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Comparison of the Ability of Different Types of Birds to Derive Energy from Corn and Soybean Meal

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Abstract

This study evaluated the ability of different types of birds to derive energy from corn and soybean meal (SBM) using the reference diet substitution method. The corn and SBM were combined into a reference diet at 60 and 30%, respectively. Other ingredients, including dicalcium phosphate, limestone, salt, minerals, vitamins, and amino acids, held constant across the reference and test diets. A total of 36 broiler breeder hens (Ross 308, 62 weeks old), 72 male broiler chickens (Ross 308, 35 days old) and 36 commercial layer hens (Hy-line W36, 40 weeks old) were used. The apparent metabolizable energy corrected for zero nitrogen retention (AMEn), apparent ileal digestible energy (AIDE) of corn and SBM and apparent ileal digestibility coefficient (AIDC) of nitrogen, crude fat and gross energy for whole diets were evaluated. The activities of intestinal digestive enzymes and intestinal morphology were measured and compared among the dietary treatments and birds. The AMEn of SBM for broiler breeder hens was significantly higher than that of broiler chickens and commercial layer hens (2525.50, 2215.10 and 2310.80 kcal/kg DM respectively; $P < 0.05$); in contrast, the corn AMEn for broiler breeder hens was lower than that of broiler chickens and laying hens (3126.67, 3382.11 and 3305.59 kcal/kg DM respectively; $P < 0.05$). The AIDE values of corn and SBM were not significantly different between the subjected birds ($P > 0.05$). Furthermore, the difference between AMEn and AIDE for both corn and SBM was not significant in any experiment ($P > 0.05$). In conclusion, the findings of this study indicate that broiler breeder hens have a distinct capacity for deriving energy from corn and SBM compared to broiler chickens and layer hens. This highlights the impracticality of using a single set of energy values for these feedstuffs in poultry feed formulations.

Introduction

The level of dietary energy is one of the main factors that influence Feed intake. Therefore, dietary nutrients must vary according to the energy content of the diet. Energy-deficient or high-energy diets can reduce performance due to nutrient imbalances in metabolism. Therefore, proper regulation of energy levels in diets is important to ensure production efficiency (Alvarenga *et al.,* 2013). Poultry diets are usually based on a fairly limited number of feed materials. In countries like the United States, Brazil, and Iran, diets typically consist primarily of corn and soybean meal, which supply the majority of the energy and protein required. Poultry nutritionists require comprehensive nutritional data on corn and soybean meal to optimize their use. Estimated metabolizable energy values for many feed ingredients are provided in published summary tables (NRC, 1994; Lesson and Summers, 2001; CVB feed table, 2011). Nevertheless, many factors can affect the energy values of ingredients that are not mentioned in the tables. Numerous studies have shown that the metabolizable energy value of feed ingredients can be influenced by several factors, including feedstuff composition and variety (Reid *et al.,* 2024; Siegert *et al.*, 2023; Zhou *et al.,* 2010;

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Stefanello *et al.,* 2016), bird age and genotype (Tancharoenrat *et al.,* 2013; Lopez and Leeson, 2008; Pishnamazi *et al.,* 2005; Spratt and Leeson, 1987), and the methodology used for energy determination (Masood *et al.,* 2011; Sales and Janssens, 2003; Scott *et al.,* 1998; Dourado *et al.,* 2010).

Limited information exists comparing the AMEn of corn and SBM across broiler breeder hens, broiler chickens, and laying hens. While Pishnamazi *et al.* (2005) examined the influence of broiler breeder and laying hen breed on the apparent metabolizable energy of feed ingredients, their results showed similar AMEn digestibility of wheat and barley in both breeds. However, white leghorn birds exhibited significantly higher AMEn digestibility of corn, SBM, and wheat bran compared to broiler breeders. It is important to note that the study of Pishnamazi *et al.* (2005) used forced feeding and the total collection method (Sibbald method) to determine metabolizable energy. The forced-feeding method, which uses only a single ingredient, does not account for potential interactions between ingredients present in practical diets. Furthermore, this method prevents birds from exhibiting natural feeding behavior, which may influence the AMEn of diets and ingredients. This study aimed to determine and compare the ability of broiler breeder hens, broiler chickens, and commercial layer hens to derive energy from corn and SBM under practical feeding conditions using two bioassay methods (partial excreta collection with a marker and ileal content collection with a marker). A secondary objective was to compare the intestinal morphology, AIDC of nitrogen, crude fat, gross energy and digestive enzyme activity among these bird types.

Material and Methods

Three experiments were conducted to compare the metabolizable energy (ME) values of corn and soybean meal (SBM) using broiler breeder hens (Ross 308), broiler chickens (Ross 308), and commercial layer hens (40 weeks old) at the Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Iran. The sources of corn and SBM were the same in all three experiments. The nutrient composition of corn and SBM was measured by AOAC (AOAC, 2000) and near infrared reflectance analysis (NIRA) methods. Table 1 gives values for corn and soybean meal based on AOAC, NIRA and NRC.

Table 1. Nutrient composition of corn and soybean meal (*As-fed* basis)

Ingredient	DM* (%)	CP* (%)	EE* (%)	GE# (Kcal/kg)	Ash (%)	CF* (%)	Total P (%)	AME# (Kcal/kg)	AMEn# (Kcal/kg)
Corn									
AOAC	90.85	7.50	4.00	3719.8	-	-	-	-	-
NIRA	89.02	7.69	3.16	-	1.36	2.34	0.25	3549	3442
NRC (1994)	89.00	8.50	3.80	-	-	2.20	0.28	-	3350
SBM									
AOAC	90.49	40.54	1.72	4258.7	-	-	-	-	-
NIRA	89.55	44.82	2.51	-	6.57	4.87	0.62	2196	2027
NRC (1994)	89.00	44.00	0.80	-	-	7.00	0.65	-	2230

*DM: Dry Matter, CP: Crude Protein, EE: Ether Extract, CF: Crude Fiber

#GE: Gross Energy, AME: Apparent Metabolizable Energy, AMEn: Apparent ME corrected for zero nitrogen retention.

Each experiment used a completely randomized design with three treatments (Table 2). The corn and SBM were combined into a corn-SBM-based reference diet at 60 and 30%, respectively, by replacing the energy-yielding ingredients using the reference diet substitution method. Other ingredients, including dicalcium phosphate, limestone, salt, minerals, vitamins, and amino acids held constant across the reference and test diets. The ingredients and nutrients composition of the diets (calculated and measured nutrients including AMEn and CP) are shown in Table 2. Measured AMEn in Table 2 is the biological assay results of the three experiments. In broiler chickens and layer hens, the biological measured AMEn was higher than predicted values which may be because of the presence of sunflower oil in the diets. The presence of oil in the diets may cause synergy. In each experiment, corn and SBM replaced energy-yielding ingredients including corn, soybean meal, and sunflower oil without altering the ratio of corn, SBM and sunflower oil (Adeola and Zhai, 2012). In each trial, the reference diet was formulated to supply the bird's requirements. Celite® was added at 10 g/kg (1%) to the diets as an indigestible marker.

Table 2. Nutrients and composition of experimental diets for broiler breeder hens, broiler chickens and commercial layer hens (%)

Ingredients (%)	Broiler breeder hens (62-week-old)			Broiler (29-35d old)			Laying hens (40-week-old)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
	Reference	SBM	Corn	Reference	SBM	Corn	Reference	SBM	Corn
Corn	72.25	48.10	23.95	60.77	41.723	22.674	52.4	34.21	16.05
SBM	17.5	11.65	5.8	31.51	21.633	11.747	29.91	19.52	9.10
SO*	0	0	0	3.43	2.354	1.279	4.08	2.66	1.24
DCP	1.23	1.23	1.23	1.39	1.39	1.39	2.08	2.08	2.08
CaCO3	7.0	7.0	7.0	0.8	0.8	0.8	9.48	9.48	9.48
salt	0.18	0.18	0.18	0.35	0.35	0.35	0.35	0.35	0.35
NaHCO3	0.22	0.22	0.22	0	0	0	0	0	0
Min.premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit. Premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Met	0.12	0.12	0.12	0.22	0.22	0.22	0.2	0.2	0.2
L-Lys-HCL				0.03	0.03	0.03	0	0	0
Celite	1	1	1	1	1	1	1	1	1
Replaced SBM	-	30	-	-	30	-	-	30	-
Replaced Corn	-	-	60	0	-	60	-	-	60
Sum	100	100	100	100	100	100	100	100	100
Calculated nutrients									
AMEn (Kcal/kg)	2775	2570	2969	3050	2794	3181	2755	2512	2887
CP (%)	13.18	22.59	9.80	18.58	26.63	12.15	17.00	24.91	10.26
Measured nutrients (biological assay)									
AMEn (Kcal/kg)	2531	2440	2882	3171	2885	3298	3499	3131	3377
CP (%)	13.49	21.53	9.41	18.60	25.70	11.71	17.78	24.33	11.27

[1]provided per kg diets of broiler chickens and broiler breeder hens: retinol, 2700µg; cholecalciferol, 75µg; DL-alpha-tocopherol acetate, 18 mg; menadione, 2mg; thiamine, 1.8 mg; pyridoxine, 3mg; cyanocobalamin, 0.015mg; biotin, 0.1 mg; pantothenic acid, 30mg; folic acid, 1mg; niacin, 10mg; Choline chloride, 500mg; manganese, 100mg; iron, 50 mg; Zinc, 85mg; Copper, 10mg; selenium, 0.2mg; Iodine, 1mg; B.H.T, 1mg.

[1]provided per kg diets of laying hens: retinol, 2640µg; cholecalciferol, 62.5µg; DL-alpha-tocopherol acetate, 11 mg; menadione, 2.2 mg; thiamine, 1.477 mg; riboflavin, 4 mg; pyridoxine, 2.462 mg; cyanocobalamin, 0.01 mg; biotin, 0.15 mg; pantothenic acid, 34.650 mg; folic acid, 0.48 mg; niacin, 7.84 mg; Choline chloride, 400 mg; manganese, 74.4 mg; iron, 75 mg; Zinc, 64.675 mg; Copper, 6 mg; selenium, 0.2 mg; Iodine, 0.88 mg; B.H.T, 1mg.

*Sunflower oil

Broiler breeder hens' experiment

A total of 36 broiler breeder hens (Ross 308, 62 weeks old) with nearly uniform BW (average: 4200±100 g) were obtained from a commercial farm. At the start of the trial, broiler breeder hens were moved to the individual cages equipped with a platform-style feeder, nipple drinker, and a plastic tray for excreta collection. All birds had *ad libitum* access to water, and the feed was given 159 g/bird/day (Based on body weight and diet's AMEn). Two adjacent cages were considered a replicate, and the three assay diets were randomly assigned to 6 replicates, with each replicate containing two birds. The lighting program was 13L: 11D and the temperature was set at 21±2°C for the entire experiment. The feeds were given in mash form once daily at 07:30 am. Egg production was recorded throughout the experiment. Based on egg production, birds were fed with a reference diet for one week to adapt to the new cages. After that, experimental diets were provided for one week.

Broiler chickens' experiment

A total of 72 Day-old male broiler chickens (Ross 308) were obtained from the local hatchery. The 3 assay diets were randomly assigned to 4 replicates of 6 birds each. The birds were raised in cages and fed commercial broiler starter (2900 kcal/kg ME, 22.2% of crude protein) and grower (2900 kcal/kg ME, 20.11% of crude protein) from d 1 to 10 and d 11 to 24 respectively. A temperature of 32±1°C was maintained for the first week, followed by a gradual decrease to approximately 23°C by the end of the third week. The lighting program was 23L: 1D for the entire experimental period. The chickens were vaccinated according to the regional vaccination schedule (Data are not shown). A commercial broiler finisher diet (3050 kcal/kg ME, 18.58% of crude protein) was offered to birds (24-28 d) until assigned to the assay diets (29-35d). Furthermore, the finisher diet served as a reference diet for subsequent analysis. Water and mash feed were offered *ad libitum*. Experimental diets were provided from d 29 to 35.

Layer hens' experiment

A total of 36 commercial layer hens (Hy-Line W36, 40 weeks old) of uniform egg production (laying rate> 90%) were obtained from the Layer Unit of the University of Tehran and allocated to cages. The 3 assay diets were randomly assigned to 4 replicates of 3 birds each. All birds were provided with *ad libitum*

access to water and received daily feed in mash form at a rate of 100 grams per bird (based on the Hy-Line W36 management guide, 2020). The lighting program was 16L: 8D for the entire experimental period. Egg production was recorded throughout the experiment. Based on egg production, birds were fed with a reference diet for one week to adapt to the new cages. After that, experimental diets were provided for one week.

Feeding and sample collection

Experimental diets (without marker) were provided for 5 days' adaptation and then birds were fasted for at least 8h before being fed with assay diets (with marker). Experimental diets with markers were used for two days and excreta were collected concurrently (24 hours' collection). After excreta collection, the birds of each replicate of each trial were anaesthetized ((broiler breeder hens (1 bird/replicate), broiler chickens (4 birds/replicate) and laying hens (3 birds/replicate)) by precise dose of thiopental sodium injection, and ileal digesta from the Meckel's diverticulum to about 2-cm cranial to the ileocecal junction were collected and for broilers and laying hens were pooled per each cage. The excreta samples were dried in a forced air oven at 55±5°C and then ground to a fine powder. The ileal digesta samples were stored in the freezer at −20°C until they were freeze-dried.

Chemical analyses and calculations

Dried samples of excreta, ileal digesta and diets were analyzed for gross energy (IKA-KALORIMETER C 400), nitrogen (KJELTEC AUTO 1030 Analyzer) and ether extract (SOXTEC SYSTEM HT 1043 Extraction Unit). The acid-insoluble ash (AIA) content of diet, excreta and ileal digesta samples was analyzed after ashing the samples, and then boiling the ash with 4 *N* HCl (Siriwan *et al.,* 1993). AMEn and AIDE of each diet and ingredients (corn and SBM) were calculated by obtained data and subsequent formulas (Equation 1 and 2 respectively; Lesson and Summers, 2001):

Equation 1:
$$\text{AMEn or AIDE}_{diet} = \text{GE}_{diet} - \left[\frac{GE_{excreta\ or\ ileal\ digesta} \times \text{AIA}_{diet}}{\text{AIA}_{excreta\ or\ ileal\ digesta}}\right] - (\text{NR} \times \text{K})$$

$$\text{NR} = \text{N}_{diet} - \left[\frac{\text{AIA}_{diet}}{\text{AIA}_{excreta\ or\ ileal\ digesta}} \times N_{excreta\ or\ ileal\ digesta}\right]$$

K = 8.73 kcal/g N retained

Equation 2:
$$\text{AMEn or AIDE}_{\text{test ingredient}} = \text{AMEn or AIDE}_{\text{reference diet}} - [(\text{AMEn or AIDE}_{\text{reference diet}} - \text{AMEn or AIDE}_{\text{test diet}}) \div \text{inclusion rat}]$$

Where GE: gross energy, NR: Nitrogen Retention, K: energy released when 1 gram of uric acid is fermented, AMEn: Apparent Metabolizable Energy corrected for zero nitrogen retention using excreta samples, AIDE: Apparent Ileal Digestible Energy corrected for zero nitrogen retention using ileal digesta samples. The apparent ileal digestibility coefficient of nitrogen, crude fat and gross energy was calculated by AIA method with the following formula (Equation 3):

Equation 3:
$$\text{AIDC} = 1 - \left(\text{N}_{ileal\ digesta} \div \text{N}_{diet}\right) \times \left(\text{AIA}_{diet} \div \text{AIA}_{ileal\ digesta}\right)$$

Where AIDC: Apparent Ileal Digestibility Coefficient, N: nutrients.

Jejunum Morphology

Jejunum Segments (midway between the endpoint of the duodenal loop and Meckel's diverticulum) were removed from one bird of each replicate in each experiment. The samples (18 samples for broiler breeder hens, 12 samples for broiler chickens and 12 samples for laying hens) were washed with distilled water and fixed in 10% formalin for 144 hours (the formalin solution changed every 48 hours). Standard paraffin embedding techniques were used to prepare two cross-sections for each sample stained with hematoxylin and eosin. Villus Width (the average of base and head) and Villus length (tip of the villus to the villus-crypt junction) were measured; crypt depth (CD) was defined as the depth of the invagination between adjacent villi. The ratio of villus to crypt (VL: CD) was estimated by dividing the villus length by the crypt depth. In addition, the surface area of the jejunal villus was calculated considering a villus as a tubular structure (Equation 4; De los *et al.,* 2005):

Equation 4: Villus surface area (VSA) = $2\pi \times$ (average villus width/2) × villus length.

Digestive enzyme activity

Jejunum segments were collected from one bird of each replicate of every experiment. The specific activities of amylase (EC 3.2.1.1), aminopeptidase (EC 3.4.11.2), and lipase (EC 3.1.1.3) in the jejunum segments were tested in homogenized tissue (Silent Crusher M, Heidolph, Schwabach, Germany). Soluble starch was used as a substrate to measure amylase activity, as described by Bernfeld (1955). L-leucine-p-nitroanilide (Sigma L-9125 Chemical Co., St Louis, MO)

was used as a substrate to measure aminopeptidase activity as described by Gal-Garber and Uni, (2000). A lipase activity assay kit (Lipase DC, Pars Azmun) was used to measure lipase activity. The protein content of the intestinal samples was measured according to the method of Bradford (1976). The enzyme activity results were expressed in units per mg of intestinal tissue protein.

Statistical analysis

The experiment used a completely randomized design (CRD). All data sets were tested for normality using the Minitab software (2016). The SAS 9.1 analysis program was used for statistical analysis (SAS Institute, 2002). The GLM (General Linear Model) procedure was used for comparing the results of three experiments. The different bird types are considered treatments. The AMEn and AIDE values of each feedstuff were compared in each experiment independently. Significantly different means were further separated using Tukey tests and all differences were considered significant at $P < 0.05$.

Results

Metabolizable energy values of corn and SBM

The AMEn and AIDE of corn and SBM for broiler breeder hens, broiler chickens and laying hens are presented in Tables 3 and 4. The results indicated that AMEn values of corn (dry matter and *as-fed* basis) for broiler breeder hens were significantly lower than broiler chickens and laying hens ($P < 0.05$). Conversely, AMEn values of SBM for broiler breeder hens were significantly greater than broiler chickens and laying hens ($P < 0.05$). Broiler chickens and laying hens obtained similar energy of corn and SBM ($P > 0.05$). The AIDE values of both corn and SBM for broiler breeder hens, broiler chickens and laying hens were not significantly different ($P > 0.05$).

Comparison of two methods in the determination of metabolizable energy

The comparison of energy values (Kcal/kg) of corn and SBM as measured by partial excreta collection with a marker (AMEn) and ileal digesta collection with a marker (AIDE) are shown in Tables 3 and 4. There were no significant differences between AMEn and AIDE values of both corn and SBM among the different bird types ($P > 0.05$). However, in broiler breeder hens the difference of AMEn and AIDE for SBM tended to be significant ($P < 0.07$).

Table 3. Effect of strain and age of birds and the method of experiment on corn metabolizable energy values (Kcal/kg)

ME Values	Broiler breeder hens (62 wk.)	Broilers (35d)	Layer hens (40 wk.)	*SEM*[1]	P-value[1]
AMEn (DM)	3126.67[b]	3382.11[a]	3305.59[a]	40.74	0.001
AIDE (DM)	3030.19	3270.40	3236.60	98.98	0.25
SEM[2]	69.43	46.63	79.58	-	-
P-Value[2]	0.36	0.14	0.56	-	-
AMEn (*As-fed*)	2845.27[b]	3077.72[a]	3008.09[a]	33.67	0.001
AIDE (*As-fed*)	2757.47	2976.10	2945.30	90.07	0.25
SEM[2]	63.18	42.44	72.42	-	-
P-Value[2]	0.36	0.14	0.56	-	-

[a–f] Values within a row with different superscripts differ significantly ($P < 0.05$).
[A–F] Values within a column with different superscripts differ significantly ($P < 0.05$).
[1] comparison between birds' type
[2]comparison between methods (partial excreta collection with a marker or ileal method)

Table 4. Effect of strain and age of birds and the method of experiment on soybean meal metabolizable energy values (Kcal/kg)

ME Values	Broiler breeder hens (62 wk.)	Broilers (35d)	Layer hens (40 wk)	*SEM*[1]	P-value[1]
AMEn (DM)	2525.50[a]	2215.10[b]	2310.80[b]	58.51	0.009
AIDE (DM)	2333.00	2317.40	2164.30	196.36	0.80
SEM[2]	62.78	101.70	183.20	-	-
P-Value[2]	0.07	0.50	0.59	-	-
AMEn (*As-fed*)	2274.75[a]	1993.60[b]	2079.70[b]	52.66	0.009
AIDE (*As-fed*)	2099.70	2085.70	1947.90	176.72	0.80
SEM[2]	56.50	91.53	164.88	-	-
P-Value[2]	0.07	0.50	0.59	-	-

[a–f] Values within a row with different superscripts differ significantly ($P < 0.05$).
[A–F] Values within a column with different superscripts differ significantly ($P < 0.05$).
[1] comparison between birds' type
[2] comparison between methods (partial excreta collection with marker or ileal method)

Jejunum morphology

The results of intestinal morphology parameters are given in Tables 5 and 6. In each experiment, treatments were compared independently (columns) and the differences among birds' species in each treatment were also compared (rows). There were no significant differences in villus width and crypt depth (data are not shown), VL: CD ratios and VSA between dietary treatments in each experiment ($P > 0.05$). Nevertheless, villus length was significantly shorter in treatment 3 in the laying hens' experiment ($P < 0.05$). No significant differences were observed

between birds' types in the reference diet for all measured parameters ($P > 0.05$). The results displayed that in treatment 3 (corn replaced diet), broiler breeder hens and broiler chickens had longer villus length than laying hens (Fig 1; $P < 0.05$). In treatment 2 (SBM replaced diet), broiler chickens had significantly higher VSA than laying hens and broiler breeder hens ($P < 0.05$). Likewise, broiler chickens had a significantly higher VL: CD ratio than laying hens in treatment 2 ($P < 0.05$).

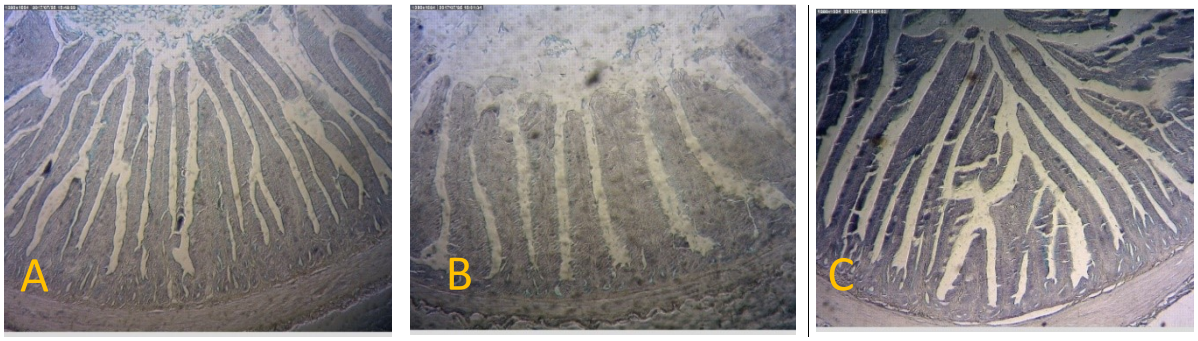

Figure 1. The jejunum morphology of A: Broiler chicken, B: laying hen and C: Broiler breeder hen

Table 5. Effect of diets and bird's type on villus length (VL) and villus length: crypt depth ratio (VL: CD) of jejunum

	VL (µm)			*SEM*	P-Value	VL: CD			*SEM*	P-Value
	B*	L*	BB*			B	L	BB		
R (reference diet)	1531.7	1393.46^{a}	1465.85	64.56	0.22	9.70	9.11	10.52	0.74	0.39
T1 (replaced SBM)	1508.4	1366.67^{a}	1504.19	65.02	0.26	10.70^{A}	8.11^{B}	9.47^{AB}	0.58	0.02
T2 (replaced corn)	1405.5^{A}	1178.14^{B-b}	1434.80^{A}	52.48	0.004	9.07	8.09	9.82	0.58	0.10
SEM	61.40	34.25	56.06	-	-	0.51	0.38	0.40	-	-
P-Value	0.44	0.003	0.74	-	-	0.85	0.23	0.34	-	-

$^{a–f}$ Values within a column with unlike superscripts differ significantly ($P < 0.05$).
$^{A-F}$ Values within a row with unlike superscripts differ significantly ($P < 0.05$).
*B: Broiler, L: Laying hens, BB: Broiler Breeder hens.

Table 6. Effect of diets and bird's type on villus width (VW) and villus surface area (VSA) of jejunum

	VW (µm)			*SEM*	P-Value	VSA (mm2)			*SEM*	P-Value
	B*	L*	BB*			B	L	BB		
T1 (reference diet)	156.25	141.37	150.40	12.02	0.70	0.74	0.61	0.68^{b}	0.07	0.52
T2 (replaced SBM)	159.18	156.72	152.50	8.86	0.86	0.75^{A}	0.59^{B}	0.58^{b-B}	0.03	0.007
T3 (replaced corn)	150.33	156.25	155.19	6.11	0.82	0.66	0.58	0.68^{b}	0.04	0.17
SEM	7.94	6.80	8.76	-	-	0.057	0.04	0.04	-	-
P-Value	0.78	0.22	0.94	-	-	0.58	0.83	0.31	-	-

$^{a–f}$ Values within a column with unlike superscripts differ significantly ($P < 0.05$).
$^{A-F}$ Values within a row with unlike superscripts differ significantly ($P < 0.05$).
*B: Broiler, L: Laying hens, BB: Broiler Breeder hens.

Digestive enzymes activity

The effect of diets and the bird's type on digestive enzyme activity is presented in Tables 7 and 8. The Intestinal amylase activity was significantly higher in laying hens compared to broiler breeder hens and broiler chickens in T1 and T3 ($P < 0.05$). Similarly, amylase activity in broiler breeder hens was significantly higher than in broiler in T1 and T2 ($P < 0.05$). Besides, amino peptidase activity was higher in broiler breeder hens compared to broiler chickens and laying hens in reference diet ($P < 0.05$). In broiler chickens and laying hens, amylase activity significantly increased with increased corn in the diet (T3; $P < 0.05$). Also, in broiler chickens, amino peptidase activity in the test diets (T2 and 3) was significantly higher than the reference diet ($P < 0.05$). Dietary treatments had no significant effect on the amino peptidase activity of laying hens and broiler breeder hens ($P > 0.05$). Lipase activity significantly increased in laying hens with increased corn in the diet (T3; $P < 0.05$). Otherwise, in T2 (SBM replaced diet), lipase activity of laying hens was significantly lower than in broiler chickens and broiler breeder hens ($P < 0.05$).

Table 7. Effect of diets and bird's type on the specific activity of the intestinal digestive enzyme (unit/mg intestinal protein)

	Amylase					Amino peptidase				
	B*	L*	BB*	*SEM*	P-Value	B	L	BB	*SEM*	P-Value
R (reference diet)	36.33^{C-c}	81.23^{A-b}	62.61^{B}	3.94	<0.0001	12.65^{B-b}	14.46^{A}	15.10^{A}	0.37	0.0002
T1 (replaced SBM)	54.92^{B-b}	85.14^{A-b}	75.72^{A}	5.83	0.001	14.86^{a}	14.45	15.42	0.47	0.38
T2 (replaced corn)	69.83^{B-a}	117.38^{A-a}	65.29^{B}	4.28	<0.0001	15.03^{a}	14.19	14.83	0.46	0.45
SEM	3.56	5.13	3.92	-	-	0.49	0.34	0.42	-	-
P-Value	<0.0001	<0.0001	0.16	-	-	0.001	0.81	0.71	-	-

[a–f] Values within a column with unlike superscripts differ significantly ($P < 0.05$).
[A-F] Values within a row with unlike superscripts differ significantly ($P < 0.05$).
*B: Broiler, L: Laying hens, BB: Broiler Breeder hens.

Table 8. Effect of diets and bird's type on the specific activity of the intestinal digestive enzyme (unit/mg intestinal protein)

	Lipase			*SEM*	P-Value
	B*	L*	BB*		
R (reference diet)	4.39	3.74^{b}	4.72	0.37	0.16
T1 (replaced SBM)	4.19^{A}	2.95^{B-b}	4.78^{A}	0.26	<0.0001
T2 (replaced corn)	4.72^{AB}	5.85^{A-a}	4.09^{B}	0.34	0.001
SEM	0.35	0.27	0.38	-	-
P-Value	0.57	<0.0001	0.26	-	-

[a–f] Values within a column with unlike superscripts differ significantly ($P < 0.05$).
[A-F] Values within a row with unlike superscripts differ significantly ($P < 0.05$).
*B: Broiler, L: Laying hens, BB: Broiler Breeder hens.

Nutrients digestibility coefficient

The apparent ileal digestibility coefficient of nutrients in dietary treatments of each experiment and different bird types are shown in Tables 9 and 10. The data demonstrated that the nitrogen and gross energy digestibility coefficient in broiler chickens and laying hens was significantly higher than in broiler breeder hens in most dietary treatments ($P < 0.05$). Crude fat digestibility was higher in broiler chickens and laying hens compared to broiler breeder hens ($P < 0.05$). Dietary treatments did not have any significant effect on crude fat digestibility in all three experiments ($P > 0.05$). Broiler breeder hens (62 weeks old) exhibited lower nutrient digestibility compared to broiler chickens ($P < 0.05$).

Table 9. Effect of diets and bird's type on ileal digestibility coefficient of nutrients

	Nitrogen					Gross Energy				
	B*	L*	BB*	*SEM*	P-Value	B	L	BB	*SEM*	P-Value
R (reference diet)	80.48^{A}	69.51^{B}	68.34^{B}	2.26	0.008	75.97^{A-ab}	66.88^{AB-b}	60.74^{B-a}	2.58	0.008
T1 (replaced SBM)	81.03^{A}	75.63^{AB}	70.85^{B}	2.40	0.03	70.09^{A-b}	62.85^{A-b}	50.37^{B-b}	3.34	0.003
T2 (replaced corn)	75.23^{A}	74.08^{A}	63.80^{B}	2.72	0.02	78.97^{A-a}	79.09^{A-a}	64.56^{B-ab}	1.71	0.0003
SEM	2.97	1.9	2.58	-	-	1.52	2.84	2.83	-	-
P-Value	0.35	0.16	0.11	-	-	0.008	0.008	0.02	-	-

[a–f] Values within a column with unlike superscripts differ significantly ($P < 0.05$).
[A-F] Values within a row with unlike superscripts differ significantly ($P < 0.05$).
*B: Broiler, L: Laying hens, BB: Broiler Breeder hens.

Table 10. Effect of diets and bird's type on ileal digestibility coefficient of nutrients

	Crude Fat				
	B*	L*	BB*	*SEM*	P-Value
R (reference diet)	99.93^{A}	99.92^{A}	99.54^{B}	0.07	0.012
T1 (replaced SBM)	99.96^{A}	99.91^{A}	99.67^{B}	0.04	0.002
T2 (replaced corn)	99.94^{A}	99.88^{B}	99.73^{C}	0.008	<0.000
SEM	0.01	0.012	0.28	-	-
P-Value	0.13	0.14	0.08	-	-

[a–f] Values within a column with unlike superscripts differ significantly ($P < 0.05$).
[A-F] Values within a row with unlike superscripts differ significantly ($P < 0.05$).
*B: Broiler, L: Laying hens, BB: Broiler Breeder hens.

Discussion

Metabolizable energy values of corn and SBM

The results indicated that AMEn values of corn for broiler breeder hens were significantly lower than those for broiler chickens and laying hens (3126.67, 3382.11 and 3305.59 kcal/kg DM, respectively; $P < 0.05$). In contrast, the AMEn values of SBM for broiler breeder hens were significantly higher than those for broiler chickens and laying hens (2525.50, 2215.10 and 2310.80 kcal/kg DM, respectively; $P < 0.05$). Also, the ratio of AIDE: AMEn for SBM was 0.923, 1.04 and 0.936 in broiler breeder hens, broiler chickens and laying hens, respectively. This indicates that cecal fermentation contributed approximately 7.6% and 6.3% to the energy value of SBM in broiler breeder hens and laying hens, respectively. Since the AIDE of SBM was not significantly different between bird groups, the higher AMEn values observed in broiler breeder hens may be attributed to differences in microbial activities beyond the ileum, such as variations in the cecal microflora and their activities. The ratio of AIDE: AMEn of corn was 0.968 for breeders, 0.966 for broilers and 0.979 for layers, subsequently, cecal fermentation added about 3.1, 3.4 and 2.1% on energy of corn, respectively. Cecal fermentation contributed more to the energy value of SBM compared to corn in broiler breeders and laying hens. The results of Barzegar *et al.* (2019) showed that the AMEn values of corn and SBM in laying hens, using a reference diet substitution method, were 3722 and 2496 kcal/kg DM, which was higher than the obtained results of the present study (3305.59 and 2310.80 kcal/kg DM respectively). The results of the present study indicated that the *As-fed* basis SBM AMEn for broiler chickens and laying hens (1993.6 and 2079.70 kcal/kg) were almost equal to the NIRA results (table 1), but the SBM AMEn value for broiler breeder hens was equal to the NRC (1994) value (2274.75 vs. 2230 kcal/kg *As-fed*). But, our results on AMEn values of corn (*As-fed* basis) in all bird types were lower than the NIRA results and the NRC (1994) table (Table 1).

In broiler chickens and laying hens, amylase activity significantly increased with increased corn in the diet (T3; $P < 0.05$). The higher amylase activity observed in broiler chickens and laying hens compared to broiler breeder hens may contribute to the higher AMEn values of corn in broiler chickens and laying hens. The results of Pishnamazi *et al.* (2005) indicated that the AMEn of corn, SBM and wheat bran for white leghorn roosters were significantly greater than broiler breeder roosters. They used a classical total collection method (Sibbald method) which differed from the method used in the present study that was conducted under practical conditions. Likewise, the results of Liu *et al.* (2022) indicated that the AMEn of expanded cottonseed meal was significantly different among broilers aged 14-16 days (2245.19 kcal/kg DM), broilers aged 28-30 days (1841.53 kcal/kg DM) and 45-week-old Hy-Line Brown hens (2629.74 kcal/kg DM). Spratt and Leeson (1987) reported that broiler breeder hens and Single Comb White Leghorn (SCWL) hens were different in deriving energy from feed, so SCWL hens were more efficient than broiler breeder hens. These results are partly in agreement with the results of the current study that indicated broiler breeder hens metabolized less energy of corn than broiler chickens and laying hens. However, broiler breeder hens were able to utilize the energy from SBM more effectively than broiler chickens and laying hens. Many studies have shown that the digestibility of feed ingredients may be dependent on the genotype, age and sex of the birds (Reid *et al.,* 2024; Adeola *et al*., 2018; Pishnamazi *et al.,* 2005; Zelenka, 1997; Roberts and Ball, 2004). Given that the ability of derive energy from feedstuffs varies among birds at different ages, using a single value of metabolizable energy for all stages of production can lead to inaccurate estimation of dietary energy values (Calderano *et al.,* 2010). Masood *et al.* (2011) explained that to accurately determine the metabolizable energy values of feed ingredients for birds, it is essential to conduct experiments under conditions that consider all factors that may influence feed intake and consequently the energy value of the feed ingredients.

Comparison of two methods in the determination of metabolizable energy

The results of the current study demonstrated that there was no significant difference between AMEn and AIDE values of both corn and SBM in each type of bird ($P > 0.05$). Our results are in agreement with those of Masood *et al.* (2011), who reported that ME values determined using marker, total, or ileal collection methods were not significantly different. The results of Scott *et al.* (1998) showed that AME determined using excreta collected and ileal digesta collected was not significantly different for wheat-based diets but differed significantly for barley-based diets. This finding may be attributed to the higher fiber content of hulled barley and the higher NSP content of hull-less barley compared to wheat. The amount of indigestible fiber provides an important food source for an adaptable population of intestinal microflora. Ten Doeschate *et al.* (1993) reported that determining metabolizable energy (ME) using ileal digesta may be more accurate than using excreta collection methods. This is because microbial fermentation in the large intestine may not always be beneficial for the bird and can result in energy loss as waste products. While Scott *et al.* (1998) concluded that although excreta and ileal samples provided similar values and accuracy, obtaining the ileal contents was time-consuming and required euthanasia

of the bird, leading to the conclusion that measurement of AME from excreta was more cost-effective. However, the difference between excreta or ileal digesta methods depends on the marker used, the number of birds and replicates, the type of the birds and age, experimental diets, marker level and other conditions. Based on the results of the present study it can be concluded that excreta and ileal samples provide comparable values of ME of corn and SBM in broiler chickens and laying hens. However, for broiler breeder hens at 62 weeks of age, the ME value of SBM determined from excreta collection tended to be higher than that determined from ileal digesta samples.

Jejunum morphology

There were no significant differences in villus width and crypt depth (data are not shown), VL: CD and VSA between treatments in each experiment ($P > 0.05$). Nevertheless, villus length was significantly shorter in corn replaced diet (T3) in the laying hens experiment ($P < 0.05$). No significant differences were observed between birds' types in the reference diet for all measured parameters ($P > 0.05$). The results displayed that in the corn-replaced diet (T3), broiler breeder hens and broiler chickens had longer villus length than laying hens (1434.8, 1405.5 and 1178.14 µm respectively; $P < 0.05$). Likewise, broiler chickens had a significantly higher VL: CD ratio than laying hens in the SBM-replaced diet ($P < 0.05$). Increased VL: CD is associated with reduced epithelial cell turnover, while this can lead to increased nutrient absorption and improved performance (Gomide *et al.*, 2004). Our results indicated that VSA of jejunum in broiler chickens were significantly higher than in laying hens and broiler breeder hens in T2 (SBM replaced diet), but the ME utilization of SBM was significantly lower in broiler chickens than in broiler breeder hens and laying hens (2215.10, 2525.50 and 2310.80 kcal/kg DM respectively). It has been shown that the ileal villi can enlarge as a compensatory mechanism in response to jejunal dysfunction. Therefore, the observed increase in villus height may be a consequence of an increased need for digestive capacity in the ileum (Svihus, 2014). Kaminska (1979) found that the gizzard weight and gut length to body weight ratio were greater in leghorn compared to broiler chicks. A larger, more muscular gizzard and a longer gut may enhance grinding and absorptive capacities in laying hens. Also, De Verdal *et al.* (2010) observed that Villus width and surface area were higher in broilers divergently selected for a low (D−) AMEn on a wheat-based diet than in broilers selected for a high (D+) AMEn in the whole intestine and concluded that this is an attempt to compensate for the low functionality of the gastric area. Furthermore, villus height was higher for D− than for D+ in the jejunum. Their results also suggest that intestinal motility may have been indirectly modified by selection in AMEn.

Digestive enzymes activity

Our results indicated that digestive enzyme activity was significantly different among birds and diets ($P < 0.05$). Studies by Kadhim *et al.* (2014), Brzek *et al.* (2013) and O'Sullivan *et al*. (1992) have shown that levels of digestive enzymes in birds are significantly influenced by genetics. It was reported that lipase levels, were unaffected by a high content of fat in the diet (Gidez, 1973). Besides, the results of the present study revealed that lipase activity was not affected by the soybean oil levels in the diets. On the other hand, lipase activity significantly increased in laying hens with increased corn in the diet (T3; $P < 0.05$). Furthermore, our results indicated that in broiler chickens and laying hens, amylase activity significantly increased with increased corn in the diet (T3; $P < 0.05$). Also, in broiler chickens, amino peptidase activity in test diets (T2 and 3) was significantly higher than in the reference diet ($P < 0.05$). In contrast to our results, Brzek *et al.* (2013) found that diet had no significant effect on mass-specific enzyme activities, nor amylase to chymotrypsin and amylase to trypsin ratios in chickens and quails. Rideau *et al.* (1983) concluded that the intestinal contents and enzyme activities in laying hens during egg formation were higher than in laying hens during the pause stage. It appears that the light-dark cycle and egg formation might influence enzyme synthesis and secretion rates. Similar to our results, the study of Nir *et al.* (1993) confirmed that the activity of digestive pancreatic enzymes is similar in both types of hens, but the activity of enzymes in the small intestine is higher in laying chicks than in broiler chickens.

Some studies confirmed that feed restriction in broiler chickens significantly affected digestive enzyme gene expression and activity, with effects varying depending on age (Duarte *et al*., 2011; Cristiane *et al.,* 2014). In fact, Shamoto and Yamauchi (2000) demonstrated that feed restriction affects intestinal villus height, cell area, cell proliferation, and mitosis rate. According to this content, feed restriction, different lighting programs (in broiler breeder hens, laying hens, and broiler chickens), age, and bird strain may all affect digestive enzyme activity.

Nutrients digestibility coefficient

The results indicated that nutrient digestibility decreased as age increased, with lower digestibility observed at 40 and 62 weeks compared to 35 days. Adedokun *et al.* (2014) found that dry matter and Met and Lys digestibility values of all SBM samples evaluated were higher ($P < 0.05$) in broilers

compared with those from laying hens. The high intestinal uptake of nutrients in young chickens may be associated strictly with their rapid growth rate as well as a larger intestinal surface area per unit weight (Uni *et al*., 1995; Ferrer *et al.,* 2003). Yaghoobfar (2013) showed that the digestibility of most amino acids in sunflower meal was greater in broiler chickens (42d) compared to adult cockerels. Similarly, Almiral *et al.* (1995) described that the crude protein digestibility of corn for roosters was lower than broiler chickens. Broilers exhibited higher digestibility of amino acids from most ingredients compared to layers and roosters. The reasons for the improved digestion in broilers are not fully understood. One potential explanation is that modern fast-growing broilers possess greater nutrient transport capacity and a larger intestinal mass compared to layers and roosters (Nir *et al.,* 1993; Uni *et al.,* 1995). In the present study, the diets of broilers and layers contained sunflower oil, which may affect the rate of feed passage and gastric emptying by increasing the secretion of cholecystokinin in the duodenum, thus improving the digestibility of nutrients compared to broiler breeders. In addition, the presence of cholecystokinin in the blood stimulates the secretion of digestive enzymes from the pancreas, thus contributing to better digestibility of proteins and carbohydrates (Huang *et al.,* 2006).

Our study revealed that in 62-week-old broiler breeder hens, aminopeptidase activity was higher compared to 35-day-old broilers and 40-week-old laying hens in reference diet. Additionally, intestinal villus length and absorptive surface area were numerically greater in broiler breeders than in laying hens. However, nitrogen digestibility was lower in broiler breeders compared to both broilers and laying hens. These findings suggest a potential decline in nutrient transporter activity with advancing age in broiler breeder hens. The results of Birds *et al.* (1994) showed that passive glucose transport was constant with age in rats, but active transport peaked at 2 months of age and subsequently declined steadily thereafter. These results indicate that age specifically modifies active transport function in mouse jejunal mucosa without concomitant changes in jejunal wall structure. According to the above explanations, the differences in nutrient absorption between broiler breeder hens, broiler chickens, and laying hens in this study may be due to differences in age and genotype.

Conclusion

In conclusion, the present study demonstrated significant variations in the physiological capacity of broiler breeder hens, broiler chickens, and laying hens to utilize energy from corn and soybean meal. As standard feed tables often list Metabolizable Energy values derived from studies conducted on White Leghorn roosters, relying solely on these values for diet formulation in other bird types may lead to energy imbalances in their diets.

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