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Metabolizable and Net Energy Values of Expanded Cottonseed Meal for Laying Hens and Broiler Chickens

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Three experiments were conducted to determine the metabolizable energy (ME) and net energy (NE) values of expanded cottonseed meal (ECSM) for broilers aged 14–16 days (Experiment 1), broilers aged 28–30 days (Experiment 2), and 45-week-old Hy-Line Brown hens (Experiment 3). Reference diets based on corn-soybean meal were used to meet the nutritional needs of the birds. The test diets contained ECSM as basis, which was used to replace 18.5% of the gross energy-yielding ingredients from the reference diet. The birds were fed a commercial feed before the experimental period. After the dietary adaptation period, six birds per replicate (Experiment 1) and two birds per replicate (Experiments 2 and 3) for each treatment group were placed in an individual open-circuit respiratory calorimetry chamber for 3 days. Daily O_2 consumption and CO_2 production were recorded, and excreta samples were collected. The ME and NE values of ECSM were determined using the substitution method. The apparent metabolizable energy (AME) values of ECSM for experiments 1, 2, and 3 were 2605.85, 2178.31, and 2782.60 kcal/kg of dry matter (DM), respectively. The NE values were 1655.23, 1196.64, and 1538.19 kcal/kg of DM, respectively. The NE:AME ratios of ECSM were 63.52%, 54.93%, and 55.29%, respectively. Our data showed that the ME and NE values of ECSM differed across various growth stages and types of chickens. These results demonstrate that the appropriate ME and NE should be used in the design of different feed formulas for specific growth stages and types of chickens.

Key words: broilers, expanded cottonseed meal, laying hens, metabolizable energy, net energy

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Introduction

Accurate evaluation of the effective energy of feed is important for the rational selection of feed raw materials, optimization of the feed formula, and reduction of feed cost. It is worth mentioning that different types of chickens at different physiological stages have different digestive and metabolic statuses. This causes differences in nutrient digestion and utilization of energy from the feed and affects the amount of available energy of feedstuffs. The metabolizable energy (ME) system is widely used in poultry nutrition, but it ignores the contribution of animal heat increment in the processes of feeding, digestion, and metabolism. Previous studies have shown that crude protein triggers the highest heat increment, and fat provokes the lowest heat increment (Hill and Anderson, 1958). Therefore, ME quantification often overestimates the effective energy of a high-protein feed (Carré and Juin, 2015). The calculation of net energy (NE) is based on the deduction of the heat increment component from the value of ME. Therefore, NE is more accurate than ME in reflecting the nutritional value of a feed, especially when evaluating protein feedstuffs. In China, cottonseed meal (CSM) is a commonly used protein feedstuff. However, it contains anti-nutritional factors, such as gossypol, which limit its application. Gossypol can be divided into free gossypol and bound gossypol according to its chemical status (Henry *et al*., 2001; Mena *et al*., 2001). CSM can be expanded to create a byproduct feed referred to in this paper as expanded cottonseed meal (ECSM). In this experiment, the processing parameters of ECSM were a screw speed of 150 rpm, an expansion temperature of 120℃, and a moisture content of CSM of 24%. The results showed that the expansion procedure could eliminate free gossypol and other harmful sub-

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stances of cottonseed. At the same time, proteins and starch in the raw materials could isomerize well, and the palatability of CSM was improved significantly, making it easier for animals to digest and utilize (Noftsger *et al*., 2000; Buser and Abbas, 2002). However, the lack of sufficient studies on the effective energy value of ECSM in poultry limits its proper usage. In this study, the ME and NE values of ECSM were determined by conducting three experiments in chickens of different ages and types.

Materials and Methods

ECSM and Diets

ECSM was obtained from the Xinjiang Province, China. The reference diet consisted of a corn-soybean meal and met the nutritional needs of the animals. The test diets contained ECSM, which was used to replace 18.5% of the gross energy (GE)-yielding ingredients from the reference diet (as basis). This substitution ratio corresponds to that used by other Chinese researchers working on CSM. The addition of vitamins, minerals, and other non-energy ingredients to the reference and test diets was adjusted for consistency. The birds had ad libitum access to food and water. The moisture content of each ingredient was determined during the preparation of the feed to calculate its contribution to each diet on a dry matter (DM) basis. The ingredients and chemical composition of the diets used in the experiments are presented in Table 1.

Equipment

An open-circuit respiratory calorimetry instrument of approximately 0.54 m^3 was used in this study. This instrument can control temperature and humidity and collect gas on a regular basis to calculate poultry O_2 consumption and CO_2 production. Inside of it, a cage of 70 cm×55 cm×70 cm was placed. The cage was equipped with nipple drinkers and feeders.

Animals and Experimental Design

The study protocol was approved by the Animal Ethics Committee of China Agricultural University (AW02501202-2-1) and the study was conducted in accordance with the guidelines of the Guide for the Care and Use of Agricultural Animals in Research and Teaching.

The number of chickens for each replicate of the experiment was determined according to bird size, the capacity of the chamber, and the concentration of CO_2 in the chamber. Birds were selected at approximately equal body weights and randomly assigned to each of the treatment groups in a fully randomized design. During the experimental period, feed intake (FI) was measured, daily O_2 consumption and CO_2 production were recorded, and total excreta were collected. Feed spillage was measured from the under-cage collection tray and subtracted from FI. Feathers were removed from the collected excreta.

For Experiment 1, Arbor Acres broilers were purchased from a commercial hatchery. Broilers were raised according to a management handbook (Aviagen, 2019) and reared in pens until 10 days of age. On the 11th day, 72 male broilers weighing 300±15 g were randomly transferred into each of the 12 respiration chambers for adaptation to feed and the environment. Each diet was administered to broilers in six replicate cages for a 3-day adaptation period followed by a 3-day experimental period (Liu *et al*., 2017). Feed and water were freely available. The lighting cycle was 20 h light: 4 h dark; lighting was switched on at 04:00 and off at 24:00. During the experimental period, FI was measured, daily O_2 consumption and CO_2 production were recorded, and total excreta were collected. ME and NE values were recorded from day 14 to day 16.

For Experiment 2, Arbor Acres broilers were purchased from a commercial hatchery. Broilers were raised according to a management handbook (Aviagen, 2019) and reared in pens until 24 days of age. On the 25th day, 24 male broilers weighing 1,200±60 g were randomly transferred into each of the 12 respiration chambers for adaptation to feed and the environment. Each diet was administered to broilers in six replicate cages for a 3-day adaptation period followed by a 3-day experimental period (Liu *et al*., 2017). Feed and water were freely available. The lighting cycle was 20 h light: 4 h dark; lighting was switched on at 04:00 and off at 24:00. During the experimental period, FI was measured, daily O_2 consumption and CO_2 production were recorded, and total excreta were collected. ME and NE values were recorded from day 28 to day 30.

For Experiment 3, Hy-Line Brown pullets were purchased from a commercial hatchery. Layers were raised according to a management handbook (Hy-Line, 2018) and reared in pens until 45 weeks of age, when they laid at a 91% hen/day production. Twenty-four Hy-Line Brown hens were randomly transferred into each of the 12 respiration chambers for adaptation to feed and the environment. Each diet was administered to hens in six replicate cages for a 7-day adaptation period followed by a 3-day experimental period (Ning *et al*., 2014; Barzegar *et al*., 2019). The lighting cycle was 16 h light: 8 h dark; lighting was switched on at 04:00 and off at 20:00. During the experimental period, FI was measured, daily O_2 consumption and CO_2 production were recorded, and total excreta were collected.

Analyses of Ingredients, Diets, and Excreta

Feed refusals and spillage were collected daily and analyzed for their DM content. For each diet, a feed sample was collected during the experimental period, and its DM content was measured and subsequently used for chemical analyses. Daily excreta samples were stored in a refrigerator at 4℃. At the end of each period, the excreta collected from each respiratory chamber over 3 days were mixed and dried in an oven at 65℃ for 72 h. Excreta were left at room temperature, crushed, and then screened. Excreta samples were analyzed for their crude protein (CP), ether extract (EE), crude fiber, ash, neutral detergent fiber, and acid detergent fiber content (AOAC, 2016). The GE contained in the test ingredients, feeds, and excreta was measured using a bomb calorimeter (C2000, IKA, Guangzhou, China) using benzoic acid as a standard. The chemical characteristics of ECSM are shown in Table 2.

Calculations

Heat production (HP) was calculated using the Brouwer

Table 1. **Ingredients and chemical composition of the diets used in the study (in %)**

Item	Reference diet			Test diet		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
Ingredient						
Corn	60.82	59.35	61.20	49.57	48.37	49.88
Soybean meal	21.42	20.60	21.50	17.46	16.79	17.52
Corn gluten meal	2.60	2.00	3.30	2.12	1.63	2.69
Peanut meal	3.00	4.00		2.45	3.26	
Soybean oil	4.50	6.00	1.00	3.67	4.89	0.82
DDGS	3.00	4.00		2.45	3.26	
Expanded cottonseed meal (ECSM)				17.64	17.75	16.10
$CaHPO_4$	1.14	0.70		1.14	0.70	
70% L-Lys, HCl	1.00	0.96		1.00	0.96	
Sodium chloride	0.25	0.24		0.25	0.24	
Sodium humic acid	0.20	0.20		0.20	0.20	
D, L-methionine	0.25	0.24		0.25	0.24	
L-threonine	0.14	0.15		0.14	0.15	
Choline chloride (60%)	0.11	0.11		0.11	0.11	
Baking soda	0.12	0.01		0.12	0.01	
Treasure fine salt	0.02	0.02		0.02	0.02	
Limestone	0.90	0.92	8.00	0.90	0.92	8.00
Compound premix (0.5%)[a)]	0.50	0.50		0.50	0.50	
Compound premix (5.0%)[b)]			5.00			5.00
L- tryptophan	0.03			0.03		
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient levels (calculated in %)						
Crude protein	20.31	20.00	17.00			
Moisture	11.63	11.58	12.33			
Crude fat	6.83	8.39	3.73			
Crude fiber	2.38	2.47	2.40			
NDF	8.46	8.75	9.66			
Starch	40.45	39.55	40.98			
Ca	0.71	0.61	3.80			
Total P	0.53	0.46	0.42			
Methionine	0.57	0.56	0.42			
Lysine	1.30	1.27	0.81			
Threonine	0.90	0.90	0.66			

[a)] Provided per kilogram of diet: Vitamin A, 10,000 IU; vitamin D3, 2,500 IU; vitamin E (DL-α-tocopheryl acetate), 20 IU; vitamin K3, 3 mg; thiamine hydrochloride, 0.01 mg; riboflavin, 8.00 mg; pyridoxine hydrochloride, 4.5 mg; vitamin B12, 0.02 mg; nicotinic acid, 34 mg; calcium pantothenate, 12 mg; folic acid, 0.5 mg; biotin, 0.2 mg; choline chloride, 1,200 mg; Fe, 80 mg; Cu, 8 mg; Zn, 80 mg; Mn, 80 mg; I, 0.7 mg; Se, 0.3 mg.
[b)] Provided per kilogram of diet: D, L-methionine, 2.8 g; L-lysine HCl (78.4%), 0.15 g; vitamin A, 20,000 IU; vitamin D3, 6,000 IU; vitamin E (DL-a-tocopheryl acetate), 60 mg; vitamin K3, 3 mg; thiamine B1, 4.6 mg; riboflavin B2, 15 mg; pyridoxine B6, 10.6 mg; vitamin B12, 0.04 mg; nicotinic acid, 90 mg; pantothenic acid, 24 mg; folic acid, 2.0 mg; biotin, 0.4 mg; Fe, 200 mg; Cu, 40 mg; Zn, 200 mg; Mn, 240 mg; Se, 0.6 mg.
Abbreviations: Expt. 1, broilers aged 14–16 days; each treatment consisted of six replicates with six broilers each; Expt. 2, broilers aged 28–30 days; each treatment consisted of six replicates with two broilers each; Expt. 3, 45-week-old Hy-Line Brown hens; each treatment consisted of six replicates with two hens each; DDGS, Dried Grains with Solubles; NDF, neutral detergent fiber.

equation (Brouwer, 1965):

$$\text{HP (kcal)} = 3.866 \times VO_2\ (L) + 1.200 \times VCO_2\ (L)$$

where VO_2 is the volume of O_2 consumed (L) and VCO_2 is the volume of CO_2 exhaled (L).

The retained energy (RE) was calculated as ME intake (MEI) minus HP.

The AME, AME corrected for zero nitrogen retention (AMEn), and NE of the experimental diets were determined using the following equations:

$$\text{AME (kcal/kg of DM)} = [(FI \times GEd) - (E \times GEe)] / FI$$

$$\text{AMEn (kcal/kg of DM)} = AME - 8.22 \times (Ni - Ne) / FI$$

$$\text{NE (kcal/kg of DM)} = (RE + FHP) / FI$$

where FI is the feed intake (kg of DM); E is the excreta output (kg of DM); GEd is the gross energy of the diet (kcal/kg of DM); GEe is the gross energy of the excreta (kcal/kg of DM); 8.22 is the nitrogen correction factor for each gram of nitrogen retained in body and eggs (kcal/g; Hill *et al*., 1958); Ni is the nitrogen intake from the diet (g/day); Ne is the nitrogen output through the excreta (g/day); RE is the retained energy and FHP is the fasting heat production. An FHP value of 450 kJ/BW$^{0.70}$ per day per bird was used for broilers (Noblet *et al*., 2015) and an FHP value of 370 kJ/BW$^{0.75}$ per day per bird was used for layers (Wu *et al*., 2016).

The values of AME, AMEn, and NE of the test ingredients were calculated according to the difference method (Barzegar *et al*., 2019).

AMEingr (kcal/kg of DM)=(AMEtest−AMEref×a%) / b%.

NEingr (kcal/kg of DM)=(NEtest−NEref×a%) / b%.

where a% is the contribution of the energy-yielding ingredients from the reference diet in the test diet and b% is the substitution level of the ingredients in the test diet. All the ingredients in the diets were converted to DM in order to express the a and b values on a DM basis. The energy-yielding ingredients in the reference and test diets included grains, meals, oils, and amino acids.

The respiratory quotient (RQ) of the birds was calculated as the ratio of the CO_2 volume exhaled to the O_2 volume consumed by the birds. The heat increment (HI) was calculated by subtracting the FHP from the HP.

Statistical Analyses

The data of production performance, nitrogen balance, and energy metabolism during the 3-day experimental period were analyzed by *t*-test and one-way ANOVA using SPSS statistical software. Differences between treatments were examined using the Tukey-Kramer test and were considered significant at $P<0.05$.

Table 2. **Chemical characteristics of expanded cottonseed meal (ECSM) on a dry matter basis**

Item	Value
Crude fat (%)	1.08
Crude protein (%)	54.11
Ash (%)	10.34
Crude fiber (%)	11.23
NDF (%)	39.11
ADF (%)	14.67
Gross energy (kcal/kg)	4702.96

Abbreviations: NDF, neutral detergent fiber; ADF, acid detergent fiber.

Results

Experiment 1

The AME, AMEn, and NE values of ECSM were determined as 2605.85, 2245.19, and 1655.23 kcal/kg of DM, respectively (Table 3). The AME:GE and NE:AME ratios were 55.39% and 63.52%, respectively.

The test diet containing ECSM significantly increased the body weight gain and Feed conversion ratio (FCR) of broilers ($P<0.01$) and significantly decreased their RQ ($P<0.01$). Nitrogen intake, excretion, and retention were also affected by the test diet. The nitrogen retained in the birds during Experiment 1 was 1.60 and 2.01 g per bird per day (Table 4). The effects of different diets on energy metabolism in Experiment 1 are shown in Table 5. To correct for the effect of body weight on energy metabolism, the energy metabolism data reported in the table were converted into metabolic body weight (BW$^{0.70}$) consumption. The two diets (reference and test) had no significant effects on GE intake (GEI), AME intake (AMEI), NE intake (NEI), total heat production (THP), and HI in broilers. The energy retained as protein was significantly higher in the test diet group than in the reference diet group ($P<0.01$), while the energy retained as fat was significantly lower in the test diet group than in the reference diet group ($P<0.01$).

Experiment 2

The AME, AMEn, and NE values of ECSM were determined as 2178.31, 1841.53, and 1196.64 kcal/kg of DM, respectively (Table 3). The AME:GE and NE:AME ratios were 46.32% and 54.93%, respectively.

The test diet increased body weight gain ($P<0.05$), but the average body weight, FI, and RQ were not affected (Table 6). The test diet significantly increased the level of nitrogen metabolism in the broilers ($P<0.01$), which showed the same pattern as that observed in Experiment 1. The energy balance data in Table 7 were converted to metabolic body weight, as those from Experiment 1. The GEI of the metabolic body weight was not influenced by the test diet, but AMEI and NEI were higher ($P<0.05$) in broilers fed the test diet than in those fed the reference diet. The RE and REfat values were significantly higher ($P<0.05$) in broilers fed the control diet than in

Table 3. **Energy value and conversion efficiency of expanded cottonseed meal (ECSM) observed in the study (on a dry matter basis)**

Item	Expt. 1	Expt. 2	Expt. 3	SEM	P-value
AME (kcal/kg)	2605.85^{a}	2178.31^{b}	2782.60^{a}	78.32	0.001
AMEn (kcal/kg)	2245.19^{b}	1841.53^{c}	2629.74^{a}	99.15	0.001
NE (kcal/kg)	1655.23^{a}	1196.64^{b}	1538.19^{a}	55.81	<0.001
AME/GE (%)	55.39^{a}	46.32^{b}	59.18^{a}	1.66	0.001
NE/AME (%)	63.52^{a}	54.93^{b}	55.29^{b}	1.41	0.010

$^{a–c}$ Different letters indicate significant differences between the means at $P<0.05$.

Abbreviations: Expt. 1, broilers aged 14–16 days; each treatment consisted of six replicates with six broilers each; Expt. 2, broilers aged 28–30 days; each treatment consisted of six replicates with two broilers each; Expt. 3, 45-week-old Hy-Line Brown hens; each treatment consisted of six replicates with two hens each; AME, apparent metabolizable energy; AMEn, AME corrected for zero nitrogen retention; NE, net energy; GE, gross energy; SEM, standard error of the mean ($n=6$).

Table 4. **Effect of diet composition on performance and nitrogen balance in Experiment 1**

Item	Reference diet	Test diet	SEM	P-value
Performance				
Body weight (g)	567.56	598.43	5.29	0.011
Feed intake (g of DM/d)	70.49	74.55	0.94	0.059
Body weight gain (g/d)	57.76	68.68	3.75	<0.001
Feed conversion ratio (g/g of DM)	1.27	1.09	0.02	<0.001
Respiratory parameters				
VO_2 (L/d/kg $BW^{0.70}$)	42.19	43.43	0.46	0.319
VCO_2 (L/d/kg $BW^{0.70}$)	41.36	40.42	0.37	0.255
Respiratory quotient (L/L)	0.98	0.93	0.01	<0.001
Nitrogen intake (g/d)	2.53	3.39	0.08	<0.001
Nitrogen excretion (g/d)	0.93	1.39	0.04	<0.001
Nitrogen retention (g/d)	1.60	2.01	0.05	<0.001

Abbreviations: DM, dry matter; Expt. 1, broilers aged 14–16 days; each treatment consisted of six replicates with six broilers each; SEM, standard error of the mean (n=6).

Table 5. **Effect of different diets on energy metabolism in Experiment 1**

Item	Reference diet	Test diet	SEM	P-value
Energy balance (kcal/kg $BW^{0.70}$/d)				
THP	212.66	216.32	2.13	0.492
HI	105.18	108.84	2.13	0.492
RE	154.82	142.35	4.48	0.261
RE as protein	84.53	102.32	2.28	<0.001
RE as fat	70.29	40.03	4.42	0.003
GEI	490.63	495.97	4.58	0.641
AMEI	367.48	358.67	3.86	0.358
NEI	262.31	249.83	4.48	0.261
Available energy (kcal/kg of DM)				
AME	3508.71	3360.62	0.12	0.024
AMEn	3496.76	3343.90	0.11	0.003
NE	2503.15	2340.73	0.15	0.060
Efficiency (%)				
AME/GE	74.93	72.35	0.57	0.063
NE/AME	71.28	69.62	0.68	0.323

Abbreviations: DM, dry matter; Expt. 1, broilers aged 14–16 days; each treatment consisted of six replicates with six broilers each; THP, total heat production; HI, heat increment; RE, retained energy; GEI, gross energy intake; AMEI, apparent metabolizable energy intake; NEI, net energy intake; AME, apparent metabolizable energy; AMEn, AME corrected for zero nitrogen retention; NE, net energy; GE, gross energy; SEM, standard error of the mean (n=6).

those fed the ECSM test diet, while the opposite was true for the REprotein value. The AME, AMEn, NE, and AME:GE ratio values were higher ($P<0.01$) in broilers fed the reference diet than in those fed the test diet, while no difference was observed for the NE:AME ratio.

Experiment 3

The AME, AMEn, and NE values of ECSM for laying hens were determined as 2782.60, 2629.74, and 1538.19 kcal/kg of DM, respectively (Table 3). The AME:GE and NE:AME ratios were 59.18% and 55.29%, respectively.

As shown in Table 8, unlike in broilers, the test diet had no significant effect on the HDP, FCR, VO_2, VCO_2, and RQ of laying hens ($P>0.05$), but FI was higher for hens on the test diet ($P<0.05$). Nitrogen intake and excretion were affected by the test diet, while this had no effect on nitrogen retention.

The test diet affected GEI ($P<0.05$) but had no effect on AMEI, NEI, THP, and RE ($P>0.05$). Similar to broilers, the test diet influenced the form of energy retained in laying hens and significantly increased the amount of energy retained as protein ($P<0.05$; Table 9). The AME:GE ratio was higher ($P<0.01$) in hens fed the reference diet than in those fed the test diet, but there were no differences in the NE:AME ratio.

Discussion

The results of the present study showed differences in ME, NE, and energy conversion efficiency in broilers and layers of different age groups. The resulting AME and AMEn values of ECSM in layers were higher than those in broilers, and the

Table 6. **Effect of diet composition on performance and nitrogen balance in Experiment 2**

Item	Reference diet	Test diet	SEM	P-value
Performance				
Body weight (g)	1659.75	1694.21	17.12	0.347
Feed intake (g of DM/d)	133.45	137.35	1.15	0.101
Body weight gain (g/d)	104.97	115.14	6.46	0.016
Feed conversion ratio (g/g of DM)	1.27	1.19	0.03	0.101
Respiratory parameters				
VO_2 (L/d/kg $BW^{0.70}$)	37.49	38.37	0.53	0.444
VCO_2 (L/d/kg $BW^{0.70}$)	37.49	36.47	0.31	0.115
Respiratory quotient (L/L)	1.00	0.95	0.01	0.055
Nitrogen intake (g/d)	4.90	6.22	0.17	<0.001
Nitrogen excretion (g/d)	1.75	2.58	0.11	<0.001
Nitrogen retention (g/d)	3.16	3.65	0.07	<0.001

Abbreviations: DM, dry matter; Expt. 2, broilers aged 28–30 days; each treatment consisted of six replicates with two broilers each; SEM, standard error of the mean (n=6).

Table 7. **Effect of different diets on energy metabolism in Experiment 2**

Item	Reference diet	Test diet	SEM	P-value
Energy balance (kcal/kg $BW^{0.70}$/d)				
THP	189.85	192.02	2.25	0.656
HI	82.37	84.54	2.25	0.656
RE	158.04	132.29	4.56	0.002
RE as protein	78.97	89.81	1.64	<0.001
RE as fat	79.07	42.48	5.30	<0.001
GEI	453.09	453.63	3.38	0.941
AMEI	347.89	324.31	4.43	0.005
NEI	265.53	239.77	4.56	0.002
Available energy (kcal/kg of DM)				
AME	3714.12	3413.17	0.18	<0.001
AMEn	3520.65	3195.81	0.19	<0.001
NE	2835.15	2524.64	0.20	<0.001
Efficiency (%)				
AME/GE	76.78	71.49	0.81	<0.001
NE/AME	76.31	73.96	0.68	0.094

Abbreviations: DM, dry matter; Expt. 2, broilers aged 28–30 days; each treatment consisted of six replicates with two broilers each; THP, total heat production; HI, heat increment; RE, retained energy; GEI, gross energy intake; AMEI, apparent metabolizable energy intake; NEI, net energy intake; AME, apparent metabolizable energy; AMEn, AME corrected for zero nitrogen retention; NE, net energy; GE, gross energy; SEM, standard error of the mean (n=6).

ME value was also greater in Experiment 1 (using broilers aged 14–16 days) than in Experiment 2 (using broilers aged 28–30 days). However, the NE value of ECSM in layers was lower than that in 14- to 16-day-old broilers. Thus, the different physiological stages and types of chickens affect the digestion and utilization of the nutrients included in the feed, thereby determining its digestion and utilization. These findings are in agreement with the results of Ryan *et al*. (1986) and Adeola *et al*. (2018), who reported that the growth stage and type of chickens influence their digestive efficiency. Adedokun *et al*. (2015) found that ileal crude protein digestibility and standardized ileal amino acid digestibility for Distillers Dried Grains with Solubles (DDGS) samples was higher ($P<0.05$) in 21-day-old broilers than in laying hens. Pishnamazi *et al*. (2005) found that for White Leghorn birds the AMEn digestibility of corn (egg-type birds, 3065 kcal/kg; meat-type birds, 2842 kcal/kg), soybean (egg-type birds, 2185 kcal/kg; meat-type birds, 2040 kcal/kg), and wheat bran (egg-type birds, 1440 kcal/kg; meat-type birds, 1333 kcal/kg) was significantly greater than that of broiler chickens. Stefanello *et al*. (2016) reported that the utilization of energy and nitrogen contained in a basal diet decreased when bakery meal was included and increased with the age of broiler chickens. Walugembe *et al*. (2015) reported that different microbes were found inside different types of chickens fed the same diet: there was a higher concentration of acetic acid and propionic acid in broiler chicks than in laying hen chicks. In a study that characterized the development of amino acid

Table 8. **Effect of diet composition on performance and nitrogen balance in Experiment 3**

Item	Reference diet	Test diet	SEM	P-value
Performance				
Feed intake (g of DM/d)	82.73	92.64	2.20	0.044
Average hen daily production (%)	88.33	91.67	3.59	0.290
Respiratory parameter				
VO_2 (L/d/kg $BW^{0.70}$)	25.94	27.76	0.45	0.107
VCO_2 (L/d/kg $BW^{0.70}$)	25.88	26.64	0.57	0.664
Respiratory quotient (L/L)	1.00	0.96	0.01	0.160
Nitrogen intake (g/d)	3.02	3.94	0.13	<0.001
Nitrogen excretion (g/d)	2.09	2.80	0.12	<0.001
Nitrogen retention (g/d)	0.94	1.14	0.04	0.090

Abbreviations: DM, dry matter; Expt. 3, 45-week-old Hy-Line Brown hens; each treatment consisted of six replicates with two hens each; SEM, standard error of the mean (n=6).

Table 9. **Effect of different diets on energy metabolism in Experiment 3**

Item	Reference diet	Test diet	SEM	P-value
Energy balance (kcal/kg $BW^{0.75}$/d)				
THP	131.72	143.41	2.32	0.098
HI	43.34	55.03	2.32	0.098
RE	22.33	23.00	2.54	0.412
RE as protein	20.46	25.00	1.00	0.029
RE as fat	1.87	−1.71	2.42	0.977
GEI	212.54	245.03	5.71	0.002
AMEI	154.05	166.49	3.40	0.074
NEI	111.14	115.63	2.54	0.412
Available energy (kcal/kg of DM)				
AME	3050.11	2925.91	0.10	0.004
AMEn	2954.57	2825.60	0.09	0.001
NE	2199.81	2037.39	0.18	0.060
Efficiency (%)				
AME/GE	72.47	67.95	0.69	<0.001
NE/AME	71.91	67.09	1.18	0.310

Abbreviations: DM, dry matter; Expt. 3, 45-week-old Hy-Line Brown hens; each treatment consisted of six replicates with two hens each; THP, total heat production; HI, heat increment; RE, retained energy; GEI, gross energy intake; AMEI, apparent metabolizable energy intake; NEI, net energy intake; AME, apparent metabolizable energy; AMEn, AME corrected for zero nitrogen retention; NE, net energy; GE, gross energy; SEM, standard error of the mean (n=6).

transport in broiler chicks using L-tryptophan, a significant reduction was observed in the uptake rate of 0.04 mM L-tryptophan in both the jejunum and ileum with increasing age (Iji *et al*., 2001). This could partly explain why the two chicken lines showed differences in digestive efficiency, as reported in our and other studies.

The AME value of ECSM in broilers was lower than the value of 2568 kcal/kg of DM reported by Zhang and Adeola. (2017). Moreover, the ME and NE of ECSM were higher by 115 kcal/kg of DM and 75 kcal/kg of DM, respectively, than those of soybean meal recorded by Liu *et al*. (2017). This shows that the feeding value of ECSM was similar to that of a regular soybean meal. Similar results were obtained by Lordelo *et al*. (2004) and Yuan *et al*. (2014).

There was a significant increase in the body weight gain of broilers fed the test diet containing ECSM compared with that of broilers fed the reference diet. Sun *et al*. (2013) obtained a positive effect on growth performance by supplementing the diet with 4% and 8% fermented CSM. However, some researchers have obtained different results. For example, Abdulrashid *et al*. (2013) added a CSM formulation to diets in various proportions, and showed that CSM did not affect the body weight gain when it was included in diets at a concentration of up to 30%. Elangovan *et al*. (2006) did not identify a negative influence of 0% to 10% CSM in diets. However, the inclusion of 20% or 30% CSM in isocaloric and isonitrogenous diets can negatively affect the body weight gain, testicular volume, semen volume, and sperm count of broilers (Villalba and Smith, 2011). In summary, diets supplemented with CSM showed contrasting results. Furthermore, the relatively low concentration of lysine may restrict the quality of proteins in CSM (Watkins and Waldroup, 1995;

Devanaboyina *et al*., 2007).

As the CP content of the test diet was higher than that of the reference diet, nitrogen intake and excretion in the test diet group were significantly higher than those in the reference diet group, and the nitrogen retained in the broilers was significantly increased. However, nitrogen retention in laying hens was not significantly affected. These results are consistent with those obtained by Wu *et al*. (2019) and Barzegar *et al*. (2020). Nitrogen retention in broilers was higher than that in laying hens; in addition, nitrogen retention in older broilers was higher than that in younger broilers. It is well known that broilers are in the growth phase and thus grow rapidly. Therefore, they require a lot of energy and protein to develop their body tissues. Conversely, laying hens are in the adult stage, show stable body weight, and mainly use their daily intake of energy and nutrients for laying and maintenance. Therefore, the energy and nutrient requirements of laying hens are stable (Shapiro and Nir, 1995; Abdulrashid *et al*., 2013; Kimiaeitalab *et al*., 2017).

Furthermore, we observed an interesting phenomenon in the experiments with broilers: the unit body weight deposition energy of the reference diet group was higher than that of the test diet group. This indicates that broilers fed the reference diet need more energy to gain the same weight. Estimating the form of energy deposition revealed that the test diet changed the form of energy deposition; in fact, it increased the proportion of energy deposited as protein and reduced the proportion of energy deposited as fat. Indeed, the energy content of protein per gram was lower than that of fat. Hence, the energy intake of the metabolic body weight in the experimental diet group did not differ from that of the reference diet group. Nevertheless, the efficiency of conversion of effective energy into body weight was improved by the test diet, with increased production performance after a week from the beginning of the experiment.

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Conflict of Interest

The authors declare no conflicts of interest.

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