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Growth Performance and Energy Utilization of broilers fed High and Low Metabolizable Energy Diets Supplemented with Multi-Enzyme

Research Article

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ABSTRACT

This experiment was determined to investigate the effects of exogenous-enzyme addition to the high and low levels of metabolizable energy (ME), on performance, energy utilization and body composition of broiler chickens fed corn-soybean meal diets from 0 to 21 days of age. 240 one-d-old male Ross 308 broilers were used in a completely randomized 2 × 2 factorial design, with six replicate cages per treatment. Two metabolizable energy level: 3150 or 2750 kcal/kg and two enzyme (with xylanase beta-glucanase, and phytase activity) level: 0 and 200 mg/kg dry matter (DM) were used. There was a main effect of energy ($P<0.05$) on feed intake of broilers and feeding 2750 kcal/kg ME diet increased feed intake (FI) of broilers in the whole period. The addition of enzyme to 2750 kcal/kg ME diet significantly ($P<0.05$) improved the average daily gain (ADG) and feed conversion ratio (FCR) of broiler chickens on 0-21 days of age ($P<0.05$). While net energy for production (NEp), NE, ME, organic matter, and dry matter digestibility were improved ($P<0.05$) by supplementing both energy level diets with enzyme at 21 d, only addition of enzyme to 2750 kcal/kg ME diet reduced heat production (HP) of broilers in the total trial ($P<0.05$). On day 21, the amount of NE improvement by enzyme supplementation was greater than ME for 2750 and 3150 kcal/kg, respectively. This study showed that NE is a more sensitive energy utilization measure than ME for evaluating the response of broilers to enzyme supplementation, and the energy retained as fat and protein in the body carcass was higher for the birds fed lower energy diet supplemented with enzyme.

KEY WORDS β-glucanase, body composition, broiler chickens, energy retention, net energy, xylanase.

INTRODUCTION

The presence of anti-nutritive compounds such as phytate and non-starch polysaccharides (NSPs) in feed can negatively affect the performance of poultry. The application of NSP degrading enzyme or phytase in numerous studies has resulted in energy retention and growth performance improvement of broiler chickens alongside with depolymerization of anti-nutritional compounds (Kiarie *et al.* 2014; Pirgozliev *et al.* 2015; Hashemipour *et al.* 2016). The energy concentration of diet is one of the major factors for the rapid growth of broiler chicks. It is reported that over a wide range of dietary energy levels, broilers tended to consume feed to fulfil their energy requirement (Hill and Dansky, 1954). In other words, feeding diets deficient in energy to broiler chickens may not provide them with their energy requirement. In contrast, at higher energy levels of diets, birds may consume more feed than is needed for the

higher growth rate, and the extra energy may be deposited in the body as fat (Spring, 1957). Zhou *et al*. (2009) reported that supplementation of xylanase, α-amylase, and protease mixture improved energy availability in corn-soybean diet with lower metabolizable energy (ME) greater than higher ME alongside with higher protein digestibility improvement.

Poultry diets are generally formulated according to the nutritive values of available feed ingredients. These ingredients are mixed in order to provide the required nutrient level at a minimum cost. Therefore, it seems more practical for poultry producers to use an optimal energy level when using enzyme to compensate for the extra cost of enzyme supplementation. Some reports indicated that enzyme supplementation is more effective in a younger broiler chicken, which may be related to the inefficient production of endogenous digestive enzymes at an earlier life period (Rotter *et al*. 1990). Olukosi *et al*. (2008) demonstrated that enzyme supplementation had more positive effects on broiler chickens at a younger age, and the contribution of enzyme to nutrient retention reduced with age in broiler chickens.

The ME system had been generally accepted and used for energy evaluation of diets for poultry, which calculate energy loss of excreta. However, this system is failed to calculate energy losses in the liquid, solid, and gaseous excreta or as heat (Pirgozliev and Rose, 1999). Another measure of energy is the net energy (NE) system in which the efficiency of ME utilization is considered and it might be more accurate than ME for determination of energy utilization efficiency in poultry (De Groote, 1974). Pirgozliev *et al*. (2011) reported a high correlation between bodyweight and NE for production. According to the definition, NE is the amount of available energy for the poultry after ME has been used to provide the heat increment of feeding (HIF). It seems that NE may be a more sensitive and accurate evaluation of energy truly used by the broilers fed diet containing exogenous enzyme since it considers the efficiency of ME utilization for growth. There are two different methods identified for NE determination including carbon-nitrogen method and comparative slaughter technique and both of them can be applied to determine NE when diet include exogenous enzyme as reported by several researchers (Olukosi *et al*. 2008; Nian *et al*. 2011; Hossain *et al*. 2012; Barekatain *et al*. 2014). However, in comparison with carbon-nitrogen, the comparative slaughter method seems to be more economical and simulates the real rearing environment (Sakomura *et al*. 2003).

It was hypothesized that the addition of xylanase, beta-glucanase and phytase cocktail is effective on performance and energy, fat and protein retention in broilers especially at the earlier age of growing period and the improvement can be truly expressed by NE system. Therefore, the current study aimed to evaluate the effects of enzyme addition on performance, ME, NEp, heat production, protein, and fat retention in broiler chickens fed corn-soy meal-based diets with low and high levels.

MATERIALS AND METHODS

Diets and enzyme

The trial diets were formulated with two ME levels (2750 and 3150 kcal/kg) representing a high and low concentration of diet energy content for broiler chickens and two enzyme inclusion rates (0 and 200 mg/kg). The basal diet was corn-soybean and birds were fed the experimental diets for 21 days. The ingredients compositions of diets are presented in Table 1. Titanium oxide was added to the diets as an indigestible marker to enable the determination of digestibility. The multi-enzyme was Rovabio Max Advance P 25 and provided Endo-1,4 beta-xylanase 6250 visco unit/g, endo-1,3(4) beta-glucanase 4300 visco unit/g, and 6-phytase 2500 FTU/g, obtained from a fermentation broth of *Talaromyces versatilis* and *Schizosaccharomyces pombe* complex.

Birds and management

A total of 264 male Ross broiler chicks with an average initial body weight of 46.5 ± 2.7 g were used in a 21-day growth assay. On the first day, 24 chicks were randomly killed before starting the trial as a first slaughter group. The remaining 240 birds were assigned into four treatment groups with six replicates of 10 chicks. Twenty-four cages were provided for the four treatment groups. Housing conditions were closely monitored to ensure similar environmental conditions in each cage (The space allocation per bird in cages was 570 cm^2). The temperature was maintained at 32 to 34 °C for the first 3 days and then gradually reduced to 23 °C at the end of the experiment (21 day of age). The birds were also provided with an 18: 6 hours lighting to darkness lightening regimen, except for the first three days when they were offered with 24-hr illumination. Feed was offered *ad libitum* and water was freely available. The other slaughter groups of 48 chicks (2 birds from each replicate) each made up the final slaughter groups that were killed by cervical dislocation at 10 and 21 day. On slaughter days, after weighing the birds, feed was withdrawn for 5 hours before cervical dislocation. The carcasses were subsequently frozen, feed intake (FI) and bodyweight of birds were measured at the end of each period. In order to determine ME, excreta were collected from each cage in the last 6 days of each period (0 to 10 d and 11 to 21 d). Before being dried, the excreta were frozen in a forced-air oven to a constant weight. Then, the samples were pooled within each cage and ground before analyses.

Table 1 Ingredient composition of experimental diets

Treatments	T_1 (A_1B_1)[1]	T_2 (A_1B_2)	T_3 (A_2B_1)	T_4 (A_2B_2)
Ingredients				
Corn	565.2	565.2	550	550
Wheat bran	29.0	29.0	-	-
Soybean meal (44% CP)	286.1	286.1	250.2	250.2
Corn gluten meal (62% CP)	44.4	44.4	78.1	78.1
Soya oil	8.2	8.2	54.2	54.2
Dicalcium phosphate (18.7% P)	18.0	18.0	17.1	17.1
$NaHCO_3$	3.8	3.8	4.0	4.0
Limestone	12.4	12.4	12.9	12.9
Sodium chloride	4.0	4.0	3.4	3.4
L-lysine-HCL	4.0	4.0	4.8	4.8
DL-methionine	2.9	2.9	3.3	3.3
Vitamin–mineral premix[2]	5.0	5.0	5.0	5.0
Titanium oxide marker	17.0	17.0	17.0	17.0
Enzyme, mg/kg of diet	-	200.0	-	200.0
Calculated nutrient contents				
ME, kcal/kg	2750	2750	13.18	13.18
Crude protein, g/kg	203	203	203	203
Calcium, g/kg	10.1	10.1	10.2	10.2
Available phosphorus, g/kg	4.9	4.9	5.1	5.1
Arginine, g/kg	12.6	12.6	12.6	12.6
Lysine, g/kg	13.2	13.2	14.8	14.8
Methionine+cyctine, %	8.9	8.9	9.5	9.5
Dietary Cation-Anion balance	250	250	250	250

[1] A: metabolizable energy (ME) concentration (A_1: 2750; A_2: 13.18); B: enzyme inclusion rate (B_1: 0; B_2: 200 mg/kg diet).
[2] Provided the following (per kg of diet): vitamin A (transretinyl acetate): 9000 IU; vitamin D_3 (cholecalciferol): 2000 IU; vitamin E (allrac-tocopherol acetate): 18 IU; vitamin K (bisulfate menadione complex): 2 mg; Riboflavin: 6.6 mg; Pantothenic acid (D-calcium pantothenate): 10 mg; Pyridoxine (pyridoxine HCl): 3 mg; Folic acid: 1 mg; Thiamin (thiamin mononitrate): 1.8 mg; vitamin B_{12} (cyanocobalamin): 15 µg; D-biotin: 0.1 mg; Niacin: 30 mg; Choline (choline chloride): 500 mg; Ethoxyquin: 0.1 mg; Se (Na_2SeO_3): 0.2 mg; I (KI): 1 mg; Cu ($CuSO_4$.$5H_2O$): 10 mg; Fe ($FeSO_4$.7H2O): 50 mg; Zn (ZnO): 85 mg and Mn ($MnSO_4.H_2O$):100 mg.

Chemical analysis

To determine ME, grab samples excreta and diet samples were analyzed for gross energy, dry matter (DM) and organic matter. Titanium concentration in the diets and excreta samples was determined using the method of Short *et al.* (1996). The whole intact chicken was frozen immediately after being killed and later processed. Frozen birds were partially thawed, coarse-ground, and mixed in a blender to obtain a homogenous subsample. Gross energy, protein, and fat contents of samples were determined according to the methods of AOAC (1990).

Calculations

A series of calculations were carried out according to Olukosi *et al.* (2008). ME (kcal/kg) for each period using the following formula:

(A) $ME = GE_d - [GEe \times Ti_d / Ti_e]$

Where:
GE_d: gross energy (kcal/kg) in diet.
Gee: gross energy (kcal/kg) in excreta.
Ti_d: concentration of Titanium oxide in the diets.
Ti_e: concentration of titanium oxide in the excreta.

Net energy for production was calculated as follows:

(B) Initial GE of carcass (kcal)= carcass GE (kcal/g) × bodyweight of bird (g)
(C) Final GE content of carcass (kcal)= carcass GE (kcal/g) × bodyweight of bird (g)
(D) NEp (kcal/)= (C) – (B)

NE (KCAL/kg) for each period calculated was subsequently as follows:
(E) NE= (NEp+FHP) / FI

Where:
FHp: fasting heat production is value of 108 kcal/$BW^{0.70}$ per day, corresponding to the asymptotic Hp (at zero activity), according to the estimation made by Noblet *et al.* (2010).
FI and NEp: feed intake and retained energy (kcal/day) of broiler in each period.

Heat production (HP), which consists of fasting HP and the heat increment of feeding, is calculated using the following formula:

(F) HP (kcal)= MEI – NEp

Where:
MEI: ME intake was calculated using the following formula:
(G) MEI (kcal)= ME (kcal/g) × feed intake (g)

The following formulas were used to calculate energy retained as fat (REf) and as protein (REp) were as follows:

(H) REf (kcal)= carcass fat (g) × 9.13 kcal/g
(I) REp (kcal)= carcass crude protein content (g) × 5.64 kcal

The values 9.13 and 5.64 kcal/g are energy values per gram of fat and protein respectively, and were according to Olukosi *et al*. (2008).

Since excreta were collected for the last 6 days of each period, ME intake was determined for each period. The MEI for birds were killed at day 10 was calculated using days 0–10 feed intake. The ME intake for birds killed at day 21 was calculated by adding the ME intakes from the 0–10, and 10–21 day periods. Metabolizable energy, DM, and OM digestibility were determined for each period (10 and 21 days).

(J) Efficiency of ME use for energy retention (K_{RE})= NEp / MEI
(K) Efficiency of ME use for lipid retention (K_{REf})= REf / MEI
(L) Efficiency of ME use for protein retention (K_{REp})= REp / MEI

Statistical analysis

The study was conducted in a completely randomized factorial with two levels of enzyme and ME. Data obtained were analyzed using the GLM procedure of SAS (2003). For all variables, when a significant difference was detected, means were separated using least-squares means the option of SAS at $P < 0.05$.

RESULTS AND DISCUSSION

The results of bird growth performance, ME and NE is presented in Table 2. There was a main effect of energy level on feed intake of birds in 11-21 and 0-21 day periods ($P<0.05$), and feeding 2750 kcal/kg energy-containing diet significantly increased feed intake of birds ($P<0.05$). The group fed enzyme had greater ADG in the whole trial ($P<0.05$).

Also, there was enzyme × energy interaction on both ADG and FCR in the 0-21 d period ($P<0.05$), and only addition of enzyme to 2750 kcal/kg energy-containing diet significantly ($P<0.05$) improved these criteria.

Not only corn contains phytate as a major anti-nutritional compound, but it also contains highly branched glucuronoar-arabinoxylans (Maisonnier *et al*. 2001), which can be degraded by exogenous enzymes into a series of oligomers. These structures have high water-capacity known as responsible for high intestinal viscosity and depressed performance in poultry (Kalmendal and Tauson, 2012). This condition would alter nutrients transportation at the mucosal surface and confined the capacity for digestive enzymes production, especially at an early age (Ribeiro *et al.* 2011). On the other hand, phytic acid in corn has the potential to bind protein and minerals at different pH of the gastrointestinal tract and therefore lead to the lower solubility of nutrients and eventually reduce their utilization. In agreement with earlier studies, multi-enzyme supplementation improved bodyweight and feed conversion ratio of birds in the whole period (Kocher *et al*. 2003; Zhou *et al.* 2009; Ribeiro *et al*. 2011). However, the improvement was significant only when birds fed lower energy concentration with the enzyme in the whole period. According to Zhou *et al*. (2009), the addition of xylanase, amylase, and protease cocktail to corn-soy-based diet improved energy availability of broiler chickens at lower ME contents more than higher ME diet. Kocher *et al*. (2003) reported an improvement in nutrient utilization and FCR of broilers when the enzyme was added to the low-energy diet. In the current study, experimental treatments were designed to provide high and low energy concentration diets in order to investigate at which certain energy level enzyme supplementation is more effective at the early growth stage for broilers. The results of the enzyme in terms of growth performance, ME and NE indicated that effect was more remarkable at lower-energy level, which is supported by earlier studies (Zanella *et al*. 1999).

There were main effects ($P<0.05$) of enzyme and energy level as well as enzyme × energy interaction on ME and NE of the diets for broilers in the whole period (Table 3). While enzyme addition significantly ($P<0.05$) improved ME only for 2750 kcal/kg diet, NE increased for both 2750 and 3150 kcal/kg containing-diet with enzyme as compared to unsupplemented diets in 0-21 d period. In 0-10 d, Enzyme supplementation enhanced ME values of 2750 and 3150 kcal/kg diet 110.8 kcal/kg (200.6 kcal/kg for NE) and 58.04 kcal/kg (128.56 kcal/kg for NE), respectively;on the hand, in 10-21 days period, the amounts of ME released from 2750 and 3150 kcal/kg containing-diet with enzyme were 114 kcal/kg (217.49 kcal/kg for NE) and 81.5 kcal/kg (141.01 kcal/kg for NE), respectively.

In comparison with ME system, the amount of energy released by enzyme at low and high energy-containing diets was higher when NE was considered as a main energy system.

Table 2 Effects of dietary treatments on feed intake (FI), average daily gain (ADG) and feed conversion ratio (FCR0 of broilers in 10 and 21 days of age

Treatments	FI (g/bird/day)			ADG (g/bird/day)			FCR		
	0-10 day	11-21 day	0-21 day	0-10 day	11-21 day	0-21 day	0-10 day	11-21 day	0-21 day
T1 (A1B1)	32.23	82.31^{a}	58.36^{a}	25.35	53.13^{b}	38.62^{b}	1.27	1.54	1.51^{a}
T1 (A1B2)	32.43	81.24^{a}	58.11^{a}	28.84	58.91^{a}	45. 1^{a}	1.12	1.41	1.3^{b}
T2 (A2B1)	30.28	77.41^{b}	55.01^{b}	25.07	56.27^{ab}	41.78^{ab}	1.23	1.38	1.32^{b}
T2 (A2B2)	30.16	78.01^{b}	54.75^{b}	27.79	57.07^{ab}	43.08^{a}	1.08	1.35	1.27^{b}
Pooled SEM	0.95	0. 9	0. 88	1.21	0.95	0.88	0.09	0.06	0.05
P for main effects and interactions									
ME	0.095	0.03	0.026	0.213	0.178	0.17	0.144	0.76	0.205
Enzyme	0.083	0.124	0.176	0.094	0.072	0.03^{v}	0.177	0.97	0.075
ME × enzyme	0.139	0.152	0.126	0.202	0.014	0.023	0.195	0.62	0.014

A: metabolizable energy (ME) concentration (A_1: 2750; A_2: 13.18); B: enzyme inclusion rate (B_1: 0; B_2: 200 mg/kg diet).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of dietary treatments on ME, NE (kcal/kg DM), DM and OM digestibility of broilers in 10 and 21 days of age

Treatments	MEI (kcal/kg DM)				NE (kcal/kg DM)				DM digestibility (%)		OM digestibility (%)	
	0-10 day	Improv.	10-21 day	Improv.	0-10 day	Improv.	10-21 day	Improv.	0-10 day	10-21 day	0-10 day	10-21 day
T1 (A1B1)	2755.73^{c}	136.14	2784.41^{c}	113.92	2021.98^{d}	201.1	2033.93^{c}	218.78	73.03^{b}	74.16^{b}	70.35^{b}	71.15^{b}
T1 (A1B2)	2889.57^{b}		2899.13^{b}		2222.75^{c}		2246.65^{b}		78.13^{a}	79.13^{a}	76.63^{a}	76.06^{a}
T2 (A2B1)	3173.99^{a}	16.71	3130.97^{a}	31.04	2456.97^{b}	114.64	2433.07^{a}	19.1	74.16^{b}	74.57^{b}	71.03^{b}	73.57^{ab}
T2 (A2B2)	3190.72^{a}		3164.43^{a}		2571.70^{a}		2452.19^{a}		76.74^{ab}	79.41^{a}	73.86^{ab}	75.16^{a}
Pooled SEM	47.8		26.29		50.19		43.02		0.814	0.851	1.01	0.82
P for main effects and interactions												
ME	0.002		0.011		0.011		0.01		0.078	0.138	0.231	0.225
Enzyme	0.084		0.003		0.012		0.013		0.008	0.003	0.013	0.005
ME × enzyme	0.056		0.0319		0.042		0.035		0.031	0.085	0.021	0.04

A: metabolizable energy (ME) concentration (A_1: 2750; A_2: 13.18); B: enzyme inclusion rate (B_1: 0; B_2: 200 mg/kg diet).
MEI: metabolizable energy; NE: net energy; DM: dry matter and OM: organic matter.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means and Improv: improvement.

A possible explanation for greater available NE for enzyme-treated groups is that enzyme reduced heat increment caused by fibre digestion in the gastrointestinal tracts of animals. Therefore, it could be concluded that not only NE system could truly estimate available energy for birds, but also it could be more cost-effective for producers to reduce the energy level of the formulated diet containing enzyme.

There were enzyme and enzyme × energy interaction (P<0.05) for the digestibility of organic matter and dry matter in 0-10 and 10-21 days periods. While supplementing both 2750 kcal/kg and 3150 kcal/kg energy diet with enzyme significantly (P<0.05) improved DM and OM digestibility on 0-10 day (P<0.05), the addition of the enzyme only to 2750 kcal/kg containing diets increased DM and OM digestibility in 10-21 days period (P<0.05). However, no significant (P>0.05) difference on the digestibility of OM and DM was observed in both energy levels.

The results of the positive effect of enzyme on DM and OM digestibility in our study is in agreement with the result of Zhou *et al.* (2009) who investigated the influence of enzyme preparation supplement on energy utilization efficiency of broilers fed with different metabolizable energy concentration and found that apparent digestibility of DM linearly decreased with the reduction of ME level and enzyme. Moreover, it is proposed that dietary NSP and phytate contents of cereals reduce feed DM and OM availability, probably due to their chemical structure and poor digestibility in broilers (Adeola *et al.* 2010). Indeed, in our experiment, supplementing the corn-soybean diet with a exogenous enzyme resulted in higher nutrient digestion at both higher and lower ME level diets. It seems that the exogenous enzyme alleviated the negative effects of anti-nutritive compounds and increased the accessibility of gastrointestinal enzymes to the nutrients.

The results of energy utilization and heat production is presented in Table 4. There were enzyme and enzyme × energy interaction (P>0.05) on NEp in 0-10 day and 0–21 day periods. Enzyme addition to 2750 kcal/kg and 3150 kcal/kg diets increased NEp in 0-21 day (P<0.05) and the greatest NEp was obtained for a diet with 2750 kcal/kg ME with enzyme. There were no effects of enzyme, energy, and enzyme × energy interaction on MEI in 0-10 and 0-21 day period (P>0.05). Contrary to our results, Olukosi *et al.* (2008) reported an improvement in MEI for broilers fed corn-wheat-soybean with a cocktail of xylanase, amylase and protease phytase or a single phytase. However, in their study, retained energy, which was expressed as net energy for production, significantly (P<0.05) improved with enzyme addition. The reason for the lack of significant effect of enzyme addition on MEI in the present study may be attributable to MEI formula (G), where the amounts of feed consumed and ME of the diet were calculated. Comparing to 3150 kcal/kg energy-containing diet (higher energy level), supplementing 2750 kcal/kg energy level (higher energy level) with enzyme improvement induced greater MEI, possibly as a result of higher feed consumption and ME digestibility of broilers.

However, when the MEI of birds was calculated, this significant difference has been disappeared. On the other hand, the NEp amount is highly dependent on the body energy content as well as the bodyweight of chicken (D). Improvement in different anti-nutritional compound degradation in our basal diet and nutrient digestion may be responsible for the enhancement in energy utilization. This result is in agreement with Barekatain *et al.* (2014) who observed exogenous xylanase improved NEp of birds fed sorghum distillers dried grains using the respiratory chamber and the comparative slaughter methods. Comparing to MEI, the greater improvement occurred in NEp with enzyme supplementation at both energy levels suggesting more reliability of bodyweight and body gross energy than ME and feed intake to predict the true amount of energy released when an exogenous enzyme is added to diet. The higher NEp of birds received lower ME level diet with enzyme could account for higher bodyweight and available energy of those chickens. It is proposed that extra energy released from nutrients retention would be deposited in the carcass and increase bodyweight and tissue energy concentration; as a consequence, this energy would be deposited as either fat or protein (Nourmohammadi *et al*. 2018).

Table 4 Effects of dietary treatments on metablisable energy intake (MEI), retained energy (RE) and heat production (HP) of broilers

Treatments	MEI (kcal/d)		NEp (kcal/d)		HP (kcal/d)	
	0-10 day	0-21 day	0-10 day	0-21 day	0-10 day	0-21 day
T1 (A1B1)	89.68	165.77	51.19^{b}	73.37^{b}	38.48	92.85^{a}
T1 (A1B2)	93.81	168.41	60.63^{a}	84.47^{a}	33.47	83.44^{b}
T2 (A2B1)	97.19	171.97	57.78^{ab}	83.29^{ab}	39.06	88.45^{ab}
T2 (A2B2)	94.81	173.25	57.32^{ab}	86.06^{a}	36.81	87.09^{ab}
Pooled SEM	2.37	2.58	1.82	2.14	3.02	2.07
P for main effects and interactions						
ME	0.212	0.115	0.13	0.107	0.125	0.092
Enzyme	0.137	0.7	0.012	0.037	0.237	0.02
ME × enzyme	0.312	0.145	0.022	0.021	0.234	0.023

A: metabolizable energy (ME) concentration (A_1: 2750; A_2: 13.18); B: enzyme inclusion rate (B_1: 0; B_2: 200 mg/kg diet).
MEI: metabolizable energy; NEp: net energy for production and HP: heat production.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of dietary treatments on retained energy as fat (REf), and retained energy as protein (Rep) of broilers

Treatments	Ref (kcal/d)		Rep (kcal/d)	
	0-10 day	0-21 day	0-10 day	0-21 day
T1 (A1B1)	19	30.77^{b}	28.93^{b}	36.12^{b}
T1 (A1B2)	21.68	38.06^{a}	34.14^{a}	43.81^{a}
T2 (A2B1)	20.41	35.32^{a}	32.64^{ab}	43.31^{a}
T2 (A2B2)	21.1	36.86^{a}	30.86^{ab}	45.47^{a}
Pooled SEM	1.29	0.93	1.17	1.07
P for main effects and interactions				
ME	0.172	0.136	0.132	0.108
Enzyme	0.156	0.025	0. 071	0.035
ME × enzyme	0.238	0.014	0.022	0.031

A: metabolizable energy (ME) concentration (A_1: 2750; A_2: 13.18); B: enzyme inclusion rate (B_1: 0; B_2: 200 mg/kg diet).
Ref: retained energy as fat and Rep: retained energy as protein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of dietary treatments on efficiency of metabolizable energy (ME) use for tissue energy deposition

Treatments	Efficiencies of ME use for energy retention					
	K_{REf}		K_{Rep}		K_{RE}	
	0-10 day	0-21 day	0-10 day	0-21 day	0-10 day	0-21 day
T_1 (A_1B_1)	0.212	0.189	0.321	0.219	0.561[b]	0.448
T_1 (A_1B_2)	0.232	0.221	0.36	0.249	0.666[a]	0.509
T_2 (A_2B_1)	0.21	0.205	0.331	0.252	0.595[ab]	0.498
T_2 (A_2B_2)	0.227	0.215	0.325	0.267	0.607[ab]	0.508
Pooled SEM	0.018	0.016	0.011	0.019	0.021	0.025
P for main effects and interactions						
ME	0.116	0.211	0.245	0.126	0. 22	0.14
Enzyme	0.155	0.61	0.081	0.118	0.022	0.163
ME × enzyme	0.196	0.211	0.15	0.126	0.014	0.13

A: metabolizable energy (ME) concentration (A_1: 2750; A_2: 13.18); B: enzyme inclusion rate (B_1: 0; B_2: 200 mg/kg diet).
K_{RE}: efficiency of ME use for carcass energy retention; K_{Ref}: efficiency of ME use for energy retained as fat and K_{Rep}: efficiency of ME use for energy retained as protein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Moreover, there was enzyme × energy interaction on HP and this criterion decreased by supplementing the 2750 kcal/kg diet with enzyme (P<0.05). These results are consistent with Olukosi *et al*. (2008), who observed that the addition of xylanase and amylase reduced the heat production of chickens fed diet wheat-corn-soybean based diet. Interestingly, in their study, the addition of phytase as the only enzyme increased the heat loss of broilers. While viscous condition resulted from NSP increase the relative size and length of the gastrointestinal tract of broiler chickens (Fan *et al*. 2009; Moftakharzadeh *et al*. 2017), enzyme inclusion in many studies has reduced relative weight of these energetically active organs (Alam *et al*. 2003). One possible reason for lower HP in enzyme treatments would be lower maintenance of active digestive organs induced by enzyme supplementation. Furthermore, it seems exogenous enzyme enhanced NEp of chickens, especially at the lower energy concentrations, which could partly explain the lower energy spending of animals. The results reported that the present study was in agreement with Barekatain *et al*. (2014), who hypothesized that the ME level of diets contain high fiber cereal and may overestimate the energy available for productive uses and may result in higher heat production and enzyme could alleviate this negative effect of these indigestible components.

The results of the energy deposited as fat or protein in the carcass is presented in Table 5. In 0-10 day period, energy retained as protein was significantly (P<0.05) affected by enzyme × energy interaction and enzyme addition improved this criterion for birds received 2750 kcal/kg diet. However, there was main effect of enzyme and enzyme × energy interaction on energy retained as protein and fat in 0-21 d period and only supplementing 2750 kcal/kg diet by enzyme increased (P<0.05) protein and fat deposition. It seems that the exogenous enzyme caused a higher quantity of nutrients and energy release from the lower energy concentration diet, possibly, resulting in higher carcass protein than fat.

Olukosi *et al.* (2008) reported that addition of phytase increased REf of broilers fed deficit ME and non-phytate phosphorous diet while REp of birds was not changed in the 21 days period. Notably, energy deposited as protein was more than fat in the 21 days period probably due to the time of our experiment which was at an early period of broilers growth when more protein is required to muscle and bone development. Similar results were reported by Bregendahl *et al.* (2002) in broilers at 21 day fed a common corn-soybean meal diet.

The data on the efficiency of use of MEI for RE, REp, or REf are presented in Table 6. There were main effects of enzyme and enzyme × energy interaction on the efficiency of MEI use for RE in 0-10 days period and addition of enzyme to 2750 kcal/kg ME level significantly (P<0.05) improved the efficiency of NEp during this period. These results are inconsistent with the result of Olukosi *et al.* (2008) who observed that addition of xylanase improved the efficiency of ME intake utilization for protein deposition, while ME efficiency for fat and energy retention remained unchanged. However, there were no enzyme, energy and enzyme × energy interaction on the efficiency of body composition and NEp in the whole period.

CONCLUSION

This study concluded that supplementing 2750 kcal/kg diet with enzyme resulted in higher NE, ME, NEp, fat and protein retaintion in the carcass along with lower HP as compared to 3150 kcal/kg diets, while MEI was not significantly changed during the whole experimental period. According to our results, it seems that NE is a more reliable system of energy to estimate the amount of energy released by exogenous enzyme for broilers fed corn-soybean diet. Furthermore, the determination of NE by the comparative slaughter method allows the partitioning of energy deposition and hence allows the evaluation of the effect of the exogenous enzyme on the efficiency of energy utilization.

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