271	0.271	0.271	0.271
L-threonine (%)	0.093	0.093	0.093	0.093	0.093

Expressed as percentage of reference diet and test ingredients

Ingredients	Ref Diet	Corn Diet	Soymeal Diet	Wheat Diet	Wheat+Xyl Diet
Reference diet (%)	0.00	65.74	65.74	65.74	65.735
Corn (%)	60.5	30.00	0.00	0.00	0.00
Wheat (%)	0.00	0.00	0.00	30.00	30.00
SBM, Arg (%)	25.8	0.00	30.00	0.00	0.00
Canola oil (%)	1.28	0.00	0.00	0.00	0.00
Limestone (%)	9.69	3.32	3.32	3.32	3.32
Dical Phos (%)	1.56	0.53	0.53	0.53	0.53
Xylanase (%)	0.00	0.00	0.00	0.00	0.005
Salt (%)	0.27	0.09	0.09	0.09	0.09
Na bicarb (%)	0.20	0.07	0.07	0.07	0.07
UNE pmx (%)	0.20	0.13	0.13	0.13	0.13
Choline Cl 60% (%)	0.078	0.027	0.027	0.027	0.027
L-lysine HCl (%)	0.079	0.027	0.027	0.027	0.027
D,L-methionine (%)	0.271	0.093	0.093	0.093	0.093
L-threonine (%)	0.093	0.032	0.032	0.032	0.032

UNE premix supplied per tonne: 12.0 MIU Vit A, 3.5 MIU Vit D, 40.0 g Vit E, 2.0 g Vit K, 2.0 g thiamine, 6.0 g riboflavin, 5.0 g pyridoxine, 0.02 g cyanocobalamin, 50 g niacin, 11 g pantothenic acid, 0.10 g biotin, 1.5 g folic acid, 60.0 g iron, 60.0 g zinc, 80.0 g manganese, 8.0 g copper, 0.30 g selenium, 1.0 g molybdenum, 0.30 g cobalt, 1.0 g iodine, 25 g Endox (antioxidant)

AME measurement

The total excreta voided daily were pooled from each replicate and weighed (wet basis). Multiple subsamples were collected and homogenized from the total at the end of the collection period. A 30-g representative sample was weighed and freeze dried. Samples of

feed and freeze-dried excreta were finely ground to ensure homogeneity. Gross energy content of feed and excreta was measured in triplicate on a 0.5 g dried sample using an adiabatic oxygen bomb calorimeter. N in feed and excreta was measured on a 0.15 g sample with a LECO nitrogen analyzer.

AME of diets was calculated according to the following equation:

$AME = (Fi \times GEf - E \times GEe)/Fi$

AMEn of diets was calculated according to the following equation:

$AMEn = [Fi \times GEf - E \times GEe - 34.39 \times (Ni - Ne)] / Fi$

Where: GEf is the gross energy of feed intake and GEe is the gross energy of excreta (kJ/g); Fi = feed intake (g); E = excreta output (g) and 34.39 kJ/g is nitrogen correction factor; Ni is nitrogen intake from the diet and Ne is the nitrogen output from the excreta (g).

Calculation of test ingredient AME or AMEn:

$AME_{test} = a\% \times AME_{ref} + b\% \times AME_{ing}$

Where AME_{test} is the measured AME of test diet

AME_{ref} is the measured AME of reference diet

AME_{ing} is the AME of ingredient under analysis

a% is the share of energy yielding ingredients from reference diet formulated in test diet over the total energy yielding ingredients in test diet (It is corrected due to the fact that it has been diluted by a small amount of minerals, amino acids and limestone)

b% is the proportion of ingredient under analysis in the test diet including minor ingredients.

Explanatory example calculation:

The reference diet AMEn is determined. The test diet AMEn is determined. Example: The reference diet is 12.34 MJ/kg. The test diet is 12.46 MJ/kg. 30% of the test ingredient is mixed with 65.74% of the reference diet and 4.26% additional minerals, vitamins and amino acids (to make the mixed test diet similar to the reference diet in terms of calcium, vitamins, trace minerals etc). The test ingredient AMEn is then $(12.46 - (0.6574 \times 12.34))/0.30 = 14.49$ MJ/kg.

Experiment II Prediction equation for net energy from nutrients

Birds

For the NE prediction equation trial, two separate hatches of sixty 16-week-old Hy-Line Brown pullets were sourced from The Glenwarrie Partnership, Tamworth, NSW. The birds were subjected to experiments during age from 32 to 62 weeks (

Table 3). They were fed a standard commercial diet from the day of arrival until the day of adaptation and then fed respective test diets during adaptation and measurement period in calorimetric chambers. Prior to heat production measurements, birds were adapted in calorimeter chambers (with lids open) for 3 days in a climate controlled room then continued on their respective test diets. Each chamber accommodated 3 birds and each run consisted of 16 chambers with 8 runs as replicates. Birds were raised and handled humanely with fresh clean drinking water available at all times. The study was approved by the Animal Ethics Committee of the University of New England and designed to follow the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2013). Table 3 shows the first 4 replicates used the first hatch of birds (then older) and the second 4 replicates used the second hatch of younger birds

Table 3 Age of birds in chamber trials

Run	Start date	Age of birds(wk)	Comments
1	7-Dec-15	51	Old
2	21-Dec-15	53	Old
3	18-Jan-16	57	Old
4	21-Feb-16	62	Old
5	21-Mar-16	30	Young
6	4-Apr-16	32	Young
7	18-Apr-16	34	Young
8	2-May-16	36	Young
validation	30-May-16	40	Young

Diet formulation and analysis of diets and excreta

Diet compositions are given in Tables 4 and 5. Sixteen diets were formulated to have similar AMEn values based on commercially used AMEn values used for corn and wheat. The final calculated AMEn values were then adjusted for the actual measured AMEn of corn, wheat and SBM obtained from Exp 1. Half of the diets were based on corn and the other half based on wheat (Table 4 and Table 5). All wheat based diets contained xylanase (Econase XT 25, AB Vista, Marlborough, UK). None of the diets contained phytase. Some of the diets contained small amounts of alpha cellulose and celite (fine silica) to allow greater additions of oil at the same calculated AMEn level. Diets ranged in crude protein content from 12% to 22% and single amino acids were added to low protein diets to ensure digestible amino acid were sufficient according to the Hy-Line standard. Diets were formulated such that levels of fat, protein, crude fibre and starch had minimal

correlations to each other as much as possible. This was to ensure higher chances of developing a robust NE prediction equation. Correlation coefficients will be described in results. As some nutrients are chemically correlated to one another, such as ADF and crude fibre, low correlations are impossible.

Respiratory chambers and measurements of O_2 consumption and CO_2 expiration

The measurement of NE followed Swick et al., (2013). The closed circuit calorimetric chambers were similar in design described by Farrell (1972) with modifications. The chambers were constructed of stainless steel and were 100 cm long × 76 cm high × 70 cm wide and equipped with a wire-mesh cage (89 cm long × 60 cm high × 60.5 cm wide). Water was used to seal the chamber according to the modifications made by Farrell (1972). The pressure was controlled by barometric sensor connected to an electronic switch to activate a solenoid valve. Temperature and humidity of each chamber were constantly monitored using temperature and humidity sensors with electronic display and memory capabilities. A 28 L/min diaphragm air pump first circulated chamber air through a screw-capped plastic bottle containing 2 L of 32% w/w potassium hydroxide with bubbler assembly to absorb CO_2 expired by the birds, after which the air was passed through a PVC trap containing approximately 3 kg of dried silica gel to absorb moisture before being returned to the chamber. Humidity was maintained at less than 70% for the entire run and CO_2 levels were maintained at less than 10 ml/L. Medical grade oxygen was provided by equipping each chamber with a 490 L cylinder fitted with a regulator and a reducing valve to replenish the consumption of O_2 in the chamber by birds.

The O_2 consumption was calculated by deduction of the weight of the oxygen cylinder at the end of each run from the weight of the cylinder at the beginning. The density of O_2 being 1.331 g/L at normal temperature and pressure (NTP, defined as air at 20°C and 101.325 kN/m^2) was used for the conversion of weight (g) to volume (L). Subsamples of KOH from each chamber were taken after all the solution from each KOH bottle was made up to 2 L. Collected KOH samples were kept at room temperature until analysed for CO_2 recoveries. The recovery of CO_2 was performed according to the method described by Annison and White (1961) based on a barium chloride ($BaCl_2$) precipitation technique. Briefly, 1 mL of KOH solution was accurately pipetted into a dried and pre-weighed 15-mL centrifuge tube in duplicate. Subsequently, 1.5 mL of NH_4Cl was added to each tube. The solution was gently swirled and mixed thoroughly. After the addition of 5 mL $BaCl_2$ to the tubes, the mixture was centrifuged for 15 min at 3500 rpm. The supernatant from each tube was carefully decanted and the carbonate pellet was then resuspended in 5 mL distilled water followed by centrifugation for 30 min at 3500 rpm. The supernatant was subsequently decanted and the tubes were dried overnight at 105˚C. Finally, tubes were cooled in a desiccator and accurately weighed to record the $BaCO_3$ recovered from 1 mL aliquot of KOH solution. The CO_2 exhaled by the birds was then calculated by multiplying the weight of $BaCO_3$ (in 2 L KOH) by 0.2229 (the fraction of molecular weight of CO_2 to the molecular weight of $BaCO_3$). The changes of O_2 and CO_2 in the calorimetric chambers were measured before the close and opening of the chambers every day during the run using the FoxBox Respirometry System (Sable Systems, Las Vegas, NV, USA). The total consumption of O_2 and expiration of CO_2 were calculated by taking into account the changes of the gases in the chambers. All the measurements were adjusted to 24 hrs each day.

ME, total heat production, and net energy

Apparent metabolisable energy was determined by the total collection method and values were corrected for N retention. The method was previously described by Bourdillon et al. (1990 b) and modified for total collection. The degree of oxidation of diets and heat production corresponded to the O_2 consumed and the amount of CO_2 produced from birds. Total heat production was measured for 3 days in sealed chambers. It was suspended for about 2 hours each day for replenishing feed, water, KOH and silica gel and collection of excreta. Heat production values were obtained by applying chamber CO_2 and O_2 data to the modified Brouwer equation (taking out measurements of methane and N in expired gas). The equation (Brouwer, 1965; McLean, 1972) is

Total heat production (kJ) = *(O_2 consumption, L × 3.866 + CO_2 generation, L X 1.2) / 0.239*

The respiratory quotient (RQ) of each 3-day run was calculated as the ratio of CO_2 volume expired to O_2 volume consumed by birds. Heat increment was calculated by subtracting fasting heat production (FHP) from total heat production. To correct for zero activity, a FHP value of 370 kJ/$BW^{0.75}$ per bird per day which corresponds to the asymptotic HP (at zero activity) over a 24 h fasting was used (Wu et al., 2016). NE was calculated as ME intake minus HI divided by feed consumed on an as-is basis.

Table 4 Ingredient composition of 16 test diets

Ingredients	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Corn (%)	66.1	57.4	51.6	39.5	54.7	54.8	64.8	52.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Wheat (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	62.5	52.2	73.0	52.9	59.8	48.0	48.9	60.4
SBM, Arg (%)	21.1	11.1	33.8	37.3	30.7	26.8	15.8	25.6	20.2	33.9	6.20	16.4	17.9	27.8	31.0	25.8
Canola oil (%)	0.14	5.20	2.40	5.90	2.03	2.96	1.67	4.26	1.77	1.98	0.75	5.99	3.23	5.27	4.14	1.26
Limestone (%)	9.96	9.96	9.94	9.93	9.96	9.95	9.97	9.95	10.0	9.97	10.0	9.99	10.0	9.98	9.98	10.0
Dical P (%)	1.60	1.78	1.50	1.54	1.55	1.60	1.70	1.63	1.52	1.39	1.62	1.63	1.56	1.52	1.47	1.46
Xylanase (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Salt (%)	0.24	0.24	0.24	0.27	0.27	0.27	0.27	0.27	0.22	0.20	0.22	0.23	0.23	0.23	0.23	0.22
Na bicarb	0.20	0.25	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Alpha cellulose (%)	0.00	3.80	0.00	3.00	0.26	1.30	1.60	2.60	0.00	0.00	3.21	4.43	1.62	2.40	1.00	0.00
Celite (%)	0.00	8.10	0.00	2.00	0.00	1.70	2.70	3.00	3.01	0.00	2.62	7.09	4.65	4.28	2.78	0.45
UNE Layer pmx (%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Choline Cl 60% (%)	0.10	0.16	0.05	0.04	0.06	0.08	0.13	0.09	0.02	0.00	0.07	0.07	0.04	0.02	0.00	0.00
L-lys HCl (%)	0.10	0.47	0.00	0.00	0.00	0.00	0.29	0.00	0.11	0.00	0.55	0.28	0.20	0.00	0.00	0.00
D,L-met (%)	0.25	0.39	0.15	0.15	0.18	0.22	0.32	0.24	0.22	0.11	0.34	0.30	0.26	0.19	0.15	0.17
L-threonine (%)	0.08	0.25	0.00	0.00	0.00	0.02	0.16	0.05	0.07	0.00	0.25	0.16	0.12	0.00	0.00	0.00
L-tryptophan (%)	0.00	0.07	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00
L-isoleucine (%)	0.00	0.24	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.25	0.11	0.05	0.00	0.00	0.00
L-arginine (%)	0.00	0.27	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.29	0.05	0.00	0.00	0.00	0.00
L-valine (%)	0.00	0.25	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.26	0.12	0.05	0.00	0.00	0.00

UNE layer premix supplied per tonne: 10.0 MIU Vit A, 3.0 MIU Vit D, 20.0 g Vit E, 3.0 g Vit K, 35.0 g nicotinic acid, 12 g pantothenic acid, 1 g folic acid, 6 g riboflavin, 0.02 g cyanocobalamin, 0.10 g biotin, 5.0 g pyridoxine, 2.0 g thiamine, 8.0 g copper, 0.20 g cobalt, 0.50 g molybdenum, 1.0 g iodine, 0.30 g selenium, 60.0 g iron, 60.0 g zinc, 90.0 g manganese, 20.0 g Oxicap E2 (antioxidant)

Table 5 Nutrient composition (% as is)

Nutrients	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
AMEn MJ/kg [1]	11.61	11.61	11.61	11.61	11.61	11.61	11.61	11.61	11.61	11.61	11.61	11.61	11.61	11.61	11.61	11.61
AMEn MJ/kg [2]	11.53	11.55	11.52	11.52	11.52	11.53	11.54	11.53	12.32	12.17	12.36	12.35	12.32	12.28	12.28	12.31
AMEn MJ/kg [3]	10.62	10.85	10.88	11.17	10.36	11.22	10.80	11.14	10.73	10.55	10.98	10.85	10.75	10.77	11.23	10.22
Crude protein [2]	16.1	11.7	20.7	21.2	19.5	17.7	13.9	16.9	16.7	21.9	12.4	14.3	15.5	18.7	20.2	19.0
Crude protein [4]	16.0	11.5	20.7	20.0	19.2	17.8	13.1	16.9	17.1	21.7	12.4	13.6	15.4	18.1	19.9	19.3
Ether extract [2]	2.52	7.11	4.56	7.83	4.23	5.08	3.91	6.28	3.47	3.70	2.43	7.39	4.82	6.78	5.73	3.01
Crude fibre [2]	1.79	4.92	1.97	4.73	2.16	3.00	3.11	4.17	2.11	2.32	4.93	5.97	3.51	4.30	3.10	2.24
d Arg [2]	0.92	0.84	1.27	1.34	1.18	1.06	0.83	1.01	0.92	1.31	0.81	0.80	0.83	1.10	1.20	1.09
d Lys [2]	0.83	0.83	1.04	1.10	0.97	0.87	0.83	0.83	0.83	1.07	0.83	0.83	0.83	0.90	0.98	0.88
d Met [2]	0.47	0.54	0.43	0.43	0.44	0.46	0.50	0.47	0.43	0.38	0.48	0.47	0.45	0.42	0.40	0.40
d M+C [2]	0.70	0.69	0.70	0.70	0.70	0.70	0.69	0.70	0.69	0.70	0.68	0.69	0.69	0.69	0.69	0.69
d Trp [2]	0.15	0.16	0.22	0.23	0.20	0.18	0.15	0.17	0.21	0.27	0.17	0.17	0.19	0.23	0.25	0.23
d Ile [2]	0.61	0.62	0.81	0.84	0.76	0.68	0.62	0.65	0.61	0.84	0.63	0.61	0.61	0.71	0.77	0.71
d Thr [2]	0.59	0.58	0.67	0.69	0.63	0.59	0.58	0.59	0.58	0.70	0.57	0.58	0.58	0.59	0.64	0.59
d Val [2]	0.67	0.69	0.87	0.89	0.82	0.74	0.68	0.70	0.68	0.91	0.71	0.68	0.68	0.77	0.84	0.78
Calcium [1]	4.20	4.21	4.20	4.21	4.21	4.21	4.21	4.21	4.21	4.20	4.21	4.21	4.21	4.21	4.21	4.21
P avail [1]	0.40	0.41	0.40	0.41	0.41	0.41	0.41	0.41	0.41	0.40	0.41	0.41	0.41	0.41	0.41	0.41
Sodium [1]	0.17	0.18	0.17	0.18	0.18	0.18	0.18	0.18	0.18	0.17	0.18	0.18	0.18	0.18	0.18	0.18

[1] As formulated
[2] Using measured values for corn, wheat and SBM, AMEn by bioassay at UNE, protein and amino acids by wet chemistry.
[3] Final diets as measured in respiration chambers at UNE.
[4] Measured in final diets as fed by wet chemistry.
d = standard ileal digestibility using coefficients from Evonik Amino Dat 5.0.

Experiment III Validation of net energy equation

For validation of the NE prediction equation, younger birds used in Experiment II at the age of 40 weeks were used. The trial procedure and measurements of ME, HP and dietary nutrients followed the same protocol as have been described in Experiment II except that two diets with low and high NE:AMEn ratios were used as shown in Table 6. Eight chambers were used for each of the diet as replicates. The study was approved by the Animal Ethics Committee of the University of New England and designed to follow the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2013).

The equation generated from Experiment II was used to formulate two validation diets: 1) low NE:AMEn and 2) high NE:AMEn. Both diets were based on corn (30%), wheat (29.8% and 32.7%), SBM (27.3% and 11.8%), canola oil (0.70% and 4.54%) and 50:50 celite and alpha cellulose blend (0 and 7%). The ingredients used were from different batches used in Experiments I and II. The ingredients were assayed in advance of formulation. The “low NE:AMEn” diet was calculated to have 18.9% crude protein and 2.74% ether extract. The “high NE:AMEn” diet had 13.4% CP and 6.34% ether extract. Both met required digestible amino acids requirements.

Experiment IV Small scale validation of NE based formulation

Sixty-two Hy-Line Brown hens were house singly in layer cages in a completely randomised design. Hens were 44 weeks old and laying at over 95% hen day production at the beginning of the 11 week experiment. The hens were previously used for runs 5 to 8 of the equation experiment run and also for the validation experiment (run 9). Two diets with high and low NE:AMEn ratio were formulated based on Hy-Line Brown nutrient specifications to meet or exceed digestible amino acids (Table 7). Wheat, corn and soybean meal were from the same lot used in previous experiments. The other main ingredients, millrun, cold pressed canola meal and meat meal were analysed for nutrient content by NIRS (Evonik AminoNIR). No xylanase, phytase or other feed enzymes were included in the two diets.

The birds were fed these two diets as an adaptation period for the first week. After the adaptation period, measurements of the following variables were conducted during the following 11 weeks: Egg production (recorded daily), feed consumption and egg weight (measured weekly). External and internal egg quality were determined fortnightly using all eggs from 31 replicates of each treatment for two consecutive days. Body weight was measured by weighing all hens at the beginning, after adaptation, and again at the end of the experiment. Egg mass and feed conversion (g of feed/g of egg) were calculated from egg production, egg weight, and feed consumption.

External and internal egg quality characteristics were measured in the UNE egg laboratory using the methods of Roberts (2016, personal communication). Egg shell reflectivity was measured as percentage on the wide tip of each egg. Eggshell breaking strength was measured was expressed as the unit of compression force (Newton) required to break a the shell. The egg was cracked carefully and the eggshell separated thoroughly. Albumen height was measured using a digital micrometre measuring one centimetre apart from yolk

perimeter. Haugh unit was calculated using the formula with the records of albumen height and egg weight: $HU = 100 \log_{10} (H - 1.7 W^{0.37} + 7.56)$, where HU = Haugh unit, H = height of the albumen (mm) and W = egg weight (g). Yolk was separated from the albumen by rolling them down to the yolk colour reader as a yolk score. Before the yolk weight was determined, the chalazae and any adhering albumen were removed and then the yolk weight measured by a digital scale.

The egg shell was washed and dried overnight. Egg shell thickness (with inner and outer shell membranes) was measured at three different points around equator of each egg using a micrometre. An average of three different thickness measurements of an egg was regarded as eggshell thickness. The dried egg shell weight was determined using a digital scale and shell percentage was calculated as its percentage of the egg weight. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight.

The PROC GLM and Duncan's multiple range test was used to separate means (SAS 2010). PROC Reg with the option "selection = stepwise cp adjrsq" was used to perform stepwise multiple linear regressions of chemical components of diets with AME, HI, NE, RQ and NE:AME (SAS 2010). After selecting appropriate predictors with PROC Reg stepwise, the regression was run again using PROC GLM with "run" as a class and as a covariate (model = NE:AME = predictor(s) run/solution).

The study was approved by the Animal Ethics Committee of the University of New England and designed to follow the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2013).

Table 6 Composition of validation diets

	Diet	
	Low NE:AMEn	High NE:AMEn
Ingredients		
Corn (%)	30.0	30.0
Wheat, no xyl (%)	29.8	32.7
SBM (%)	27.3	11.8
Canola oil (%)	0.70	4.54
Limestone (%)	9.97	9.98
Dical Phos (%)	1.48	1.66
Salt (%)	0.22	0.22
Na bicarb (%)	0.20	0.20
Celite (%)	0.00	3.50
Alpha cellulose (%)	0.00	3.50
UNE Layer pmx (%)	0.10	0.10
Choline Cl 60% (%)	0.03	0.10
L-lysine HCl (%)	0.00	0.41
D,L-methionine (%)	0.18	0.34
L-threonine (%)	0.00	0.21
L-tryptophan (%)	0.00	0.03
L-isoleucine (%)	0.00	0.19
L-arginine FB (%)	0.00	0.30
L-valine (%)	0.00	0.20
Calculated nutrients [1]		
AMEn, MJ/kg	11.15	11.79
NE layer, MJ/kg	8.44	9.41
Crude protein	18.9	13.4
Ether extract	2.74	6.34
C. fibre	2.08	4.96
d Arg	1.11	0.93
d Lys	0.90	0.82
d M+C	0.69	0.68
d Trp	0.21	0.16
d Ile	0.72	0.61
d Thr	0.59	0.57
d Val	0.79	0.69
Calcium	4.20	4.20
P. avail	0.40	0.40
Sodium	0.646	0.587
Chloride	0.17	0.17
Linoleic acid	0.19	0.27

[1] as is basis

d = standard ileal digestibility using coefficients from Evonik Amino Dat 5.0

UNE layer premix supplied per tonne: 10.0 MIU Vit A, 3.0 MIU Vit D, 20.0 g Vit E, 3.0 g Vit K, 35.0 g nicotinic acid, 12 g pantothenic acid, 1 g folic acid, 6 g riboflavin, 0.02 g cyanocobalamin, 0.10 g biotin, 5.0 g pyridoxine, 2.0 g thiamine, 8.0 g copper, 0.20 g cobalt, 0.50 g molybdenum, 1.0 g iodine, 0.30 g selenium, 60.0 g iron, 60.0 g zinc, 90.0 g manganese, 20.0 g Oxicap E2 (antioxidant)

No xylanase or phytase were used in these diets.

Nutrients calculated from matrix values shown in appendix.

Table 7 Composition of experimental diets used in small scale performance validation

Ingredient	Ing price $/t	NE:AMEn low 0.764 %	NE:AMEn high 0.792 %
Corn	310	15.0	15.0
Wheat	310	44.6	22.1
Wheat millrun	240	5.00	24.0
SBM	630	13.1	13.7
Canola meal (cold pressed)	400	10.0	10.0
Meat meal	600	1.10	0.00
Canola oil	885 or 1250	0.44	4.06
Limestone	75	9.90	10.1
Dical Phos	600	0.18	0.38
Xylanase (Econase)	30000	0.005	0.005
Phytase (Axtra)	17500	0.005	0.005
Salt	230	0.117	0.138
Na bicarb	450	0.20	0.20
UNE Layer pmx	4000	0.10	0.10
Choline Cl 60%	1400	0.043	0.039
L-lysine HCl	1900	0.093	0.05
D,L-methionine	4500	0.130	0.142
L-threonine	2400	0.021	0.018
Jabiru Red pigment	85000	0.004	0.004
Jabiru Yellow pigment	82000	0.003	0.003
Nutrients			
AMEn MJ/kg (kcal/kg)		11.55 (2760)	11.63 (2780)
NE layer MJ/kg (kcal/kg)		8.82 (2108)	9.21 (2201)
Crude protein		18.67	18.63
Ether extract		3.47	7.27
Crude fibre		3.15	3.96
d Arg		0.98	1.02
d Lys		0.83	0.81
d M+C		0.71	0.70
d Ile		0.63	0.62
d Thr		0.58	0.57
d Val		0.79	0.73
Calcium		4.20	4.20
Phosphorus avail		0.37	0.35
Sodium		0.17	0.17
Chloride		0.15	0.16
Linoleic		1.20	2.10
$/t with oil at $885/t		360.27	366.15
$/t with oil at $1250/t		361.90	380.96

d = standard ileal digestibility using coefficients from Amino Dat 5.0 (Evonik, Frankfurt, Germany)

NE layer premix supplied per tonne: 10.0 MIU Vit A, 3.0 MIU Vit D, 20.0 g Vit E, 3.0 g Vit K, 35.0 g nicotinic acid, 12 g pantothenic acid, 1 g folic acid, 6 g riboflavin, 0.02 g cyanocobalamin, 0.10 g biotin, 5.0 g pyridoxine, 2.0 g thiamine, 8.0 g copper, 0.20 g cobalt, 0.50 g molybdenum, 1.0 g iodine, 0.30 g selenium, 60.0 g iron, 60.0 g zinc, 90.0 g manganese, 20.0 g Oxicap E2 (antioxidant)

Nutrients calculated from matrix values shown in appendix.

Results

Experiment I. Determination of AMEn of test ingredients

AME and AMEn values of corn, soybean meal, wheat and wheat supplemented with xylanase were measured and the values are shown in Table 8. The AMEn values of the ingredients calculated according to the EU prediction are also listed in the Table 8. Compared to the corn, wheat had lower AME and AMEn. However, when xylanase was supplemented, the AME and AMEn of wheat were not different from corn ($P > 0.05$) and were higher than the values of wheat not supplemented with xylanase ($P < 0.05$). As expected, correction of AME to zero N retention decreased AMEn of SBM to a greater extent than maize or wheat. Interestingly, xylanase supplementation resulted in greater decrease of AMEn relative to AME indicating higher N retention in birds. Relative to the EU prediction values using chemical analysis, measured AMEn values of corn and wheat supplemented with xylanase were 0.41 and 0.74 MJ/kg higher, while those of SBM and wheat not supplemented with xylanase were only 0.04 and 0.11 MJ/kg higher.

Table 8 Measured AME and AMEn values of ingredients (as is basis)

Ingredients		Corn	SBM	Wheat	Wheat + xyl
AME	(MJ/kg)	14.47[a]	10.10[c]	13.70[b]	14.89[a]
	(kcal/kg)	3457	2413	3273	3559
AMEn	(MJ/kg)	14.19[a]	9.60 [c]	13.35 [b]	14.40[a]
	(kcal/kg)	3391	2294	3190	3442
AMEn calc*	(MJ/kg)	13.78	9.59	13.24	13.66
	(kcal/kg)	3293	2292	3164	3265

* EU prediction using chemical analysis, 1989

(with 0.41 MJ/kg added AMEn per xylanase manufacturer's recommendations)

Experiment II. Prediction equation trial

Correlations of the nutrients of the diets

The 16 diets used to generate the NE prediction equation were formulated intentionally to minimise relationships among nutrient and chemical components. Correlations between nutrients in the diets were analysed as shown in Table 9. Low correlations between nutrients and chemical components were expected to improve the prediction and ability of the regression to determine independent relationships between HI, NE and each nutrient or chemical component. Relationships for some nutrients and chemical components however were difficult or impossible to minimise as their values are intrinsically related. For example starch and fat are difficult due to the fact they both contain energy; ADF and crude fibre cannot be separated as they both contain fibre; and fat and ash are correlated as fat is high in energy and ash has zero energy.

Table 9 Correlation coefficients of nutrients in test diets

	Protein	Fat	Fibre	Ash	ADF	NDF	Starch	Sugars	NSPt	NSPs
Fat	0.1									
Fibre	-0.5	0.7								
Ash	-0.4	0.8	0.8							
ADF	-0.6	0.3	0.9	0.4						
NDF	-0.5	0.7	1.0	0.8	0.8					
Starch	-0.6	-0.8	-0.1	-0.4	0.3	-0.1				
Sugars	0.9	-0.1	-0.7	-0.5	-0.8	-0.7	-0.6			
NSPt*	0.4	-0.3	-0.3	-0.6	0.1	-0.3	0.2	0.3		
NSPs*	0.2	-0.1	0.0	-0.3	0.4	0.0	0.3	0.0	0.9	
NSPi*	0.5	-0.3	-0.4	-0.7	0.0	-0.4	0.2	0.4	1.0	0.9

*NSPt = total NSP, NSPs = soluble NSP, NSPi = insoluble NSP

Performance of the birds in calorimetric chambers

Performance of the birds fed 16 different diets during 8 runs is given in Table 10. Egg weight, egg mass, hen day production and feed intake were not affected by diet and were reasonably and close to industry standard (Hy-Line Brown Management Guide, 2014). The lack of significant differences indicated that diets were adequate in nutrient content. The short 3 day collection period resulted in hen day production ranging from 85 to 103%. In some cases the last or next day's egg was laid during placement or when birds were being removed from the chambers.

Table 10 Performance of layers fed experimental diets

Diet	N	BW average, g/hen	HDP, %	Egg wt, g	Egg mass, g/hen/d	Feed intake as is, g/d	FCR
1	7	2074	100.0	61.2	61.5	94.6	1.547
2	8	2056	94.4	58.5	55.3	97.1	1.872
3	8	2035	93.1	61.6	57.5	95.0	1.670
4	7	2063	96.8	62.0	60.0	99.9	1.674
5	7	2022	95.2	61.2	58.3	99.0	1.720
6	7	2002	92.1	62.1	57.1	91.9	1.647
7	8	1960	84.7	58.5	49.9	94.3	2.076
8	8	2042	106.9	61.3	65.5	94.9	1.459
9	7	2030	90.5	61.5	55.5	99.5	1.848
10	7	1999	96.8	61.1	59.1	95.9	1.649
11	7	2017	98.4	60.2	59.3	99.9	1.697
12	8	2030	98.6	61.0	60.3	104.3	1.771
13	7	2047	101.6	61.1	62.0	100.1	1.618
14	8	2021	95.8	61.5	58.6	102.0	1.741
15	8	1977	84.7	61.2	52.4	96.5	3.351
16	7	1995	85.7	61.1	53.0	98.6	2.000
SEM		101	15.0	2.9	9.6	14.7	1.333
CV%		5.00	15.8	4.77	16.7	15.0	72.4
P > F		0.648	0.215	0.320	0.224	0.983	0.639

N = number of chambers with valid data used for equation generation.

Table 11 Performance RQ, HP and energy partitioning of the two hatches of hens of different age ranges

Age	BW g	HDP %	Egg wt g	Egg mass g/hen/d	Feed int as is, g/hen/d	FCR
Old (51-62 weeks)	2098	96.0	63.1	60.6	98.5	1.665
Young(30-36 weeks)	1948	93.3	58.7	55.0	96.9	2.018
P > F	<0.001	0.441	<0.001	0.003	0.634	0.178

Age	RQ	HP kJ/kg$^{0.75}$/d	HI kJ/kg$^{0.75}$/d	AMEn diet MJ/kg	NE diet MJ/kg	HI diet MJ/kg	NE:AMEn
Old (51-62)	0.963	530.8	160.8	11.89	9.27	3.09	0.779
Young (30-36)	0.955	550.3	180.3	11.60	8.87	3.32	0.765
P > F	0.287	<0.001	<0.001	<0.001	<0.001	<0.001	0.031

Performance of hens sourced in two hatches and used at different ages was also measured. Younger birds had lower HDP, egg weight, egg mass, feed intake and poorer

FCR than the older birds (Table 11). In addition younger hens had higher HP per bird $BW^{0.75}$, HI per bird $BW^{0.75}$, obtained lower AMEn and NE from the diet and produced higher HI from the diet. The NE:AMEn ration was lower in young birds as compared to older birds. No bird age by diet interactions were observed for any parameter measured ($P > 0.05$).

Respiratory quotient, heat production and NE intake

Respiratory quotient (RQ) and energy balance in the birds were measured and the results are shown in

Table 12. While RQ showed difference among the birds fed different diets ($P < 0.001$) due at least partially to the dietary EE levels (Pearson correlation r = -0.55), HP, HI and NE intake were not altered in response to the feeding of different diets. ($P > 0.05$) indicating nutritionally balanced diets and normal physiological status of the chickens.

Table 12 RQ, heat production and heat increment in chambers

Diet	RQ	HP kJ/kg$^{0.75}$/d	HI kJ/kg$^{0.75}$/d	NE intake kJ/kg$^{0.75}$/d
1	0.99^{ab}	538	168	441
2	0.95^{cde}	526	156	483
3	0.95^{bcde}	541	171	463
4	0.91^{f}	550	180	500
5	0.96^{bcde}	552	182	451
6	0.95^{bcde}	535	165	478
7	0.98^{abc}	530	160	479
8	0.94^{def}	527	157	492
9	0.98^{abc}	543	173	482
10	0.95^{bcde}	550	180	451
11	1.01^{a}	549	179	494
12	0.96^{bcde}	534	164	531
13	0.96^{bcde}	543	173	480
14	0.94^{ef}	541	171	508
15	0.95^{cde}	544	174	499
16	0.97^{abcd}	553	183	448
P > F	< 0.001	0.835	0.835	0.622
CV%	3.16	5.84	18.49	14.95

dry matter basis

Energy partitioning in hens fed various test diets is shown in Table 13. Diet 6 had highest and diet 16 had the lowest AMEn ($P < 0.001$), while diet 8 had the highest ($P < 0.001$) and 16 had lowest NE ($P < 0.001$). Heat increment was highest in diet 10 and the lowest in diet 12 ($P < 0.03$). The NE:AMEn ratio of diet 8 tended to be higher than diet 16 ($P < 0.09$). The differences in calculated AMEn (based on results of Experiment I) and measured

AMEn were possibly due to interactions between nutrients and differences between measurements in the chambers in the current experiment and cages in experiment I. The corn based diets 1 to 8 had measured values very close to calculated AMEn values. Diet 6 had the highest measured AMEn value yet was formulated to be moderate in crude protein (17.7%) with moderately high ether extract (2.96% added canola oil). The lowest corn based diet AMEn was diet 1 which also had the smallest amount of canola oil added. The wheat based diets add had higher calculated AMEn compared to measured AMEn. The wheat based diets were all lower in measured AMEn as compared to calculated based on results of Experiment 1. The reason is unknown but requires follow up. The effect oil canola oil on AMEn in the wheat based diets was less apparent than in corn based diets.

Table 13 Energy partitioning of the 16 diets

Diet	AMEn diet MJ/kg	NE diet MJ/kg	HI diet MJ/kg	NE:AMEn
1	11.63^{cde}	8.86^{cde}	3.35^{ab}	0.763
2	11.71^{bcd}	9.21^{abc}	2.96^{bc}	0.786
3	11.88^{abc}	9.08^{bcd}	3.34^{ab}	0.764
4	12.14^{a}	9.37^{abc}	3.35^{ab}	0.772
5	11.29^{ef}	8.48^{ef}	3.35^{ab}	0.752
6	12.24^{a}	9.57^{ab}	3.26^{abc}	0.782
7	11.72^{bcd}	9.22^{abc}	3.02^{abc}	0.787
8	12.13^{a}	9.65^{a}	3.05^{abc}	0.796
9	11.66^{bcde}	8.93^{cde}	3.20^{abc}	0.765
10	11.49^{de}	8.59^{def}	3.42^{a}	0.747
11	11.94^{abc}	9.07^{bcd}	3.30^{abc}	0.760
12	11.66^{bcde}	9.29^{abc}	2.88^{c}	0.796
13	11.63^{cde}	8.86^{cde}	3.22^{abc}	0.762
14	11.60^{cde}	9.11^{bcd}	3.03^{abc}	0.785
15	12.02^{ab}	9.34^{abc}	3.28^{abc}	0.778
16	11.09^{f}	8.25^{f}	3.41^{a}	0.744
P > F	<0.001	<0.001	0.034	0.096
CV	2.72	4.86	10.80	4.84

dry matter basis

Prediction equation of NE by nutrients

Following the regression analysis, a prediction equation of NE by nutrients in the diets was generated:

NE (MJ/kg) = 0.786 x MEn (MJ/kg) + 0.0844 x ether extract (%) – 0.0295 x crude protein (%)

The coefficient of variance (CV) was 4.71%, residual standard deviation (RSD) was 0.427 and correlation of determination (r^2) was 0.998.

Experiment III. Validation of the prediction equation in the calorimetry chambers

Bird performance results are given in Table 14. No differences of feed intake, FCR, hen day production, egg size, egg mass or RQ were observed between treatments (P > 0.05). Performance of birds in chambers was acceptable with 98% hen day production and 107 grams per day of feed intake.

Table 14 Performance of hens fed high and low NE:AMEn diets

	Diet			
Performance	Low NE:AMEn	High NE:AMEn	P>F	CV%
Feed intake, g/b/d as is	107.5	107.1	0.916	6.53
FCR, (feed/eggs)	1.762	1.760	0.981	8.78
HDP, %	97.2	98.6	0.554	4.67
Egg weight, g	63.1	61.8	0.283	3.78
Egg mass, g/hen/ d	61.3	60.9	0.786	5.55
Average hen wt, g	2048	2036	0.834	5.50

Heat production and heat increment expressed to a metabolic body weight were greater in birds fed the low NE:AMEn diet compared to the high NE:AMEn diet (P < 0.05). NE of the feed and NE:AMEn were also lower in the low NE:AMEn fed birds (P < 0.05 and 0.001). The NE:AMEn was different between treatments (P < 0.01) (Table 15). The predicted vs expected NE, AMEn and NE:AME are also shown in Table 16. The differences between low and high NE:AMEn are in agreement with a 0.041 difference in the predicted group and 0.045 difference in the measured group.

Table 15 Energy partition in the birds fed high and low NE:AMEn diets

	Diet			
Measurement	Low NE:AMEn	High NE:AMEn	P>F	CV%
RQ	0.982	0.986	0.754	2.27
Heat prod kJ/kg$^{0.75}$	573.4	550.7	0.040	3.56
Heat incr kJ/kg$^{0.75}$	347.5	307.4	0.018	9.15
NE MJ/kg feed*	7.64	8.71	<0.001	3.49
NE:AME	0.702	0.752	0.002	3.52
NE:AMEn	0.732	0.777	0.005	3.58

Table 16 Energy measured and predicted in the birds fed high and low NE:AMEn diets

Energy	Diet	Predicted	Measured
AMEn	Low NE:AMEn		10.45
	High NE:AMEn		11.21
NE	Low NE:AMEn	7.89	7.64
	High NE:AMEn	8.95	8.71
NE:AMEn	Low NE:AMEn	0.755	0.732
	High NEA:MEn	0.798	0.777

Table 17 Performance and egg quality of high and low NE:AMEn diets during 11 weeks of measurements

Measurement	Diet		P>F
	Low NE:AMEn	High NE:AMEn	
0 - 6 weeks			
Average BW (g/hen)	2133	2085	0.347
Feed intake, g/hen/d as is	124.0	121.7	0.429
FCR, (feed/eggs)	2.141	2.021	0.007
HDP, %	95.8	95.2	0.667
Egg wt, g	60.6	63.4	0.004
Egg mass, g/d	58.0	60.4	0.054
Cost per kg egg, $ (oil $885/t)	0.7712	0.7400	0.049
Cost per kg egg, $ (oil $1250/t)	0.7747	0.7700	0.768
0 - 11 weeks			
Average BW (g/hen)	2141	2094	0.369
Feed intake, g/hen/d as is	123.1	120.5	0.321
FCR, (feed/eggs)	2.124	2.007	0.004
HDP, %	95.9	94.9	0.323
Egg wt, g	60.5	63.4	0.002
Egg mass, g/d	58.0	60.1	0.050
Cost per kg egg, $ (oil $885/t)	0.7652	0.7350	0.037
Cost per kg egg, $ (oil $1250/t)	0.7687	0.7647	0.786
Shell colour	18.34	19.03	0.003
Haugh unit	90.20	92.88	0.000
Breaking strength	41.5	42.0	0.337
Yolk %	26.7	25.7	0.000
Albumen %	63.5	64.7	0.000
Shell %	9.75	9.61	0.003
Yolk colour score	11.37	11.70	0.000

Experiment IV. Comparison of performance of the birds between NE and AME based formulations and cost efficiency

The results measured from start to 6 weeks indicated that birds fed the high NE:AMEn diet had higher egg weight, lower FCR (feed/eggs), smaller yolks and larger albumens (expressed as percent) and higher yolk colour when compared to birds fed the lower NE:AMEn diet (Table 17). The actual yolk size expressed as egg size × percent yolk was larger in the high NE:AMEn fed birds compared to the low NE:AMEn fed birds. From 0 to 11 weeks, birds fed the high NE:AMEn diet had larger egg weight, greater egg mass, lower FCR, greater shell colour, Haugh units, smaller percentage yolks, greater percentage albumins, smaller percentage shell and higher yolk colour score as compared to those fed the low NE:AMEn diets (Table 17).

The cost per kg egg in birds fed the high NE:AMEn diet was lower than those fed the low NE:AMEn diets from both 0 to 6 ($P < 0.05$) and 0 to 11 weeks ($P < 0.05$) although the high NE:AMEn diet was more expensive than the low NE:AMEn diet per tonne using a feed-grade fat market price of $885/t. With oil prices increased to $1250/t the benefit of cost per kg egg was lost from 0 to 6 weeks ($P > 0.05$) and from 0 to 11 weeks ($P > 0.05$).

Discussion of Results

Determination of AMEn of test ingredients

The AMEn values obtained in layers at peak production were slightly higher than those obtained using prediction equations for broilers published by Janssen (1989) except for soybean meal. The excess protein and ratio of protein to energy of the soybean meal test diet is the likely reason for this. Further work is required to examine results of this ingredient at lower or multiple inclusion levels and the validity of correction to zero N retention as layers in peak production retain around 50% of N intake. It should be noted that supplementation of xylanase significantly increased AME of wheat, i.e., 1.19 MJ/kg or 284 kcal/kg. This is a substantial increase of AME. This result is in contrast to that reported by Pirgozliev et al., (2015) showing only a tendency of xylanase (from *Trichoderma reesei*) to increase AMEn (by 0.19 MJ/kg) in broilers fed wheat based diets. It was of interest that such a large improvement in wheat AME was observed in layers. Adult birds are usually considered to be less affected by arabinoxylans than younger growing birds. It may be of interest to compare xylanase in low and high AME wheat in both broilers and layers in the same experiment.

During this exercise, calculations for the substitution method of determining AME were questioned (Adeola et al., 2010). The way the procedure works is that a reference diet is tested for metabolisable energy. This requires measuring feed intake and excreta output on a dry weight basis during the trial period and then measuring gross energy consumed and excreted by the bird. The metabolisable energy is then calculated as the total energy retained divided by the amount of feed consumed. To determine the energy of a test ingredient, a new diet is fed consisting of an amount of the already defined reference diet and the test ingredient. Typically this would be 70% reference diet and 30% test ingredient. The metabolisable energy of this mix is then determined. The difference between the AME of the original reference diet and this new test diet is due to the ingredient in question. This difference is subtracted from 70% of the AME of the reference diet and the result is then divided by 30% (0.3) to obtain the AME of the test ingredient. Many researchers have reported this method and have attempted to improve it. One “improvement” is making adjustments to the test diet so that minerals, vitamins and amino acids are the same as in the original reference diet. However, these adjustments are at the expense of energy yielding ingredients in the portion of the test diet (the 70%) that is supposed to be reference diet. When this happens, the AME of the reference portion is no longer 70%. However, this change has never been considered in the literature, while ignoring this gives in lower AME value of the ingredients. In the case of layer diets, this difference is even larger due to higher calcium content in the diet.

Prediction equation of NE for layers

This is the first report of a NE prediction equation for laying hens based on nutrient composition. Fat content of the feed was positively correlated to NE and protein was negatively correlated to NE. The contributions of AME (or AMEn), fat and protein showed similar trends relative to the equation produced in broilers (unpublished data). The effects of fat and protein were greater in layers compared to broilers. Coefficients of 0.0844 for fat and -0.0295 for protein were determined for layers being larger those found for broilers

(0.031 and -0.017 respectively). This suggests that formulation on an NE basis for layers may be more beneficial than formulating on an NE basis in meat chickens as heat increment contributed by feed may be greater in hens than meat chickens.

The prediction equation enables nutritionists to predict not only the NE of diets following their proximate analysis but also of ingredients with known ether extract and crude protein contents so that diets can be formulated based on NE rather than AME. It has been long considered that feeding animals based on NE can improve feed efficiency and cost effectiveness which have been realised in swine and ruminants as have been reported (Noblet et al., 1994; Ferrell and Oltjen, 2008; van der Klis et al., 2010).

Predicted vs measured NE

It has been shown in the current study that the predicted NE values were close to that predicted for both diets used in the experiment. Similarly, the predicted and measured NE:AMEn ratios were also close. This suggests that NE values or NE:AMEn ratios can be confidently predicted using the equations obtained from this study and applied to diets or ingredients with known ether extract and crude protein contents. Essentially, the prediction of NE:AMEn is more important than the prediction of NE values *per se*, as the ratio indicates the energy efficiency of the diet in production and maintenance.

Feed efficiency and cost effectiveness of formulation based on NE

The major differences between the diets were the level of refined canola oil added, being 0.45% in the low NE:AMEn diet and 4.06% in the high NE:AMEn diet. In addition, wheat millrun was 5.0% in the low NE:AMEn diet and 24.0% in the high NE:AMEn diet. Wheat inclusion level in the high NE:AMEn diet was about half that of the low NE:AMEn diet (44.6 v 22.1%). These results show that addition of fat in the form of refined canola oil is highly beneficial even when extra fibre is added.

Ingredient prices used in this study will not be the same throughout Australia and will vary over time. In this least cost formulation, an oil price of $885/t was used and the high NE:AMEn diet showed benefit in the cost of per kg egg. This is close to the current price of feed grade oil or tallow in the market. It is of interest to evaluate tallow and other feed grade fat sources to see if they would give the same results as refined canola oil. Tallow is likely to have lower AME than canola oil and because of its different fatty acid profile, egg weight and unsaturation of lipids in egg yolk may be affected.

The results show that increasing the levels of canola oil in the feed increased the NE:AMEn and also improved FCR, egg weight, egg mass and yolk colour score. The higher yolk colour scores are likely due to the additional oil that improved the absorption of pigment from the diet. As eggs increased in size, there was more relative albumen and less relative yolk. The economic benefit is dependent on the price of oil in the feed. A larger evaluation using feed grade oil or tallow is warranted.

Implications

The experiments conducted indicated that NE values of ingredients can be predicted from AMEn, fat and protein. These can then be used to formulate diets. When evaluated in calorimeter chambers lower total heat production, lower heat increment and higher NE and NE:AMEn were observed in hens fed diets with high NE:AMEn when compared to low NE:AMEn control diets.

A small scale 11 weeks feeding study using 62 Hy-Line Brown hens was conducted under commercial conditions at the UNE Laureldale cage facility. It examined performance of birds fed low NE:AMEn vs high NE:AMEn diets. The low NE:AMEn diet contained 2.99% total fat whereas the high NE:AMEn diet contained 6.33% total fat. Refined canola oil was used to increase fat levels. Both diets had similar calculated MEn with the high fat diet containing higher levels of lower energy wheat millrun. The study showed that hens fed the high fat (high NE:AMEn) diet had lower FCR than the control. Although the high fat diet was more expensive, feed cost per kg eggs was lower in hens fed this diet. An improved cost per kg eggs in birds fed the high NE:AMEn diet (with addition of oil) was observed assuming a price of $885/t for oil. In practice, refined canola oil would be too expensive ($1250/t) for feed use and at market price there would be no benefit for its inclusion.

If lower priced feed grade fat sources such as crude vegetable oil or tallow had the same impact as refined canola oil, large potential savings for the Australian egg industry may be possible. Formulating on an NE basis may also increase the usage of supplemental amino acids such as isoleucine, arginine and valine that are currently becoming more available and economic. The use of these amino acids would reduce the crude protein level in the feed. NE formulation will also increase the relative value and therefore use of higher oil / fat ingredients such as expeller canola meal, MBM and whole seeds (canola seed, processed soybeans). Using the equation generated to predict NE of ingredients will allow feed to be formulated on a net energy basis.

Recommendations

- Conduct larger controlled experiment with greater number of birds using tallow or feed grade blended vegetable oil as the fat source.
- Compare tallow to refined canola oil in a performance study.
- Publish results in peer reviewed journals.
- Further evaluate AME levels of commonly used feed ingredients in layers and compare results to broilers and adult cockerels.
- Investigate ramifications of correcting AME values to zero N retention in laying hens.
- Evaluate the impact of using protein and energy matrix values for supplemental amino acids and their effect on AME vs NE formulation.
- Determine and compare the additivity of AMEn and NE values for ingredients to determine if there is an advantage to NE of AMEn in linear programming (least cost formulation)

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Appendix

Appendix Table 1 Nutrient matrix values used for ingredients in Tables 4, 6, 7.

Nutrient		Corn	Wheat no xyl	Wheat xyl	Wheat xyl	Millr un	Canola ml cold	SBM	Meat meal	Canol a oil	L-Thr	D,L-Met	L-Lys HCL	L-Trp	L-Ile	L-Val
DM	%	88.0	89.6	89.6	89.7	88.0	93.0	89.9	94.7	99.0	99.0	99.0	99.0	99.0	99.0	99.0
AMEn	kcal/kg	3391	3190	3442	3255	2260	2550	2294	1973	9260	3150	3680	3990	5460	5650	4990
AMEn	MJ/kg	14.19	13.35	14.40	13.62	9.46	10.67	9.60	8.26	38.74	13.18	15.40	16.69	22.84	23.64	20.88
NE layer	MJ/kg	11.14	10.36	10.90	10.50	7.27	8.21	6.31	5.52	38.85	8.19	10.37	10.35	15.42	16.61	14.20
Cr. protein	%	9.0	10.7	10.7	12.74	16.5	35.6	47.1	51.2	0.0	73.5	58.7	93.9	85.8	66.8	74.8
Ether extract	%	3.03	2.15	2.15	2.00	3.80	10.39	1.80	6.40	99.50	0.00	0.00	0.00	0.00	0.00	0.00
d Arg	%	0.37	0.43	0.43	0.49	0.89	1.94	3.20	3.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00
d Lys	%	0.29	0.31	0.31	0.29	0.47	1.79	2.64	2.03	0.00	0.00	0.00	78.4	0.00	0.00	0.00
d Met	%	0.15	0.15	0.15	0.18	0.20	0.64	0.58	0.56	0.00	0.00	99	0.00	0.00	0.00	0.00
d M+C	%	0.31	0.36	0.36	0.44	0.44	1.29	1.15	0.76	0.00	0.00	99	0.00	0.00	0.00	0.00
d Trp	%	0.05	0.14	0.14	0.13	0.20	0.37	0.57	0.20	0.00	0.00	0.00	0.00	99	0.00	0.00
d Ile	%	0.30	0.34	0.34	0.40	0.40	1.12	1.92	1.10	0.00	0.00	0.00	0.00	0.00	99	0.00
d Thr	%	0.26	0.29	0.29	0.31	0.39	1.22	1.57	1.20	0.00	99	0.00	0.00	0.00	0.00	0.00
d Val	%	0.38	0.45	0.45	0.48	0.57	1.46	1.99	1.62	0.00	0.00	0.00	0.00	0.00	0.00	99
Calcium	%	0.03	0.05	0.05	0.05	0.09	0.67	0.26	13.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P avail	%	0.08	0.12	0.12	0.10	0.12	0.35	0.24	5.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sodium	%	0.01	0.04	0.04	0.04	0.03	0.09	0.02	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix Table 2 Suggested NE layer values for common ingredients, as fed

	DM	NE layer	AMEn	Crude protein	Ether extract	Crude fibre
	%	MJ/kg	MJ/kg	%	%	%
Corn 8.6	87.0	11.17	14.10	8.60	4.00	2.70
Corn 9.0	88.0	11.14	14.19	8.97	3.03	1.65
Sorghum 10	86.8	10.54	13.49	10.20	2.84	2.17
Wheat 11	86.0	10.18	13.18	11.40	1.90	2.40
Wheat 12	86.0	10.30	13.39	11.80	1.43	2.43
Wheat 13	86.0	9.48	12.34	13.00	1.90	2.40
Oats 11.5	90.0	8.40	10.67	11.60	4.20	10.80
Groats 10.5%	90.0	12.20	15.00	10.50	8.50	3.99
Rice Brkn 8	88.0	11.21	14.44	8.00	1.22	1.06
Barley 10	89.0	8.75	11.30	9.70	1.80	5.10
Peas 24	90.0	7.84	10.75	23.80	1.04	5.18
Cassava 3.5	90.0	9.82	12.55	3.40	0.62	4.38
DDGS corn 28	93.0	7.68	9.91	27.80	8.40	11.40
DDGS sorghum 29	93.0	8.96	11.51	28.70	9.00	9.10
Bakery 9	90.0	12.06	14.64	9.30	9.80	0.90
Pasta 13	89.0	9.96	12.97	13.10	1.80	0.30
Wheat millrun	87.0	6.04	7.95	14.40	2.57	6.06
Rice Bran ext 14	90.1	6.18	8.33	14.00	0.60	9.60
Rice bran unex 14	90.1	9.82	11.44	13.80	14.56	6.15
SBM 44	90.8	6.03	9.21	44.00	1.10	6.00
SBM 45	87.9	6.73	10.04	45.20	1.97	4.44
SBM 48	87.5	6.87	10.46	48.00	0.80	3.50
FFSBM 39	87.8	9.92	12.55	39.10	14.33	4.24
Soycomil 65	91.0	9.70	13.41	65.00	12.73	3.50
Canola ml sol 35	89.0	5.82	8.37	35.30	3.31	12.06
Canola ml 37	88.3	6.04	8.66	36.20	3.60	7.60
Canola ml cold 36	93.0	8.21	10.67	35.60	10.39	11.70
Canola expeller 37	97.8	9.20	12.13	37.00	8.90	13.50
Sunflower ml 33	88.0	6.79	9.71	33.40	1.70	21.21
Cottonseed ml 45	90.0	6.06	9.21	45.40	2.00	5.00
Meat meal 49	97.0	7.70	10.46	49.00	11.00	0.00
Meat meal 52	88.0	6.36	9.08	52.00	9.00	2.60
Meat meal 55	97.0	7.81	10.88	55.80	10.70	0.00
Meat meal 59	95.5	7.76	10.88	58.60	11.10	0.00
Blood meal 88	96.1	7.65	12.89	92.40	2.98	0.28
Poultry ml 54	93.0	8.29	11.17	54.00	13.00	0.00
Poultry ml 60	93.0	9.02	12.55	60.00	11.00	0.00
Soybean Oil	99.0	38.00	37.66	0.00	99.50	0.00
Canola oil	99.0	38.85	38.74	0.00	99.50	0.00
Tallow	99.0	35.90	35.15	0.00	98.00	0.00
Veg oil blended	97.0	34.58	33.47	0.00	98.00	0.00