Energy efficiency and net energy prediction of feed in laying hens

S. Barzegar,* S.-B. Wu,* J. Noblet,† M. Choct,*,‡ and R. A. Swick*,[1]

*School of Environmental and Rural Science, University of New England, Armidale NSW 2351, Australia; †INRA, UMR1348 Pegase, F-35590 Saint Gilles, France; and ‡Poultry Cooperative Research Centre, University of New England, Armidale NSW 2351, Australia

ABSTRACT Using accurate nutrient values for ingredients is of vital importance for efficient diet formulation. The net energy (NE) system accounts for the real available amount of feedstuff energy for body maintenance and production as it considers energy dissipated as heat increment. The NE content of diets for pigs and broilers has been estimated from their nutrient contents. However, such estimates have not been made specifically for laying hens. This study reports the development of equations to predict NE for laying hens based on the chemical composition of 16 different diets meeting minimum nutrient specifications but varying in nutrient composition. Heat production and energy metabolism were measured in layers ranging from 32 to 62 weeks of age in closed-circuit calorimetry chambers with 8 replicates per diet in a randomized design. Each replicate consisted of a chamber with 3 layers that were adapted for 4 D to diets and chambers prior to measurement. The measurements included feed intake, metabolizable energy (ME) content, nitrogen balance, egg production, gas exchange, heat production, energy efficiency, and energy partition for a 3-D period. The average AME/GE and NE/AME ratios of the 16 diets were 77 and 74%, respectively. The latter ratio increased with energy efficiency (EE) content and decreased with CP content of diets. The results indicate that diet NE content can be predicted from AME, CP, and EE contents and the NE/AME ratio varied positively with EE and negatively with CP. A validation experiment with 2 diets fed to layers in calorimetry chambers confirmed the estimation from NE prediction equations. In conclusion, NE of diets can be predicted in laying hens from equations based on AME and CP and EE contents in laying hens being similar to those reported in broilers.

Key words: net energy, prediction equation, validation, calorimetry, laying hens

2019 Poultry Science 98:5746–5758
http://dx.doi.org/10.3382/ps/pez362

INTRODUCTION

Feed represents 65 to 75% of the total egg production cost with energy representing at least 60% of total cost for feed. Hence, accurate estimates of the real available amount of feedstuff energy are necessary to meet nutrient requirements and performance objectives at optimal efficiency. The pig and dairy industries have the ability to formulate feed using a net energy (**NE**) system. However, the poultry industry uses true or apparent metabolizable energy, usually corrected to zero-nitrogen retention (**TMEn**, **AMEn**), to formulate feed and as such does not distinguish maintenance from production or heat increment (**HI**) losses that would depend on nutrients.

Each feed energy system deals with energy utilization in the body and the calculation method for energy values. The ME is the attainable portion of feedstuff gross energy present after deducting the excreta energy. Heat increment is the heat produced in fed animals in excess of their maintenance (or fasting) metabolism, and it can be calculated by subtracting fasting heat production (**FHP**) from total heat production (**THP**). And NE is equal to ME minus HI. Net energy can be used for maintenance, body weight gain, and egg production. Total heat production can be measured by the calorimetry gas exchange or the comparative slaughter technique. The simple relationship of NE, ME, and HI as NE = ME – HI was originally introduced by Armsby and Fries (1915). They found the HI was dependent on the nutrient specification and independent on the level of feeding. This was a false assumption as later studies revealed that HI could be altered according to feed intake (**FI**) as a result of nutrient digestion and metabolism (Chudy et al., 2003; Ning et al., 2013, 2014; Liu et al., 2017).

Taking the NE as the most available portion of feed energy for animals, the NE system could be expected to overcome limitations of the current TMEn or AMEn systems because it represents the energy content of feed that is used for maintenance and production. De Groote (1974) examined the relative efficiency of energy utilization of different nutrients and found it to be 100,

---

Received February 25, 2019.
Accepted June 25, 2019.

[1] Corresponding author: rswick@une.edu.au

Table 1. Ingredients composition of diets (g/kg; as-is basis).

Diet	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ingredients																
Corn	661	574	516	395	547	548	648	520	0	0	0	0	0	0	0	0
Wheat	0	0	0	0	0	0	0	0	625	522	730	529	598	480	489	604
Soybean meal	211	111	338	373	307	268	158	256	202	339	62	164	179	278	310	258
Canola oil	1	52	24	60	20	30	17	43	18	20	7	60	32	53	41	13
Alpha cellulose	0	38	0	30	3	13	16	26	0	0	32	44	16	24	10	0
Celite	0	81	0	20	0	17	27	30	30	0	26	71	47	43	28	5
Others [1]	122	125	120	121	121	122	124	122	121	119	122	122	121	120	120	120
Supplemented amino acids[2]	4.4	19.5	1.5	1.5	1.8	2.5	11.3	2.9	4.0	1.1	19.6	10.1	6.7	1.9	1.5	1.7

[1] Others provided as (as-is, g/kg): 100 limestone, 16 dicalcium phosphate, 2.4 salt, 2.0 Na bicarbonate, 1.0 UNE vitamin & mineral premix, 0.6 choline 60%. Xylanase (Econase XT 25) added in wheat diets 9–16 at 0.075 g (12,000 BXU) per kg. 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).

[2] Supplemental amino acids (as-is, g/kg): 2.3 D, L-methionine for all diets; 1.2 L-lysine HCL, 78.4% for diets 1, 2, 7, 9, and 11–13; 0.7 L-threonine, 99% for diets 1–2 and 6–9 and 11–13; 0.1 L-tryptophan for diets 2, 7, and 11; 0.5 L-isoleucine for diets 2, 7, and 11- 13; 0.4 L-arginine for diets 2, 7, 11, and 12; 0.5 L-valine for diets 2, 7, and 11–13.

113, and 78% for carbohydrate, fat, and protein, respectively, relative to carbohydrate utilization in growing chicks. Pirgozliev and Rose (1999) compared the NE and AME values of 62 different feedstuffs and found the AME system overestimated the NE of high-protein animal-based feed compared to cereal grains, cereal by-products, and underestimated high-protein vegetable-based feedstuffs. It was also reported by Carré et al. (2014) that NE/AMEn ratios for CP, lipids, and starch were 76, 86, and 81% in broilers. Similarly, Wu et al. (2019) obtained NE/AMEn ratios of 73% for soybean meal (**SBM**), 88% for soy oil, and 81% for corn in growing broilers. These results indicate that the ME system would underestimate the energy value of dietary fat and overestimate the energy value of high-protein ingredients.

Net energy prediction equations for poultry have been proposed by several researchers (Schiemann et al., 1972; De Groote, 1974; Carré et al., 2002; Wu et al., 2019). The objective of this study was to measure the NE values of 16 diets in laying hens and to determine whether NE of compound feed and ingredients in laying hens could be predicted from their chemical composition.

MATERIALS AND METHODS

Birds and Experimental Design

The study was approved by the Animal Ethics Committee of the University of New England (UNE) and designed to follow the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2013). Hy-Line Brown pullets were sourced from the Glenwarrie Partnership, Tamworth, NSW, Australia, and reared following the Hy-Line Brown recommendations (Hy-Line, 2016) in a curtain-sided shed. They received 16 h of light and 8 h of darkness during their production period. A completely randomized design was used to evaluate 16 different diets in 16 calorimetry chambers (1 diet per chamber) with 3 birds per chamber. There were 8 repeat runs per diet. The same birds were kept together in chambers and shed cages between each run. Birds were assigned to diets randomly. The birds were 51 to 62 wk of age for the first 4 runs and a different batch of birds, 29 to 37 wk of age, was used in the last 4 runs. Birds were moved from shed to chambers and fed test diets for a 4-D in-chamber adaptation period with chamber lids open and running air pumps in a climate-controlled room. During the following 3 D, gas exchange was measured and HP was calculated as described below. Birds were fed a standard commercial diet when housed in the shed. Feed and water were provided ad libitum at all times.

Diets and Experiments

A total of 16 diets were formulated to have the same AMEn of 2,775 kcal/kg (as is basis) using the values for AMEn of corn, wheat, and SBM determined in a previous experiment with laying hens (Barzegar et al., 2017). This was done in an attempt to minimize the effect of dietary AMEn on FI. Half of the diets were based on corn and the other half based on wheat (Table 1). All wheat-based diets contained xylanase (Econase XT 25; AB Vista, Marlborough, UK). None of the diets contained phytase. Some diets contained small amounts of alpha cellulose and celite (fine silica) as fillers such that diets could be formulated with various levels of fat and protein at the same calculated AMEn level. Diets ranged in protein content from 13 to 24% (DM) using single crystalline amino acids added as necessary to ensure digestible amino acids met requirements recommended by Hy-Line (Hy-Line, 2016) (Table 2). Diets were formulated such that levels of fat, protein, and starch had no or minimal correlations to each other (Table 3) to improve the probability of developing robust NE prediction equations.

Table 2. Nutrient composition of experimental diets (g/kg DM basis; unless noted).

Diet	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Nutrients assayed																
DM%[1]	91.3	92.6	91.6	92.0	91.8	91.7	91.6	91.8	92.0	91.8	92.0	93.0	92.4	92.9	92.4	92.2
CP	185	132	229	226	212	198	154	190	189	244	144	156	171	200	224	215
EE	31	63	57	82	52	55	33	58	35	42	32	66	42	63	51	30
Crude fiber	82	88	59	41	55	67	75	44	69	66	63	74	43	68	66	46
Ash	156	214	165	171	197	166	189	177	204	182	163	208	186	201	169	190
NSP total	66	83	73	98	71	76	69	85	81	89	101	108	87	104	98	88
NSP soluble	6	4	6	6	6	6	5	5	15	13	15	12	13	13	13	14
NSP insoluble	60	79	67	92	65	70	64	80	66	76	86	96	74	91	85	74
NDF	76	85	81	102	82	96	78	93	92	98	116	122	118	97	93	97
ADF	39	57	47	61	43	53	56	56	38	44	47	57	49	39	38	31
Starch	429	395	338	277	321	378	431	360	372	308	460	351	399	302	338	359
Sugars	34	21	43	44	41	38	29	35	39	47	28	29	33	40	42	43
Nutrients calculated, as-is[2]																
Calcium	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42
Phosphorus, available	4.0	4.1	4.0	4.1	4.1	4.1	4.1	4.1	4.1	4.0	4.1	4.1	4.1	4.1	4.1	4.1

[1] DM measured at the feed distribution time.

[2] Total digestible amino acids calculated (g/kg as is): 4.5 methionine, 8.8 lysine, 6.8 methionine + cysteine

Table 3. Correlations between nutrient compositions and energy values of the 16 diets used for the prediction of net energy values.

		Ash	CP	EE	Starch	Free sugars	CF	ADF	NDF	NSP	GE	AME
CP	R	–0.378**										
EE	R	0.194*	0.119									
Starch	R	–0.166	–0.738**	–0.642**								
Free sugars	R	–0.330**	0.981**	0.057	–0.728**							
CF	R	0.323**	–0.579**	0.595**	0.023	–0.577**						
ADF	R	0.321**	–0.599**	0.562**	0.053	–0.593**	0.999**					
NDF	R	0.325**	–0.511**	0.178	0.126	–0.440**	0.800**	0.820**				
NSP	R	0.230*	–0.065	0.396**	–0.320**	–0.010	0.678**	0.684**	0.870**			
GE	R	–0.509**	0.661**	0.620**	–0.641**	0.604**	0.025	–0.006	–0.240**	0.138		
AME	R	–0.453**	0.067	0.355**	–0.028	0.019	0.234*	0.221*	–0.006	0.069	0.597**	
NE	R	–0.106	–0.166	0.341**	0.030	–0.197*	0.358**	0.349**	0.140	0.124	0.302**	0.749**

ADF, acid detergent fiber; CF, crude fiber; CP, crude protein; EE, ether extract or fat; GE, gross energy; AME, apparent metabolizable energy; NDF, neutral detergent fiber; NE, net energy; NSP, non-starch polysaccharides; NS, not significant.

[*] Pearson correlation significance level ($P < 0.05$).

[**] Pearson correlation significance level ($P < 0.01$).

Calorimetry Chambers Measurements

Closed-circuit calorimetric chamber design, gas exchange, and HP measurements have been previously described by Swick et al. (2013) and Wu et al. (2019) in broilers. The lighting program provided 16 h of light per day with the darkness from 10:00 pm until 6:00 am. The temperature and relative humidity were maintained within 22 to 24°C and 50 to 70% in chambers. Temperature and humidity of each chamber were monitored with electronic sensors including display and memory capabilities. A 28 L/min diaphragm air pump circulated chamber air through a plastic CO_2 trap containing 2 L of a 320 g/kg KOH solution with bubbler assembly to maximize CO_2 absorption followed by a moisture trap containing approximately 3 kg of dried silica gel before being returned to the chamber. A barometric sensor was connected to a solenoid valve to backfill the chamber with medical grade oxygen as CO_2 was absorbed. The O_2 consumption was determined gravimetrically by weighing cylinders before and after each day run. The recovery of CO_2 from KOH was performed according to the method described by Annison and White (1961) based on a barium chloride ($BaCl_2$) precipitation technique. The changes of O_2 and CO_2 in the calorimetric chambers were measured before the closing and opening of the chambers every day during the run using a FoxBox Respirometry System instrument (Sable Systems, Las Vegas, NV). The total consumption of O_2 and expiration of CO_2 were calculated by taking into account the residual proportion of gases in the chambers. The daily gas exchanges were measured for 3 consecutive days. However, it was suspended for about 2 h each day for replenishing feed, water, KOH, and silica gel and collection of excreta. Feed intake was measured and total excreta collected daily in each calorimetry chamber. Excreta collections were cumulated and weighed for 3 D. All variables were adjusted to a total of 72 h for calculation of HP. Performance measurements over 3 D included initial and final body weight of each laying hen, the daily FI per chamber, and the eggs mass and number per chamber.

Analyses of Diets and Excreta

Feed and excreta were thoroughly homogenized with subsamples taken for analysis. Feed samples were analyzed on an as-is basis, and results were expressed on a DM basis. Approximately 2 g of diet and 3 g of freeze-dried excreta samples were dried in crucibles in a forced air oven at 105°C to constant weight to determine DM. Excreta were freeze-dried for gross energy and N analysis. Wet excreta DM was calculated by correction for the loss of moisture during both freeze- and oven-drying. Gross energy was analyzed using an adiabatic bomb calorimeter (IKA Werke, C7000, GMBH and CO., Staufen, Germany). Feed samples were analyzed for CP, energy efficiency (**EE**), crude fiber, ash, neutral detergent fiber and acid detergent fiber (AOAC, 2016), starch (Megazyme Total Starch Kit, Megazyme International Ireland Ltd., Bray, Ireland), free sugars (mono- and disaccharides) (Annison et al., 1996), total non-starch polysaccharides (**NSP**), soluble NSP and insoluble NSP (Englyst and Hudson, 1987; Theander and Westerlund, 1993).

Calculations

The AME was determined using the total collection method of Bourdillon et al. (1990). The AME values were converted to AMEn (AME for zero N retention in body and eggs) and AMEs (AME corrected for a retention of N equal to 50% of nitrogen intake) (Cozannet et al., 2010) values using a GE value of 8.22 kcal per gram of N as the correction factor (Hill and Anderson, 1958). The THP corresponded to the O_2 consumed and the amount of CO_2 produced from birds according to the modified Brouwer equation: THP (kcal) = 3.866 × liters of oxygen consumed + 1.200 × liters of CO_2 expired (Brouwer, 1965; McLean, 1972). The respiratory quotient (**RQ**) of each 3-D run was calculated as the ratio of liters of CO_2 expired to liters of O_2 consumed. Heat increment was calculated by subtracting FHP from THP. An FHP value of 88 kcal/kg $BW^{0.75}$ (370 kJ/kg $BW^{0.75}$) per bird per day was used. This corresponds to the asymptotic HP (at zero activity) after a 24-h fasting period as reported for laying hens by Wu et al. (2016). The NE content was calculated as AME intake minus HI (per bird per day) divided by feed consumed on a DM basis. The N balance data were expressed per gram per bird per day.

$$\mathrm{TNR} = \mathrm{Nint} - \mathrm{Nexc} \tag{1}$$

where TNR is total nitrogen retained, Nint is N intake, and Nexc is N in excreta (g/bird/d).

$$\mathrm{NRegg} = 1.936 \times \mathrm{Egg\ mass} \tag{2}$$

where NRegg is total nitrogen retained in egg (g/bird/d), 1.936 is N% in egg (Miranda et al., 2015).

$$\mathrm{NRbody} = \mathrm{TNR} - \mathrm{NRegg} \tag{3}$$

where NRbody is total nitrogen retained in body (g/bird/d).

$$\mathrm{RE} = \mathrm{MEI} - \mathrm{HP} \tag{4}$$

where RE is total retained energy, MEI is ME intake, and HP is THP (kcal/kg/d).

$$\mathrm{REprot} = \mathrm{TNR} \times 6.25 \times 5.7 \tag{5}$$

where REprot is retained energy as protein, 6.25 is the protein equivalent of 1 g nitrogen, and 5.7 is the energy equivalent of 1 g protein (kcal/kg/d).

$$\mathrm{REfat} = \mathrm{RE} - \mathrm{REprot} \tag{6}$$

where REfat is retained energy as fat (kcal/kg/d).

$$\mathrm{REegg} = -19.7 + 1.81 \times \mathrm{egg\ weight\ (Sibbald, 1979)} \tag{7}$$

where REegg is retained energy in egg (kcal/d).

$$\mathrm{REegg\ prot} = (1.936 \times \mathrm{Egg\ mass}) \times 6.25 \times 5.7 \tag{8}$$

where REegg prot is retained energy in egg as protein (kcal/d).

$$\mathrm{REegg\ fat} = \mathrm{REegg} - \mathrm{REeggprot} \tag{9}$$

where REegg fat is retained energy in egg as fat (kcal/d).

$$\mathrm{REbody} = \mathrm{RE} - \mathrm{REegg} \tag{10}$$

where REbody is retained energy in body (kcal/kg $BW^{0.75}$/d).

$$\mathrm{REbody\ prot} = \mathrm{NRbody} \times 6.25 \times 5.7 \tag{11}$$

where REbody prot is retained energy as protein in body (kcal/kg/d).

$$\mathrm{REbody\ fat} = \mathrm{REbody} - \mathrm{REbody\ prot} \tag{12}$$

where REbody fat is retained energy in body as fat (kcal/d).

Energy balance data as AME intake, HP, retained energy (**RE**), and its partition between protein and fat and between body and egg production were further expressed per bird per kg $BW^{0.75}$ per day. Energy values of diets were expressed per kg DM, and energy utilization data were expressed as percent. The performance data were expressed per bird per day, with FI and FCR reported on a DM basis. Egg mass calculated as a product of percent hen day production (**HDP**) and average egg size (g/bird/d) and FCR as FI (g) divided by egg mass (g).

Statistical Analyses

All the performance, nitrogen balance, energy balance, energy values, and utilization data were analyzed using PROC GLM and Tukey's multiple-range test to separate means when appropriate (SAS, 2010). The model included the effects of diet (n = 16) and run (n = 8). As explained above, the so-called run effect includes the effect of batch of birds per se but also their age, and any environmental conditions that differed between successive runs. Multiple linear regression equations were calculated for estimating ME for maintenance (**MEm**), FHP, and mean utilization of ME for NE, on one hand, and efficiencies of ME for egg and body energy or protein and fat gains, on the other hand. Finally, the stepwise procedure of PROC REG was applied with or without intercept to calculate prediction equations for energy values and energy efficiencies according to linear effects of dietary characteristics.

For the validation of the NE equation, regression equations obtained from the 16 diets were applied to 2 further diets differing markedly in the NE: AME ratio. Furthermore, the AME and NE of the 4 major ingredients were calculated by regression with the AME and NE values of 16 diets against the inclusion rates of the ingredients.

RESULTS

A total of 13 observations were removed from the 128 total measurements taken because of low FI (4), low HDP (3), or technical issues with chambers (6). The calorimetry measurements were performed at different times, and each measurement (3 D) was regarded as a run. Body weight, FI, HDP, FCR, N intake, N excreted, total N retained, N retained in egg, N retained in body, AME intake, HP, HI, and RE were all affected by run ($P < 0.05$), while RE in egg as protein was not affected by run ($P > 0.05$) (Tables 4 and 5). Variance due to run includes bird age, bird individuality, and environmental conditions during the pre-measurement period.

Metabolic Utilization of Energy in Laying Hens

The average AME intake and HP of the 115 groups of 3 hens used in the study were 157 and 130 kcal/kg $BW^{0.75}$/d, respectively (Table 5). The amount of energy retained or exported to eggs averaged 54 kcal/kg $BW^{0.75}$/d, meaning that the laying hens in the present study had to mobilize 27 kcal/kg $BW^{0.75}$/d energy from body reserves. Thus, AME intake was insufficient to meet the energy requirements for both maintenance

Table 4. Effect of diet composition on performance and N balance in layers.

Diet	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	SEM	*P*-value (diet)[1]
Performance[2]																		
BW, g	2,074	2,056	2,035	2,063	2,022	2,002	1,992	2,052	2,030	1,999	2,017	2,030	2,047	2,036	1,983	2,042	11	0.645
Daily feed intake, g DM	86	90	87	92	91	84	89	91	92	88	92	97	93	98	89	92	1	0.208
Hen day production, %	100	94	93	97	95	92	92	106	90	97	98	99	102	95	95	97	1	0.482
Egg mass, g/bird/d[3]	61	59	62	62	61	62	59	62	62	61	60	61	61	62	62	63	1	0.103
FCR (g/g)	1.41	1.73	1.52	1.54	1.57	1.50	1.64	1.38	1.69	1.51	1.56	1.64	1.49	1.65	1.52	1.56	0.03	0.206
Dig Lys intake mg/bird/d	877	846	1097	1108	973	941	839	816	811	1103	817	777	811	841	1015	866	16	< 0.001
Nitrogen balance (g/bird/d)																		
Intake	2.59	1.96	3.06	3.41	3.11	2.70	2.29	2.77	2.78	3.487	2.22	2.51	2.55	3.16	3.25	3.22	0.05	< 0.001
Excreta	1.31	0.88	1.98	2.25	1.95	1.49	1.08	1.45	1.55	2.173	1.06	1.26	1.34	1.88	1.97	1.92	0.04	< 0.001
Retained:																		
Total[4]	1.28	1.07	1.08	1.16	1.16	1.21	1.21	1.32	1.24	1.31	1.16	1.25	1.22	1.28	1.28	1.30	0.02	0.340
Egg[5]	1.19	1.13	1.19	1.20	1.19	1.20	1.15	1.20	1.19	1.18	1.17	1.18	1.18	1.21	1.19	1.22	0.01	0.103
Body[6]	0.09	–0.06	–0.11	–0.04	–0.03	0.01	0.06	0.12	0.05	0.13	–0.01	0.07	0.04	0.07	0.09	0.08	0.02	0.461

[1] From the analysis of variance with diet and run effects, run effect was significant ($P < 0.001$) for all the performance parameters and N balance components; SEM as standard error of the mean.

[2] Each value represents the mean of 8 replicates (runs) for each treatment (diet) (n = 115) during 3 D respiratory measurements (3 layers per calorimetry chambers).

[3] Egg mass HDP × average egg size (g/bird/d). FCR (g/g) calculated as feed intake (g) divided by egg mass (g)

[4] Total N retained (g/bird/d) calculated as N intake – N in excreta.

[5] Retained N in egg (g/bird/d) calculated as 1.936 (N% in egg) × egg mass (Miranda et al., 2015).

[6] Retained N in body (g/bird/d) calculated as total N retained – retained N in egg.

Table 5. Effect of diet composition on energy balance, energy values, and energy utilization in layers.

Diet	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	SEM	*P*-value (diet) [1]
Energy balance (kcal/kg BW$^{0.75}$/d) [2]																		
AME intake	146	153	151	162	151	154	156	161	156	151	161	166	156	167	161	152	2	0.221
HP	128	126	129	131	132	128	128	128	130	131	131	128	130	131	131	133	1	0.668
HI	40	37	41	43	43	39	40	40	41	43	43	39	41	42	42	45	1	0.668
RE [3]																		
Total	17	27	22	31	19	26	28	33	27	19	30	39	26	36	30	19	2	0.011
As protein	26	22	22	24	24	26	26	27	26	28	25	26	25	27	27	27	1	0.286
As fat	–9	5	0	7	–5	0	2	6	1	–8	5	12	1	10	2	–8	1	< 0.001
RE$_{egg}$																		
Total	53	50	54	54	54	55	52	54	54	54	53	53	53	55	55	55	1	0.111
As protein	24	23	25	25	25	25	24	25	25	25	24	25	25	25	25	25	1	0.143
As fat	28	27	29	29	29	30	28	29	29	29	28	29	29	29	30	30	1	0.096
RE$_{body}$																		
Total	–36	–23	–32	–23	–34	–29	–24	-21	–27	–35	–23	–15	–27	–18	–25	–37	2	0.008
As protein	2	–1	–2	–1	–1	0	1	2	1	3	0	1	1	2	2	2	1	0.465
As fat	–37	–22	–29	–22	–34	–30	–26	-23	–28	–38	–23	–16	–28	–20	–27	–38	1	< 0.001
RQ	0.987	0.948	0.954	0.914	0.958	0.950	0.984	0.947	0.985	0.953	1.009	0.958	0.963	0.945	0.947	0.978	0.003	< 0.001
Energy values, kcal/kg DM																		
AME	2,918	2,909	2,971	3,040	2,830	3,066	2,928	3,047	2,899	2,870	2,956	2,906	2,887	2,901	3,023	2,808	8	< 0.001
AMEn	2,780	2,799	2,839	2,901	2,698	2,925	2,818	2,928	2,787	2,746	2,854	2,787	2,780	2,794	2,904	2,694	9	< 0.001
AMEs	2,894	2,880	2,988	3,045	2,835	3,052	2,913	3,050	2,909	2,901	2,942	2,885	2,890	2,923	3,045	2,832	9	< 0.001
NE	2,118	2,201	2,170	2,239	2,027	2,287	2,189	2,299	2,134	2,053	2,168	2,220	2,118	2,173	2,220	1,969	13	< 0.001
Energy utilization, %																		
AME/GE	77.4	78.8	74.7	73.8	74.3	77.1	78.4	77.3	77.8	74.3	79.2	77.1	77.9	74.7	75.8	75.7	0.1	< 0.001
NE/AME	72.6	75.6	73.1	73.6	71.6	74.6	74.8	75.5	73.6	71.5	73.3	76.3	73.3	74.9	73.5	70.1	0.1	0.0145

GE, gross energy; AME, apparent metabolizable energy as [(Fi × GEf) – (E × GEe)]/FI (kcal/kg DM of diet); AMEn, AME corrected for zero N retention as = [AME – [8.22 × (Ni—Ne)]]/Fi (kcal/kg DM of diet); AMEs, AME corrected for a N retention equal to 50% of nitrogen intake = AMEn + 8.22 × Ni % × 10 × 50% (kcal/kg DM of diet), where GEf and GEe are the gross energy of feed and excreta (kcal/g DM); FI = feed intake (g DM/d/hen); E = excreta output (g DM/d/hen); 8.22 as nitrogen correction factor (kcal/g); HI, heat increment as HP – FHP (FHP = 88.4 kcal/kg BW$^{0.75}$/d); HP, heat production (kcal) as 3.866 × oxygen consumed (L) + 1.200 × CO_2 expired (L) (Brouwer, 1965); respiratory quotient (RQ; CO_2/O_2); net energy (NE) values expressed based on DM of the feed (total collection method); NE (kcal/d) calculated as fasting heat production + RE.

[1,2] From the analysis of variance with diet and run effects, run effect was significant ($P < 0.05$) for all the parameters in Table 4, except RE egg as protein ($P > 0.05$). Each value represents the mean of 8 replicates (runs) for each treatment (diet) (n = 115) during 3 D respiratory measurements (3 layers per calorimetry chambers).

[3] Total retained energy (RE) calculated as ME intake – HP; RE as protein (kcal) calculated as total retained N × 6.25 × 5.7; RE as fat calculated as total RE – RE as protein; total retained energy in egg (RE egg; kcal) calculated as –19.7 + 1.81 × egg weight (Sibbald, 1979); RE egg as protein (kcal) calculated as retained N in egg × 6.25 × 5.7; protein content of an egg assumed as 12.1% (Miranda et al., 2015); RE egg as fat calculated as total RE egg – RE egg as protein; RE body calculated as total RE – RE egg; RE body as protein calculated as retained N in body × 6.25 × 5.7; RE body as fat calculated as total RE body – RE body as protein.

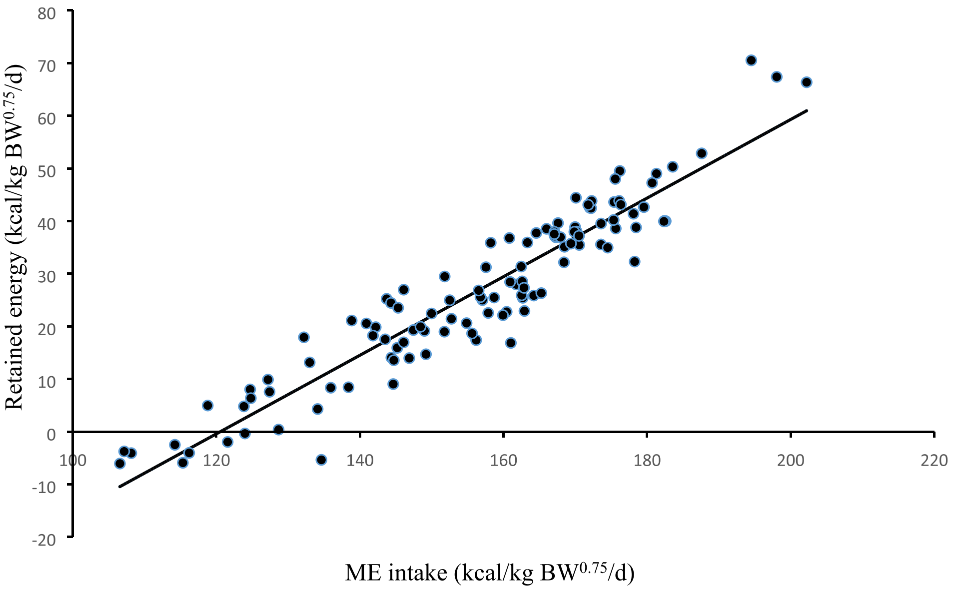

Figure 1. Relationship between energy retained and ME intake in laying hens (n = 115; kcal/kg BW$^{0.75}$/d).

Table 6. Energy utilization prediction equations in laying hens (kcal/kg BW$^{0.75}$/d).

Equation no	Equation[1]	r2	RSD
1	$RE = -90 + 0.75 \times \text{ME intake}$	0.89	5.3
2	$\text{ME intake} = 105 + 1.98 \times RE_{prot} + 0.98 \times RE_{fat}$	0.92	5.8
3	$RE_{body} = -75 + 0.75 \times \text{ME intake} -1.28\ RE_{egg}$	0.90	5.3

[1] Multiple linear regression with means of the 115 measurements on 16 diets.

RE, total retained energy; RE_{body} retained energy in body; RE_{egg} retained energy in egg; RE_{prot}, retained energy as protein; RE_{fat}, retained energy as fat; RSD, residual standard deviation.

and egg production. In fact, for all of the 16 treatment groups of laying hens, the mean body energy balance was negative, which means that dietary NE measurements were obtained under a metabolic situation where energy required for maintenance and egg production was met by both feed and body reserves. With regard to N utilization, the data presented in Table 4 indicate that N retained on average was 1.22 g/bird/d of which 1.19 g/bird/d was exported to egg with a subsequent close to zero N deposition in body (0.03 g/bird/d, on average). Unlike energy, protein and amino acid supplies were sufficient to meet the requirements of laying hens for egg production and maintenance. This also means that the mobilization of body energy corresponds to fat exclusively. In the present study, this mobilization averaged 28 kcal/kg BW$^{0.75}$ or 48 kcal/bird/d being equivalent to about 5 g/bird/d of fat.

Retained energy was related to ME intake ($r^2 = 0.89$) as depicted in Figure 1 and Model 1 in Table 6. It was then possible to estimate parameters for energy utilization in laying hens by regression modeling. Different models were tested in order to estimate the MEm (ME requirements for maintenance) and the energy cost of energy gain in egg or as protein and fat (Table 6). Model 1 indicates that MEm (for RE = 0) and FHP (for MEint = 0) equal 120 and 90 kcal/kg BW$^{0.75}$/d, respectively and the efficiency of ME for energy gain is 75%. Model 2 describes the total recovered energy from ME intake into both protein and fat for body and egg combined. Model 2 illustrates that the energy cost of depositing 1 kcal of AME intake as protein (in both egg and body) was 1.98 kcal. Also, the energy cost for retaining 1 kcal of energy intake as fat (in both egg and body) was 0.98 kcal. Therefore, the efficiencies of energy gain as protein and as fat from AME were approximately 50 and 100%. Model 3 examined how energy balance in body is dependent on ME intake and energy exported to egg. Model 3 showed that deposition of 1 kcal of egg energy required 1.28 kcal of body energy (i.e., 78% efficiency).

The Effect of Diet Composition on Performance and Energy Utilization in Laying Hens

Dietary treatment did not change FI, HDP, egg mass, and FCR ($P > 0.05$) as shown in Table 4. Total N retained, N retained in egg, and N retained in body were not affected by dietary protein level ($P > 0.05$) as shown in Table 4. Body N retention was on average 0.1 g/bird/d, while that retained in egg was much higher at 1.2 g/bird/d. The amount of lysine consumed by the birds averaged 909 mg/bird/d with a range between 777 and 1,108 mg/bird/d.

Diet composition did not alter the AME intake, HP, and HI (kcal/kg $BW^{0.75}$/d; $P > 0.05$) as shown in Table 5. However, birds fed the highest AME levels (diets 12 and 14) showed the highest total RE and the lowest mobilization of body energy. The energy gain as protein in eggs and protein in body were not affected by diet composition ($P > 0.05$). The RQ values ranged from 0.914 to 1.009 with the lowest values observed in birds fed high-fat diets (diets 4 and 14), and the highest values in birds fed the high starch diets (diets 1 and 11) (Table 5). The measured AMEn values among diets ranged from 2,694 to 2,928 kcal/kg DM as shown in Table 5 ($P < 0.05$). This was unexpected as diets were formulated to have a constant AMEn of 2,750 kcal/kg (as is basis). The average energy metabolizability (AME/GE) was 77% (range: 74 to 79%) with the highest ratio in birds fed diet 11 that contained the highest level of starch with low CP. The AMEs averaged 2,937 kcal/kg DM (ranging from 2,832 to 3,052 kcal/kg DM), and were similar to the average AME values of 2,935 kcal/kg DM (ranging from 2,808 to 3,066 kcal/kg DM). AMEn values were lower than AMEs or AME and averaged 2,815 kcal/kg DM.

The average efficiency of AME for NE was 74% and ranged from 70 to 76%. The NE/AME was lowest in birds consuming diet 16 with an EE of 29 g/kg DM (the lowest) and CP of 215 g/kg DM. The NE values ranged from 1,969 to 2,299 kcal/kg DM in connection with combined differences in ME content and efficiency of ME for NE. The lowest NE/AME value (70%) was observed in birds fed diet 16 that had the lowest AME content (2,808 kcal/kg, DM) and lowest NE of 1,969 kcal/kg DM, while the highest NE/AME value (76%) was observed in birds fed diet 12 that had an AME content of 2,906 kcal/kg DM. Birds fed diet 16 also had the lowest NE of 1,969 kcal/kg DM, while birds fed diet 8 had the highest NE of 2,299 kcal/kg DM in connection with an AME value of 3,047 kcal/kg DM and an efficiency of AME for NE of 75.5%.

Prediction of GE, AME, NE, and Energy Efficiency From Dietary Chemical Composition

Linear regression was conducted to evaluate the contributions of energy-yielding nutrients to GE, AME, AMEs, AMEn, and NE as shown in Table 7. For that purpose, organic matter was partitioned between CP, EE, starch, and residue (Res). The Res component contained fiber, including soluble and insoluble NSP, and sugar. The ratios between the AME and GE coefficients for each nutrient provide an estimate of the energy metabolizability of that nutrient, while the ratios of nutrient coefficients between the NE equation and AME equation provide an estimate of the efficiency of ME for NE of each nutrient. The ratios for metabolizability of energy were 60, 78, 95, and 65% for CP, EE, starch, and Res, respectively. The ratios for metabolizability of CP increased from AME/GE (60%) to AMEs/GE (65%) and decreased for AMEn/GE (52%). Using the same method, the efficiencies of AME for NE were 49, 104, 78, and 70% for CP, EE, starch, and Res, respectively. Thus, the NE content of 1 g of starch was equivalent to 1.84 g of CP and 0.37 g of EE.

Table 7. Contribution of energy yielding nutrients (% of DM) to GE, ME, and NE contents of layers diets.

Equation no	Energy (kcal/kg, DM)	Equation[1] CP	EE	Starch	Res1	RSD
4	GE	52.7	94.7	39.2	44.1	36
5	AME	31.8	74.2	37.1	28.7	67
6	AMEs	34.2	67.1	35.2	31.6	80
7	AMEn	27.3	66.8	35.2	32.2	80
8	NE	15.7	77.1	28.9	20.2	124

[1] Linear regression without intercept (n = 115 measurements on 16 diets).

GE, gross energy; AME, apparent metabolizable energy (as measured); AMEn, AME corrected for zero N retention; AMEs, AME corrected for N retention equal to 50% of N intake; CP, crude protein; Res1 for diet organic matter minus CP, EE and starch; EE, ether extract or fat; NE, net energy; RSD, residual standard deviation.

Table 8. Prediction of AME, AMEn, AMEs (kcal/kg, DM), and metabolizability (%) in layers from diet composition (% of DM).

Equation no	Energy	Equation [1] Intercept	CP	EE	Starch	Ash	RSD
9	AME	3,033	–	39.6	5.6	–27.1	67
10	AMEn	2,820	–	37.8	6.3	–23.1	79
11	AMEs	3,328	–	27.5	–	–28.8	79
12	AME/GE	74.3	–0.25	–	0.19	–	1.5
13	AMEn/GE	72.6	–0.30	–	0.18	–	1.8
14	AMEs/GE	72.3	–0.13	–	0.19	–	1.8

[1] Stepwise multiple linear regression (n = 115 measurements on 16 diets).

AME, apparent metabolizable energy; AMEn, AME corrected for zero N retention; MEs, AME adjusted corrected for 50% N retention; CP, crude protein; EE, ether extract or fat; GE, gross energy; NE, net energy; RSD, residual standard deviation.

Multiple stepwise regression was used to predict the AME, AMEn, and AMEs values and metabolizability of energy from diet composition (Table 8). The results showed that AME and AMEn can be estimated by dietary nutrients and were positively related to EE and starch and negatively related to ash. The AMEs values were predicted by EE and ash. Energy efficiency ratios of AME/GE, AMEs/GE, and AMEn/GE were positively related to starch and negatively related to CP.

Stepwise multiple linear regression was also used to estimate diet NE, NE/AME, NE/AMEn, and NE/AMEs from dietary nutrients as shown in Table 9. The equation shows NE to be positively related to AME (0.781) and EE (16.4), while CP was negatively related (–11.0) when AME was used as a predictor. Using AMEn as a predictor, NE is also positively related to AMEn (0.796) and EE (18.5) and negatively related to

Table 9. Prediction of NE and HI feed (kcal/kg DM) and energy efficiencies (%) in layers from diet composition (CP, EE, and starch as % of DM) and ME content (kcal/kg DM).

Equation no	Energy	Equation[1]							RSD
		Intercept	AME	AMEn	AMEs	CP	EE	Starch	
15	NE	–	0.781	–	–	–11.0	16.4	–	93
16		–	–	0.796	–	–8.8	18.5	–	98
17		–	–	–	0.798	–14.2	18.4	–	98
18	HI feed	212	–	–	–	16.4	–	6.8	88
19	NE/AME	76.7	–	–	–	–0.3	0.7	–	3.2
20	NE/AMEn	78.9	–	–	–	–0.3	0.7	–	3.5
21	NE/AMEs	78.8	–	–	–	–0.5	0.7	–	3.4

[1] Stepwise multiple linear regression (n = 115 measurements on 16 diets); if the intercept is not significantly different from zero, it is then fixed to zero.

AME, apparent metabolizable energy; AMEn, AME corrected for N retention; AMEs, AME corrected for 50% N retention; CP, crude protein; DM, dry matter; EE, ether extract or fat; NE, net energy; RSD, residual standard deviation.

CP (–8.80). Similarly using AMEs as a predictor, NE is positively related to AMEs (0.798) and EE (18.4) and negatively related to CP (–14.2). The intercept was not significant in the equations to predict NE from AME, AMEs, or AMEn ($P > 0.05$). Also using multiple linear stepwise regression, the equations to predict the ratio of NE/AME were generated with significant intercepts in each case ($P < 0.001$). The equation shows the NE/AME ratio was positively related to EE (0.66) and negatively related to CP (–0.33) with an intercept of 76.7%; NE/AMEn ratio was positively related to EE (0.72) and negatively related to CP (–0.29) with an intercept of 78.9%; and NE/AMEs ratio was positively related to EE (0.71) and negatively related to CP (–0.45) with an intercept of 78.8%.

DISCUSSION

In the current study, digestible lysine and other essential amino acids were sufficient for maintenance and egg production since the body N balance was close to zero for all diets. Digestible lysine averaged 909 mg/bird/d and ranged from 777 to 1,108 mg/bird/d across treatments as compared to the Hy-Line recommended value of 780 mg/bird/d (Hy-Line, 2016). Recently, Pastore et al. (2018) reported a minimum requirement of standardized ileal digestible lysine for layers in peak production of 813 mg/bird/d. With regard to protein intake, it ranged from 11.8 to 21.3 g/bird/d, the 17.4 g/bird/d average value being quite close to the 17.0 g/bird/d Hy-Line (2016) recommendation. Although CP intake of several of the diets in the current study were below this Hy-Line recommendation, these diets were sufficient in digestible lysine and other essential amino acids for getting a zero N balance in body and maintaining egg production as they were formulated with inclusions of essential amino acids in lieu of intact protein. This situation was achieved despite an insufficient energy supply and a subsequent mobilization of body fat. As in the study of Roberts et al. (2007), our study indicates that a sufficient supply of essential amino acids despite a shortage of energy is able to maintain egg production in laying hens, at least on a short-term basis. This also suggests that depot fat can be easily mobilized to supplement dietary energy to maintain egg production when laying hens are in negative energy balance (Waring and Brown, 1967).

In the current study, an FHP value of 88 kcal/kg $BW^{0.75}$/d in layers was applied; this value originates from a previous study using Hy-Line Brown laying hens at 27 wk of age in an open-circuit respiratory calorimetric chamber system (Wu et al., 2016). This is close to the estimated FHP obtained by regressing RE with ME intake (90 kcal/kg $BW^{0.75}$/d; Table 6) in the present study. The values are also consistent with those reported in White Leghorn layers by Farrell (1975) (94 kcal/kg $BW^{0.75}$/d) but higher than those reported by Reid et al. (1978) (69 kcal/kg $BW^{0.75}$/d). Differences in genetics of birds, in methodological approaches, and in housing conditions may explain this variability. Nonetheless, this suggests that the FHP used in the current study for calculating the NE value of diets was rather accurate and representative. Apart from FHP, another indicator of energy requirement for maintenance is represented by the ME requirement for maintenance (MEm). The estimated MEm for the current experiment (120 kcal/kg $BW^{0.75}$/d, Model 1) is identical with the 120 kcal/kg $BW^{0.75}$/d value reported for Rhode Island Red laying hens (Jadhao et al., 1999) but slightly higher than the reported value of 112 kcal/kg $BW^{0.75}$/d in caged layers at 22°C (Sakomura, 2004) or the value that can be calculated from the other models applied to our data (105 and 100 kcal/kg $BW^{0.75}$/d according to Models 2 and 3, respectively; Table 6). Again, differences in methodologies, statistical models, genetics, and environment may explain these variations.

In the present study, the utilization of dietary AME intake for fat deposition was more efficient compared to being deposited as protein (100 vs. 50%). This corroborates with other research (Spratt et al., 1990) indicating fat deposition to be more energetically efficient compared to protein deposition. However, the 100% efficiency for fat is not fully relevant, probably in connection with the structure and characteristics of the data set including a depot of fat in eggs from both feed energy and body fat energy. A 90% efficiency of ME for fat energy gain (in eggs) would be more relevant. This is because in the specific case of laying hens being on a zero N balance and zero or negative fat balance in their body, the efficiencies obtained above for protein and fat correspond to efficiencies of deposition of fat and energy in the egg. Assuming then that 54 and 46% of egg energy are retained as fat and as protein, respectively, the overall efficiency of ME for egg energy gain would be about 72% (i.e., (90 × 54+50 × 46)/100)). In our study, the energy retained in egg averaged 54 kcal/kg $BW^{0.75}$/d. The corresponding ME requirement would then be about 75 kcal/kg $BW^{0.75}$/d. This should be added to the ME requirement for maintenance (105 kcal/kg $BW^{0.75}$/d) in order to calculate the total ME requirement of the laying hens in this study (about 180 kcal/kg $BW^{0.75}$/d). For a 2 kg laying hen, that is equivalent to 303 kcal ME per day or 110 g of a conventional feed containing 2,750 kcal ME per kg (as is). According to a rather comparable model of energy utilization in laying hens and using Hy-Line inputs for bird weight, egg production, age, and egg mass, the energy requirements were calculated as 305 and 288 kcal/bird/d at 28 and 64 wk (Sakomura, 2004). The average AME intake in the current study ranged from 252 to 284 kcal/bird/d (average 266 kcal/bird/d) across the dietary treatments. This lower AME intake of layers in the current study is likely due to reduced FI caused by the change in diet and respiration chamber environments, even though there was a 4-D adaptation period.

As expected, the present study showed that fat contributed highest to gross energy, followed by CP and starch as shown in Table 7. These results are consistent with reported values of energy-yielding nutrients in food or feed (WHO, 1985; Tran et al., 2004; Carré et al., 2013; Wu et al., 2019), confirming the quality of laboratory measurements and proximate analysis in the current study. Higher AME/GE ratios were observed in diets with high starch and low CP indicating starch to be efficiently absorbed and used as an energy source. The AME/GE ratios for EE, starch, and CP in the current study were 78, 95 and 60%, respectively. Those reported values were 113, 93, and 57% for the same nutrients in broilers by Wu et al. (2019) using similar methods and facilities. Murugesan et al. (2017) confirmed that due to the higher ability of broilers to utilize more energy from dietary supplemental lipid source, the AMEn/GE ratio of corn oil was 109% in broilers compared to 82% in layers. Logically, the highest AME/GE ratios in the present study were then observed in diets with high starch ($r = 0.705$, $P < 0.001$) and low CP ($r = -0.691$, $P < 0.001$). The positive coefficient values of starch and negative coefficient of CP were also confirmed in AME/GE prediction equation in our current study (Table 8). The EE and starch as predominating dietary nutrients for layers in AME prediction equation in the current experiment were confirmed in broilers (Wu et al., 2019). As in the study of Wu et al. (2019) in broilers, this suggests that EE and starch are more digestible than other energy containing components such as non-starch polysaccharides and other fibrous components in layers and broilers. It should be noted that the corresponding ratio calculated for CP is quite difficult to interpret since it includes both undigested and endogenous protein energy and urinary energy, the latter component being highly dependent on the protein supply as compared to protein gain.

The NE/AME of nutrients in the current study were 104, 78, and 49% for EE, starch, and CP, respectively. In broilers, the NE/AME efficiency ratios were 85, 79, and 50% for EE, starch, and CP, respectively, using a similar estimation method (Wu et al., 2019). The efficiencies of AME for NE from digestible EE, starch, and CP were reported to be 84, 78, and 68% in broilers (Carré et al., 2002). In growing pigs, the efficiencies of AME for NE were found to be 90, 82, and 60% for digestible EE, starch, and CP (Noblet et al., 1994). It is clear that NE/AME is positively affected by dietary fat and negatively by dietary CP in all these studies. Similarly, as for Wu et al. (2019) or Noblet et al. (1994), NE can be predicted from ME (or DE), fat, and CP contents. In addition, based on the recent prediction equations reported in broilers (Wu et al., 2019) and the equation developed for layers in the current study, it can be concluded that layers are more responsive than broilers to levels of dietary EE and CP levels for estimating NE. Thus, formulation on a NE basis may have greater benefit in layers than broilers.

The energy values of ingredients used in the 16 diets were estimated by multiple linear regression according to their inclusion rates (Table 10). The AME/GE were 82, 81, 62, and 79% for corn, wheat, soybean meal, and canola oil, respectively, in our current study. The efficiencies of GE for AME in the current study are similar to those reported previously for corn (85%), wheat (82%), and soybean meal (56%) (Barzegar et al., 2017) or those that can be calculated from table values (Rostagno et al., 2011). According to the same calculation method, the NE/AME ratios were 75, 74, 62, and 92%, respectively, for the corn, wheat, SBM, and canola oil used in the current study. These efficiency values show that evaluation of ingredients by a NE system compared to an AME system favors high EE ingredients and demotes high-protein ingredients for energy ranking. The NE prediction equations developed in our study (Table 9) confirm this.

The 2 diets used in the validation study used the NE prediction equations generated from the main study (Table 11). The 2 diets were rather extreme in terms of theoretical NE/ME ratios, one being low in CP and high in EE and the other, high in CP and low in

Table 10. Estimated energy values (kcal/kg DM) of layer feed ingredients.

Nutrients	Corn	Wheat	Soybean meal	Canola oil	RSD
Composition, % DM (measured)					
Protein	9.6	12.4	52.7	0	
Fat	3.1	1.7	2.0	99.9	
GE, kcal/kg DM	4,455	4,373	4,659	9,446[1]	
Energy values, kcal/kg DM[2]					
GE	4,922	4,782	4,173	14,666	77
AME	4,024	3,885	2,566	11,608	87
AMEs	3,955	3,854	2,734	11,255	96
AMEn	3,869	3,753	2,391	11,323	95
NE	3,008	2,854	1,579	10,640	130
NE predicted[3]	3,089	2,925	1455	10,712	
Energy efficiency, %					
AME/GE	81.8	81.2	61.5	79.1	
NE/AME %	74.7	73.5	61.5	91.7	
NE/AMEs %	76.1	74.0	57.8	94.5	
NE/AMEn %	77.7	76.0	66.0	94.0	

[1] Based on tabulated values (Rostagno et al., 2011).

[2] Estimated by multiple regression (zero intercept) of GE, AME, AMEs, AMEn, and NE values of diets (n = 16; kcal/kg DM) on the inclusion rates (DM/DM) of ingredients as corn, wheat, soybean meal, and canola oil in the main experiment (kcal/kg, DM) (n = 115 observations).

[3] NE predicted based on the equation 15 (Table 9) with estimated AME of each ingredients based on their inclusion rate in 16 diets.

Table 11. Ingredient and nutrient compositions of diets of the validation experiment (as-is).

Diet	High NE/AMEn	Low NE/AMEn
Ingredient, g/kg		
Corn	300	300
Wheat	327	298
Soybean meal	118	273
Canola oil	45	7
Others[1]	192	120
Supplemented amino acids[2]	17	2
Nutrients assayed, g/kg		
DM %	89	88
CP	138	194
EE	66	31
Starch	281	263
Nutrients calculated, g/kg		
Calcium	42	42
Phosphorus, available	4.0	4.0

[1] Others provided as (the average as-is, g/kg): 100 limestone, 16 dicalcium phosphate, 2.2 salt, 2.0 Na bicarbonate, 1.0 UNE vitamin and mineral premix, 0.6 choline 60%. Alpha cellulose and Celite added in High NE/AMEn diet both at 35 gr/kg. 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).

[2] Supplemental amino acids (In high NE/AMEn and low NE/AMEn diet as-is, g/kg): 3.4 D, L-methionine; 4.1 L-lysine HCL, ; 2.1 L-threonine, 99%; 0.3 L-tryptophan; 2 L-isoleucine; 3 L-arginine; 2 L-valine. Low NE/AMEn diet added with only 2 g/kg D, L-methionine.

Table 12. Effect of diet composition on performance and energy utilization in layers; comparison of measured and calculated energy values.[1]

Diet	High NE/AMEn	Low NE/AMEn	SEM	*P*-value
Energy balance, kcal/kg $BW^{0.75}$***/d***				
AME intake	174	163	3	0.044
HP[2]	132	137	1	0.040
HI	43	49	1	0.040
RE[3]				
Total	42	26	3	0.002
As protein	25	28	1	0.010
As fat	18	-2	3	< 0.001
RQ[4]	0.986	0.982	0.005	0.754
Energy values (kcal/kg DM; measured)[5]				
AME	3121	2946	24	< 0.001
AMEn	3019	2829	25	< 0.001
AMEs	3120	2974	20	< 0.001
NE	2346	2070	40	< 0.001
Predicted NE[6]	2389	2117		
NE/AME % (measured energy values)	75.2	70.3	0.1	0.002
NE/AME % (predicted energy values)[6]	76.5	71.8		

[1] Two diets, 8 replicates per diet in a single run with 16 calorimetry chambers containing 3 laying hens in each; the same ingredients as in the main experiment were used. The birds in this experiment were the same young birds used in the main experiment at 40 wk of age and average BW = 2,036 g. The trial procedure and measurements of ME, heat production (HP), and dietary nutrients followed the same protocol as have been described in the main experiment.

[2] HP, heat production; heat production (HP) (kcal) = 3.866 × oxygen consumed (L) + 1.200 × CO_2 expired (L) Brouwer (1965) equation.

[3,4] Total retained energy (RE) calculated as ME intake – HP as (kcal/kg $BW^{0.75}$/d); RE as protein calculated as total retained N × 6.25 × 5.7 as (kcal/kg $BW^{0.75}$/d); RE as fat calculated as total RE – RE as protein (kcal/kg $BW^{0.75}$/d). RQ, respiratory quotient.

[5] Based on measured values in respiration chambers (n = 8 per dietary treatment).

[6] NE predicted based on the equation 15 (Table 9) with measured AME.

EE. Eight replicates per diet in a single run with the 16 calorimetry chambers were conducted with 3 laying hens per chamber; the same ingredients as in the main experiment were used (Table 12). The experimental procedure and measurements of ME, HP, HI, and NE followed the same protocol as described in the main study. The results indicate that the partition of ME intake between HP, HI, and retention of energy in this validation study is comparable to the values measured in the main experiment performed for calculating the prediction equations. The NE/AME values differed significantly between the diets, being markedly higher in the one with low CP and high EE. In connection with differences in AME content and in efficiencies of AME for NE, the difference in NE content of the 2 diets was accentuated, which justifies the use of an NE system for laying hens, especially if the diets have rather unconventional composition. Finally, the application of equation 15 (Table 9) to the measured AME values generates calculated NE values of the 2 diets that are quite close to the measured ones and with the same hierarchy. Also, the comparison between measured and predicted NE/AME ratios showed similar rankings.

In conclusion, the main study measured energy partition of diets in layer hens and generated prediction equations for AME, NE, and corresponding efficiencies. By using NE prediction equations, the NE content of feedstuffs and compound feeds can be estimated according to their levels of AME, EE, and CP. This gives an opportunity for layer nutritionists to formulate feeds based on NE rather than AME. A validation study was conducted using the generated equations to compute NE content of major ingredients used. The results confirmed quality and applicability of the prediction equations. Additional NE validation experiments and commercial studies are recommended using the new NE-based formulation system to assess applicability benefit to the industry.

ACKNOWLEDGMENTS

The authors acknowledge Poultry Hub Australia and the Australian Eggs for supporting this study. Also, we acknowledge Shuyu Song and Nikki Yue Zhang for their technical support.

REFERENCES

Annison, G., R. Hughes, and M. Choct. 1996. Effects of enzyme supplementation on the nutritive value of dehulled lupins. Br. Poult. Sci. 37:157–172.

Annison, E., and R. White. 1961. Glucose utilization in sheep. J. Agric. Sci. 80:1222–1234.

AOAC. 2016. Official Methods of Analysis of AOAC International. 20th ed. Rockville, MD, USA.

Armsby, H. P., and J. A. Fries. 1915. Net energy values of feeding stuffs for cattle. J. Agric. Res. 3:435–491.

Barzegar, S., S. B. Wu, and R. A. Swick. 2017. Metabolizable energy of ingredients in peak layers. Aust. Poult. Sci. Symp. 28:200.

Bourdillon, A., B. Carré, L. Conan, J. Duperray, G. Huyghebaert, B. Leclercq, M. Lessire, J. McNab, and J. Wiseman. 1990. European reference method for the in vivo determination of metabolisable energy with adult cockerels: reproducibility, effect of food intake and comparison with individual laboratory methods. Br. Poult. Sci. 31:557–565.

Brouwer, E. 1965. Report of sub-committee on constants and factors. In Proceedings of the 3rd Symposium on Energy Metabolism. K. L. Blaxter. ed. Academic Press, London, UK.

Carré, B., M. Lessire, and H. Juin. 2002. Development of the net energy system for broilers. Proc. East. Nutr. Conf. 38:140–149.

Carré, B., M. Lessire, and H. Juin. 2013. Prediction of metabolisable energy value of broiler diets and water excretion from dietary chemical analyses. Animal. 7:1246–1258.

Carré, B., M. Lessire, and H. Juin. 2014. Prediction of the net energy value of broiler diets. Animal. 8:1395–1401.

Chudy, A., W. Souffrant, S. Kuhla, and H. Peters. 2003. Energy and protein (AA) metabolism of high productive laying hens in dependence on exogenous factors. Publ. Eur. Assoc. Anim. Prod. 109:339–344.

Cozannet, P., M. Lessire, C. Gady, J. Metayer, Y. Primot, F. Skiba, and J. Noblet. 2010. Energy value of wheat dried distillers grains with solubles in roosters, broilers, layers, and turkeys. Poult. Sci 89:2230–2241.

De Groote, G. 1974. A comparison of a new net energy system with the metabolisable energy system in broiler diet formulation, performance and profitability. Br. Poult. Sci. 15:75–95.

Englyst, H., and G. Hudson. 1987. Colorimetric method for routine measurement of dietary fibre as non-starch polysaccharides. A comparison with gas-liquid chromatography. Food. Chem. 24:63–76.

Farrell, D. 1975. A comparison of the energy metabolism of two breeds of hens and their gross using respiration calorimetry. Br. Poult. Sci. 16:103–113.

Hill, F., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64:587–603.

Hy-Line. 2016. Hy-Line Brown commercial layers 2016 - Management Guide. Accessed 16 July 2019. http://www.specialisedbreeders.com.au/wp-content/uploads/2016/04/BRN-COM-AUS.pdf.

Jadhao, S., C. Tiwari, and M. Khan. 1999. Energy requirement of Rhode Island red hens for maintenance by slaughter technique. Asian-Australas. J. Anim. Sci. 12:1085–1089.

Liu, W., C. H. Lin, Z. K. Wu, G. H. Liu, H. J. Yan, H. M. Yang, and H. Y. Cai. 2017. Estimation of the net energy requirement for maintenance in broilers. Asian-Australas. J. Anim. Sci. 30:849.

McLean, J. 1972. On the calculation of heat production from open-circuit calorimetric measurements. Br. J. Nutr. 27:597–600.

Miranda, J. M., X. Anton, C. Redondo-Valbuena, P. Roca-Saavedra, J. A. Rodriguez, A. Lamas, C. M. Franco, and A. Cepeda. 2015. Egg and egg-derived foods: effects on human health and use as functional foods. Nutrients. 7:706–729.

Murugesan, G. R., B. J. Kerr, and M. E. Persia. 2017. Energy content of select dietary supplemental lipids for broilers, turkeys, and laying hens1. J. Appl. Poult. Res. 26:536–547.

NHMRC. 2013. Australian code of practice for the care and use of animals for scientific purposes. Australian Government Publ. Service. Canberra, ACT, Australia.

Ning, D., Y. Guo, Y. Wang, and Y. Peng. 2013. Earlier metabolizable energy intake level influences heat production during a following 3-day fast in laying hens. Asian-Australas. J. Anim. Sci. 26:558.

Ning, D., J. Yuan, Y. Wang, Y. Peng, and Y. Guo. 2014. The net energy values of corn, dried distillers grains with solubles and wheat bran for laying hens using indirect calorimetry method. Asian-Australas. J. Anim. Sci. 27:209.

Noblet, J., H. Fortune, X. Shi, and S. Dubois. 1994. Prediction of net energy value of feeds for growing pigs. J. Anim. Sci. 72:344–354.

Pastore, S. M., P. C. Gomes, G. da Silva Viana, E. A. da Silva, W. P. de Oliveira, L. V. S. Barbosa, A. Z. Fraga, and W. J. Alves. 2018. Standardized ileal digestible lysine requirement of white commercial layers in peak egg production. Biosci. J. 34:186–193.

Pirgozliev, V., and S. Rose. 1999. Net energy systems for poultry feeds: a quantitative review. World's. Poult. Sci. J. 55:23–36.

Reid, B., M. Valencia, and P. M. Maiorino. 1978. Energy utilization by laying hens I. Energetic efficiencies of maintenance and production. Poult. Sci. 57:461–465.

Roberts, S. A., H. Xin, B. J. Kerr, J. R. Russell, and K. Bregendahl. 2007. Effects of dietary fiber and reduced crude protein on nitrogen balance and egg production in laying hens. Poult. Sci. 86:1716–1725.

Rostagno, H. R., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. d. Olveira, D. C. Lopes, A. S. Firiera, and S. L. Barreto. 2011. Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements. Universidade Federal de Viçosa, Departamento de Zootecnia.

Sakomura, N. K. 2004. Modeling energy utilization in broiler breeders, laying hens and broilers. Rev. Brasil. Ciênc. Avíc. 6:1–11.

SAS. 2010. SAS users guideSAS Institute, Cary, NC.

Schiemann, R., K. Nehring, L. Hoffmann, W. Jentsch, and A. Chudy 1972. Energetische Futterbevertung und Energienormen. VEB Deutscher Landwirtschatsverlag. Berlin.

Sibbald, I. 1979. The gross energy of avian eggs. Poult. Sci. 58:404–409.

Spratt, R., H. Bayley, B. McBride, and S. Leeson. 1990. Energy metabolism of broiler breeder hens. 1. The partition of dietary energy intake. Poult. Sci. 69:1339–1347.

Swick, R. A., S.-B. Wu, J. Zuo, N. Rodgers, M. R. Barekatain, and M. Choct. 2013. Implications and development of a net energy system for broilers. Anim. Prod. Sci. 53:1231–1237.

Theander, O., and E. Westerlund. 1993. Determination of individual components of dietary fiber. Pages 77–98 in Dietary Fiber in Human Nutrition. G. A. Spiller, ed. CRC Press, Boca Raton, FL.

Tran, G., D. Sauvant, and J. Pérez. 2004. Chemical Data and Nutritional Value. Pages 17–24. Wageningen Academic Publishers, Wageningen, The Netherlands.

Waring, J., and W. Brown. 1967. Calorimetric studies on the utilization of dietary energy by the laying White Leghorn hen in relation to plane of nutrition and environmental temperature. J. Agric. Sci. 68:149–155.

WHO. 1985. Energy and protein requirements: report of a joint FAO/WHO/UNU expert consultation. Pages 1–67. WHO, Geneva, Switzerland.

Wu, S., Y. Huaming, B. Zhibin, Y. Xiaogang, and Z. Yumin. 2016. Heat production estimated from fasting layer hens at peak lay. Pages 197 in World's. Poult. Congr. 25, Beijing, China.

Wu, S.-B., R. A. Swick, J. Noblet, N. Rodgers, D. Cadogan, and M. Choct. 2019. Net energy prediction and energy efficiency of feed for broiler chickens. Poult. Sci. 98:1222–1234.