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Flock Uniformity, Blood Indices, and Nutrient Retention of Broiler Chickens Fed Low Energy and Protein Diets Supplemented with Multi-Enzyme

Emmanuel Uchenna Ahiwe, Innocent Ejiofor, Tobechukwu Chijioke Iwuji, Ifeanyi Solomon Ahamba & Ajeh Lawrence Giwa

Department of Animal Science and Technology, Federal University of Technology, Owerri – PMB 1526. Owerri, Imo State, Nigeria

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Corresponding author
Ahiwe Emmanuel Uchenna
emmanuel.ahiwe@futo.edu.ng

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Abstract

A feeding trial was conducted using two-hundred-day-old male Ross-308 broiler chickens to evaluate the effect of multi-enzyme (Natuzyme®) on flock uniformity, some haematological and serum biochemical indices, and nutrient retention in broiler chickens fed low energy and protein (LEP) diets. The birds were randomly assigned to four experimental groups of 50 birds each in a completely randomized design. There were five replicates for each treatment group, with 10 chicks per replicate. The first group (positive control/PC) received a standard diet without multi-enzyme supplementation; whereas the LEP0, LEP0.25 and LEP0.50 groups received low-energy-protein diet (LEP) supplemented with multi-enzyme at 0 (negative control), 0.25, and 0.50 g/kg feed, respectively. The PC group received a standard diet having energy and protein of (3000 kcal/kg and 23%) and (3200 kcal/kg and 20%) at starter and finisher phases respectively. Other groups received LEP diets having energy and protein of the standard diet decreased by 100 kcal/kg and 0.60% both at starter and finisher phases. The feeding trial lasted for 42 days. On days 21 and 42 of the experiment, birds under the LEP0 and LEP0.25 groups had lower ($P < 0.05$) flock uniformity compared to those of PC and LEP0.50 groups. There was no significant effect of the dietary treatments on haematological and serum biochemical indices of broiler chickens. Birds fed LEP0 and LEP0.25 diets had significantly ($P < 0.05$) low metabolizable energy (ME), crude fiber (CF) and crude protein (CP) retention compared with the PC group. On the other hand, Broiler chickens offered the LEP0.50 diet had improved ($P < 0.05$) apparent ME, CF and CP retention. Results of the study suggest that multi-enzyme supplementation at 0.50 g/kg to low energy and protein broiler chicken diet improved flock uniformity as well as metabolizable energy, CF and protein retention without adverse effects on haematological indices and serum, metabolites.

Introduction

In recent decades, the high cost of broiler chicken feed has continued to negatively affect the profit margin of most poultry farmers in different parts of the world (Thirumalaisamy *et al.*, 2019). Maize and soybean apart from being the most commonly used broiler chicken feed ingredients, also constitute the highest portion of a balance conventional poultry feed (Esonu, 2015; Ahiwe *et al*, 2018). The food-feed competition between humans and animals for available maize and soybean is one of the several reasons why prices of these energy and protein feed ingredients are on a continuous increase thereby making it difficult for broiler farmers to maximize profit from the venture (Schader *et al.*, 2015).

This persistent increase in broiler chicken feed cost has forced several farmers to suspend their broiler chicken operations while some resort to the

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use of various unconventional feeding strategies. A common unconventional feeding strategy used by some farmers to reduce the high cost of feed is the use of low nutrient density diets to feed their broiler chickens. Reducing the nutrient content (energy, protein, etc.) below the recommended level has been reported to reduce the cost of feed but may result in lesser nutrients being available/retained, poor flock uniformity, and reduced growth rate of broiler chickens (Ahiwe *et al*., 2018).

In recent decades, various researchers have reported the ability of various supplements to improve nutrient availability, blood indices, and flock uniformity when low nutrient density diets are fed to animals (Salami & Odunsi, 2019; Zhao *et al*., 2019; Thanapal *et al*., 2021). A supplement that has been reported to improve the performance and flock uniformity of poultry by enhancing nutrient availability is exogenous enzymes (Anjum & Chaudhry, 2010; Salami & Odunsi, 2019, Cowieson & Kluenter, 2019).

Enzymes are catalysts that are produced within an animal or supplemented exogenously with the aim of improving nutrient availability, viscosity, and productivity (Dosković *et al*., 2013). There are various types of enzymes and each of these enzymes is specific in its mode of action (Ravindran, 2013; Alabi *et al*., 2019). In poultry nutrition, mono-enzyme products (containing just a type of enzymes such as protease or carbohydrase) can only act effectively when a single related nutrient is reduced below the recommended level (Jackson *et al*., 2010; Ravindran, 2013). But when mono-enzymes are supplemented into a broiler chicken diet having several low-density nutrients, results have been inconsistent (Mohammadigheisar & Kim, 2018; Moftakharzadeh *et al.*, 2019). Hence it is hypothesized that supplementing multi-enzyme to broiler chickens having several low-density nutrients may be more effective in releasing several bound nutrients. This may lead to better nutrient digestion and retention that may result in better flock uniformity without having any deleterious/health effects as can be identified in various blood parameters.

After an extensive search, we observed that there is no research that has considered the effect of multi-enzyme on the flock uniformity, blood indices, and nutrient retention of broiler chickens fed low energy and protein diets. We selected a multi-enzyme enzyme known as Natuzyme® because the product contains protease, carbohydrase, and enzymes that can degrade Non-Starch Polysaccharides (NSPs). The mechanism of action of the selected multi-enzyme is based on its potential to improve nutrient availability and release additional amino acids and energy in broiler chicken. Therefore, this research considers the effect of multi-enzyme on the flock uniformity, haematological indices, serum biochemistry, and nutrient retention of broiler chickens fed low energy and protein diet.

Materials and Methods

Experimental site

This study was conducted between mid-August – and September 2021 at the Poultry Unit of the Teaching and Research Farm, School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology Owerri (FUTO), Imo State, Nigeria. The experimental site is situated in the tropical rainforest zone of Nigeria and it lies between latitude and longitude of 5º 29᾽ 06s and 7º 02᾽ 06s with an elevation of 90.91m as well as an average rainfall of 2641mm (AC-Chukwuocha *et al.*, 2017; Iwuji *et al*., 2017). The experimental area experiences a daily tropical temperature range of 26.5-32°C, and average relative humidity of up to 80% (AC-Chukwuocha *et al*., 2017; Iwuji *et al*., 2017).

Source of test materials

Natuzyme® (Bioproton Pty Ltd, Sunnybank, Australia) that was used in this study was gotten from a commercial feed supplement shop located in Owerri, Imo State. Nigeria. Natuzyme® is a multi-enzyme containing, α-amylase: 1,800,000 unit/kg, protease: 6,000,000 unit/kg, cellulase: 5,000,000 unit/kg, β glucanase: 1,000,000 unit/kg, phytase: 500,000 unit/kg, xylanase: 10,000,000 unit/kg. Other feed ingredients (maize, soybean, etc.) used in the study were purchased from a commercial feed ingredient supplier located in Owerri, Imo State. Nigeria.

Experimental diets

Four experimental starter and finisher diets were formulated. The positive control (PC) group received a standard diet without multi-enzyme supplementation, while groups LEP0 (negative control), LEP0.25, and LEP0.50 were fed LEP with multi-enzyme supplementation at 0, 0.25, and 0.50 g/kg feed respectively. As shown in Tables 1 and 2, the PC diets were formulated to meet or exceed Ross 308 nutrient specification (Aviagen, 2019), with the starter PC diet having energy and protein values of 3000 kcal/kg and 23% respectively. The PC finisher diet had an energy and protein content of 3200 kcal/kg and 20% respectively. The low energy and protein (LEP) diets had energy and protein of the standard diet reduced respectively by 100 kcal/kg and 0.60% both at the starter and finisher phases.

Experimental diets were in mash form throughout the experiment. The birds were given feed and water *ad libitum* for 6 weeks (comprising 3 weeks each for starter and finisher phases). Metabolisable energy (ME), crude protein (CP), crude fiber (CF), calcium, available phosphorous, thronine, methionine, and

lysine contents of the diets were calculated using the NRC (1994) nutrient composition table. In addition, the diets were analyzed for Dry matter, ash, CP, CF, and ether extract (EE) according to AOAC (2007) procedure. The ME was derived using the equation described by Palic *et al.*, 2012.

Table 1. Composition of the experimental starter diets (0-21 days)

Item	Control	LEP0	LEP0.25	LEP0.50
Feed Ingredients (%)[a]				
White corn, ground	52.00	48.00	48.00	48.00
Soybean meal, 44% CP	29.00	26.00	26.00	26.00
Wheat offal	3.00	8.00	8.00	8.00
Palm oil	1.00	0.50	0.50	0.50
Palm kernel cake	5.00	7.00	7.00	7.00
Fish meal	3.00	3.00	3.00	3.00
Brewer's spent grain	2.84	6.34	6.34	6.34
Bone meal	3.00	3.00	3.00	3.00
Common salt	0.25	0.25	0.25	0.25
L-Threonine	0.16	0.16	0.16	0.16
L-Lysine HCl	0.25	0.25	0.25	0.25
DL-Methionine	0.25	0.25	0.25	0.25
Vitamin/mineral premix[b]	0.25	0.25	0.25	0.25
***Analyzed composition* (%)**[c]				
Dry matter	92.36	92.08	92.08	92.08
Metabolizable energy (kcal/kg)	3008	2909	2909	2911
Ether extract	3.39	3.21	3.22	3.22
Crude protein	23.09	22.41	22.43	22.42
Crude fiber	4.41	7.23	7.23	7.23
Ash	2.28	2.27	2.27	2.27
***Calculated analysis* (%)**[d]				
Metabolizable energy (kcal/kg)	3000	2900	2900	2900
Crude protein	23.00	22.40	22.40	22.40
Crude fiber	4.30	7.19	7.19	7.19
Calcium	0.93	0.93	0.93	0.93
Phosphorous, available	0.48	0.48	048	0.48
Methionine	0.52	0.51	0.51	0.51
Lysine	1.28	1.27	1.27	1.27
Threonine	0.87	0.87	0.87	0.87

[a]Feed ingredient composition is according to NRC, 1994.
[b]Supplied per kg diet: Retinol 12000 IU; thiamine 1.43 mg; cholecalciferol 3500 IU; Niacin 40.17 mg; α-tocopherol 44.7 IU; riboflavin 3.44 mg; pantothenic acid 6.46 mg; pyridoxine 2.29 mg; biotin 0.05 mg; folic acid 0.56 mg; cyanocobalamin 0.05 mg; menadione 2.29 mg; Iron 120 mg; Zinc 120 mg; copper 15 mg; manganese 150 mg; cobalt 0.4 mg; selenium 0.3 mg; iodine 1.5 mg.
[c,d]Nutrients were analysed and calculated to meet or exceed Ross 308 nutrition specifications (Aviagen, 2019).
[c]Chemical analysis was performed according to AOAC (2007).

Birds, experimental design, housing, management, and duration of the study

Two hundred, one-day-old, male broiler chickens (Ross-308) were procured from a commercial hatchery with a mean bodyweight of about 40±1 g. The chicks were weighed, allotted to four dietary groups of 50 chickens each, and replicated five times with 10 broiler chickens per replicate. The broiler chickens were housed in a half-walled open wire mesh pen constructed on the concrete floor inside a poultry house. The replicate floor pen had a dimension of 2m x 2m. The concrete floor of each experimental pen was covered with fresh wood shavings (about 1-2 cm high from the surface of the floor pen). On day one of the experiment, the birds were provided with uninterrupted 24 hours light. Subsequently, a lighting regime of 18 hours and 6 hours of darkness was provided all through the feeding trial.

Each experimental pen was equipped with a drinker, feeder, and brooder. Broiler chicks irrespective of the treatment group were raised under similar management and hygienic conditions. A complete randomized design was used for the study. The experiment design and procedures were approved by the Animal Ethics Committee at the School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri (FUTO), Imo State, Nigeria. Furthermore, the trial was performed following international accepted standard ethical guidelines stipulated for animal use and care. The experiment lasted for 42 days.

Table 2. Composition of the experimental finisher diets (22-42 days)

Item	Control	LEP0	LEP0.25	LEP0.50
Feed Ingredients (%)[a]				
White corn, ground	56.00	54.00	54.00	54.00
Soybean meal, 44% CP	23.00	21.50	21.50	21.50
Wheat offal	2.50	5.00	5.00	5.00
Palm oil	3.00	1.00	1.00	1.00
Palm kernel cake	4.00	5.00	5.00	5.00
Fish meal	3.00	3.00	3.00	3.00
Brewer's spent grain	3.89	6.89	6.89	6.89
Bone meal	3.50	2.50	2.50	2.50
Common salt	0.25	0.25	0.25	0.25
L-Threonine	0.11	0.11	0.11	0.11
L-Lysine HCl	0.25	0.25	0.25	0.25
DL-Methionine	0.25	0.25	0.25	0.25
Vitamin/mineral premix[b]	0.25	0.25	0.25	0.25
***Analyzed composition* (%)**[c]				
Dry matter	93.54	93.28	93.28	93.28
Metabolizable energy (kcal/kg)	3212	3106	3108	3109
Ether extract	3.50	3.20	3.21	3.20
Crude protein	20.07	19.40	19.40	19.40
Crude fiber	4.20	7.20	7.30	7.30
Ash	1.60	2.20	2.21	2.20
***Calculated analysis* (%)**[d]				
Metabolizable energy (kcal/kg)	3200	3100	3100	3100
Crude protein	20.00	19.40	19.40	19.40
Crude fiber	4.10	7.17	7.17	7.17
Calcium	0.75	0.75	0.75	0.75
Phosphorous, available	0.38	0.38	0.38	0.38
Methionine	0.40	0.40	0.40	0.40
Lysine	0.96	0. 96	0.96	0.96
Threonine	0.64	0.64	0.64	0.64

[a]Feed ingredient composition is according to NRC, 1994.
[b]Supplied per kg diet: Retinol 12000 IU; thiamine 1.43 mg; cholecalciferol 3500 IU; Niacin 40.17 mg; α-tocopherol 44.7 IU; riboflavin 3.44 mg; pantothenic acid 6.46 mg; pyridoxine 2.29 mg; biotin 0.05 mg; folic acid 0.56 mg; cyanocobalamin 0.05 mg; menadione 2.29 mg; Iron 120 mg; Zinc 120 mg; copper 15 mg; manganese 150 mg; cobalt 0.4 mg; selenium 0.3 mg; iodine 1.5 mg.
[c,d]Nutrients were analysed and calculated to meet or exceed Ross 308 nutrition specifications (Aviagen, 2019).
[c]Chemical analysis was performed according to AOAC (2007).

Sampling procedure and analyses

Flock uniformity

Each treatment group's individual broiler chicken was weighed on days 1, 21, and 42 of age. The equation described by Jackson *et al* (2004) and Aviagen (2018) was used to calculate the flock uniformity of the birds using the coefficient of variation of the individual bodyweight of the birds.

Flock uniformity (%) = 100 – ((STDEV/ABW) × 100)

Where:

STDEV = standard deviation of a replicate.

ABW = average live body weight.

Blood parameters

On d 42, 2 birds per replicate (10 birds per group) were randomly selected and a dispensable 10 mL syringe was used to draw blood via the wing vein. To determine various haematological parameters, about 5mL of blood was collected and put into sterilized glass tubes containing ethylene diamine tetra-acetic acid (EDTA) an anticoagulant. The determination of the haematological parameters (pack cell volume (PCV), Haemoglobin (HB), Red blood cell (RBC), white blood cell (WBC)), was carried out according to the procedure of Ochei & Kolhatkar (2007). Another 5mL of blood was put into tubes without anticoagulants for serum biochemistry. Blood samples for serum assay were centrifuged, separated, and freeze stored at -10ºC. The serum total protein, albumin, and creatinine analyses were carried out according to the procedure outlined by Ochei & Kolhatkar (2007). Globulin concentration was obtained by subtracting Albumin from Total protein.

Nutrient retention trial

At the end of the feeding trial (d 42), two birds per replicate (10 birds from each group) were randomly selected and kept in metabolic cages for total fecal collection following a standard method (Low, 1990). The ABW of the birds selected for the nutrient retention trial was similar to the ABW of the remaining broiler chickens left in each treatment. During the nutrient retention trial, the selected broiler

chickens were fed the respective experimental diets. The birds were allowed 5 days to acclimatize or adjust. After the acclimatization or adjustment period, fecal collection was made daily for the next 7 days at 7 am. Each day's collection was pooled for each treatment group, thoroughly mixed, weighed, dried, and stored in screw-capped bottles for analysis. In addition, feed samples were collected and weighed from each replicate and stored for analysis.

The fecal and feed samples were taken to the Department of Animal Science and Technology Laboratory of the SAAT, FUTO, Imo State, Nigeria for analysis. The proximate composition Dry matter (DM), crude protein (CP), ether extract (EE) or crude fat (Cfat), crude ash (CA), and crude fibre (CF) of the feed and fecal samples were determined according to the procedures of AOAC (2007). The enzyme digestibility of organic matter (EDOM) was determined using the procedure described by Palic *et al*., 2012. Furthermore, the metabolizable energy (ME) in the diet and fecal samples were calculated using the equation described by Palic *et al*., 2012 and shown below:

ME (MJ/kg DM) = 5.46 – 0.2166 × CA - 0.0946× CF + 0.2219 CFat + 0.1054 × EDOM

Thereafter, the nutrient retention of the measured nutrient composition (DM, ME, CP, EE, CA, CF), was calculated using the following formula (Abdel-Daim *et al*., 2020)

$$\text{Nutrient Retention} = \frac{\text{Nutrient in feed} - \text{Nutrient in excreta}}{\text{Nutrient in feed}} \times 100$$

Statistical analyses

Data obtained on flock uniformity, haematological indices, serum chemistry, and nutrient retention were subjected to one-way analysis of variance (ANOVA) in the general linear model of Minitab 17 statistical package (Minitab Inc. state college, PA, and USA, 2013). The difference between the mean values was set to be significant at $P < 0.05$, and these mean values were also separated using Turkey's range test contained in Minitab 17 software**.**

Results

Flock uniformity

The flock uniformity at days 1, 21, and 42 of broiler chickens fed the dietary treatments are presented in Table 3. The flock uniformity of broiler chicks on day 1 was similar ($P > 0.05$) across all treatment groups. At d 21, birds in the LEP0 and LEP0.25 groups had poor flock uniformity compared to the PC group. However, compared to the control group, supplementation of multi-enzyme at 0.50 g/kg to the broiler diet having LEP resulted in improved flock uniformity. At d 42 (finisher phase), a similar trend observed (poor flock uniformity) at the starter phase continued for birds fed LEP. Though the LEP0.25 group had better flock uniformity than birds fed the LEP0 diet, they were still inferior to birds fed the PC diet. However, Birds in the LEP0.50 group had a flock uniformity that was comparable to those in the PC group.

Table 3. Flock uniformity (%) of broiler chickens fed the experimental diets at different ages[1]

Age (days)	PC[2]	LEP0 [3]	LEP0.25 [4]	LEP0.50 [5]	*SEM* [6]	*P*-values
1	91.2	91.8	91.5	91.0	0.24	0.10
21	91.5[a]	78.2[b]	79.4[b]	90.6[a]	1.42	0.004
42	91.9[a]	76.8[c]	80.7[b]	91.0[a]	1.51	0.003

[a,b,c.] Means in the same row not sharing a common superscript are significantly different ($P < 0.05$)

% Flock uniformity ± standard deviation (SD); 5 replicates of 50 birds each.

[1]Data presented the means flock uniformity based on 5 replicate pens per treatment

[2]PC: Positive control

[3]LEP0: low energy and protein without multi-enzyme supplementation.

[4]LEP0.25: low energy and protein supplemented with 0.25 g/kg multi-enzyme.

[5]LEP0.50: low energy and protein supplemented with 0.50 g/kg multi-enzyme.

[6]*SEM*: standard error of the mean.

Blood indices

Effects of dietary treatments on blood parameters are presented in Table 4. . There was no significant effect ($P > 0.05$) of feeding broiler chickens with diets having LEP or multi-enzymes on the haematological parameters considered in the present study. The dietary treatments did not have a significant effect ($P > 0.05$) on selected serum biochemical indices (albumin, globulin, total protein, and creatinine) of broiler chickens.

Nutrient retention

The apparent nutrient retention (%) values of broiler chickens fed the respective dietary treatments are shown in Table 5. There was no significant difference ($P > 0.05$) in the apparent nutrient retention of the DM, EE, and CA, in the present study. Compared to birds fed the PC diet, those fed the LEP0 and LEP0.25 diets had poor ($P < 0.05$) ME, CP, and CF, retention. While the LEP0.25 group had better ME, CP, and CF retention than those in the LEP0 group. However, it was noticed that broiler chickens in the

LEP0.50 group had improved ($P < 0.05$) ME, CP, and CF, retention that was superior to those in the LEP0.25 group but similar to birds in the PC group.

Table 4. Haematological indices of broilers fed low energy and low protein diets supplemented with multi-enzyme [1]

Item	PC[2]	LEP0 [3]	LEP0.25 [4]	LEP0.50 [5]	*SEM* [6]	*P*-value
Hematological indices						
PCV (%)	33.20	32.50	32.87	32.99	0.34	0.21
Hb (g/dL)	11.25	11.11	11.18	11.20	0.12	0.14
WBC (x10^6/mL)	12.82	12.76	12.80	12.81	0.82	0.09
RBC (10^6/mL)	3.06	2.94	2.98	3.02	0.07	0.12
MCH (%)	36.44	36.31	36.39	36.41	1.06	0.14
Serum indices						
Albumin (g/dL)	2.34	2.27	2.30	2.32	0.15	0.10
Globulin (g/dL)	3.97	3.92	3.94	3.96	0.22	0.09
Total protein (g/dL)	6.31	6.19	6.24	6.28	0.36	0.12
Creattinine (mg/dL)	0.48	0.41	0.44	0.46	0.04	0.11

[1]Data presented the means based on 5 replicate pens per treatment and 2 broiler chickens per replicate.
[2]PC: positive control
[3]LEP0: low energy and protein without multi-enzyme supplementation.
[4]LEP0.25: low energy and protein supplemented with 0.25 g/kg multi-enzyme.
[5]LEP0.50: low energy and protein supplemented with 0.50 g/kg multi-enzyme.
[6]*SEM*: standard error of the mean.
PVC-Pack cell volume; Hb- haemoglobin, WBC-white blood count; RBC- red blood cell count; MCH-mean corpuscular haemaglobin

Table 5. Nutrient retention of broiler chickens on the experimental diet[1]

Item (%)	PC[2]	LEP0 [3]	LEP0.25 [4]	LEP0.50 [5]	*SEM* [6]	*P*-value
Dry matter	82.07	82.04	82.10	82.09	0.34	0.16
Crude protein	85.25^a	64.46^c	76.46^b	82.30^{ab}	2.40	0.001
Ether extract	74.00	74.13	74.58	74.70	0.14	0.16
Ash	80.69	79.76	80.36	80.46	0.16	0.21
Crude fiber	81.92^a	74.81^c	78.99^b	83.62^a	0.69	0.01
ME (Mcal/kg)	2896.74^a	2703.15^c	2801.11^b	2860.89^{ab}	23.21	0.01

[a,b,c]: Means within a row with different letter superscript differ significantly ($P < 0.05$). *SEM* = Standard error mean.
[1]Data presented the means based on 5 replicate pens per treatment and 2 broiler chickens per replicate.
[2]PC: positive control
[3]LEP0: low energy and protein without multi-enzyme supplementation.
[4]LEP0.25: low energy and protein supplemented with 0.25 g/kg multi-enzyme.
[5]LEP0.50: low energy and protein supplemented with 0.50 g/kg multi-enzyme.
[6]*SEM*: standard error of the mean.

Discussion

In a broiler chicken flock, flock weight uniformity can be defined as the percent of individuals within 10% of the mean body weight (Aviagen, 2018; Vasdal *et al*., 2019). Poor flock uniformity of broiler chickens has been reported to be a reflection of poor performance and an indication of welfare issues caused by several factors including stocking density, heat stress, disease condition, nutrition, etc. (Ao and Choct, 2013; Ahiwe *et al*., 2019) . According to Vasdal *et al*. (2019), poor nutrient availability and utilization affect flock uniformity of farm animals. Thus, the decrease in flock uniformity of broiler chickens in the LEP0 and LEP0.25 groups at both d21 and d42 could be attributed to the reduction in protein and energy content of the diet as well as the poor nutrient retention or availability observed among the two groups. But the LEP0.50 group had better flock uniformity (as indicated by the high mean % flock uniformity) which could be ascribed to the observed improvement in nutrient digestion and availability that may be associated with the action of the multi-enzyme in the LEP diet. Though there is limited research on the effect of multi-enzymes on the flock uniformity of broiler chickens fed low energy and protein diet. However, various feed supplements such as amino acids, oligosaccharides, antibiotics, etc. have been reported to enhance flock uniformity of various farm animals (Ao and Choct, 2013; Ahiwe *et al*., 2019). Contrary to the observation of the present study, Souza *et al.* (2014) reported that a mono-enzyme (xylanase) did not have any effect on the flock uniformity of Hy-line laying hens fed a diet containing low energy content. This observed discrepancy in research findings could be linked to differences in the type of enzyme (mono-enzyme

versus multi-enzyme), the number of reduced nutrients in the diet, age, type, breed, and strain of the animals used.

Blood indices are good indicators of the nutritional, health, physiological, status of farm animals (Meluzzi *et al*., 1992). The non-significant effect of the dietary treatments on both haematological and serum biochemical indices considered in the present study indicates that the birds were all in a healthy state irrespective of the dietary treatment administered (LEP diet or multi-enzyme supplementation). It is also important to note that the haematological and serum biochemical indices values obtained in the present study fall within the normal reference values of healthy broiler chickens (Meluzzi *et al*., 1992; Bounous & Stedman, 2000).

The decrease in protein retention among birds fed LEP diet may be associated with the low protein content of the broiler chicken diet that may have resulted in a decrease in protein availability in the gastrointestinal tract. In addition to the preceding explanation, it is important to note that not all nutrients in a diet are retained and utilized. Some nutrients are excreted via feces, thereby reducing nutrient retention. The reduced protein level in the LEP diet coupled with protein loss via feces may be part of the reason why there is poor protein availability observed in the present study. This observation is in line with the finding of Sarica *et al*. (2020), who also noted that broiler chickens fed a low mono-nutrient protein diet had reduced protein retention and utilization. However, multi-enzyme supplementation at 0.50 g/kg to LEP broiler diet resulted in an improvement in protein retention. This marked improvement in protein retention may be due to the ability of the multi-enzyme to improve protein digestion, availability, and utilization. According to Ravindran (2013), enzymes aid in the breakdown of plant cell walls (of the feed ingredient) and subsequently release the nutrients encapsulated by the cell wall. Furthermore, the author explained that enzymes through adequate viscosity increased nutrients retained while reducing the nutrient excreted via feces. The finding of the present study is in agreement with the results of Abudabos (2012), who reported that mono-enzyme (protease) supplementation improved the protein digestion/retention in broiler chickens fed a low-density corn-soybean meal. Contrary to our findings, Mohammadigheisar & Kim (2018) reported that protease supplementation in a low protein and high fiber broiler chicken diet did not affect CP retention. The reason for this discrepancy could be attributed to several factors such as the type of enzyme used (mono-enzyme versus multi-enzyme), the number of low-density nutrients, enzyme dosage, breed of bird, and type of feed ingredient used. In the same vein, the supplementation of multi-enzyme at 0.50 g/kg to broiler chicken fed LEP diet [high in PKC (contain cellulose and hemicellulose), wheat offal (high in non-starch polysaccharides), and Brewer's spent grain (mostly high in hemicellulose)] could have resulted in improved fiber digestibility. The multi-enzyme supplementation at 0.50 g/kg to birds fed LEP diet may have resulted in the hydrolysis/break-down of non-starch polysaccharides, cellulose, hemicellulose, and/or release of other bound nutrients. The breakdown/digestion of CF to a more absorbable/utilizable form (protein and energy) may have resulted in better fiber retention observed in the present study. The result of this study is in agreement with those of Iyayi & Davies (2005), who reported that apparent digestibility of fiber was significantly higher in broiler chickens fed a high fiber diet (high in PKC and brewer's dry grain) supplemented with an exogenous enzyme. The decreased ME retention observed in the LEP0 group may be associated with the low energy and protein content of the LEP0 diet. Likewise, the decreased ME content noticed among the LEP0.25 g/kg multi-enzyme supplementation may be due to the lower dose of the enzyme that was not sufficient enough to break down feed ingredients to aid in improving the ME availability intestine. On the other hand, the improved ME retention in birds fed LEP 0.50 g/kg multi-enzyme could be linked to better ME digestion, availability, and less ME excreta loss as a result of the multi-enzyme inclusion level of 0.50 g/kg diet used in the present study. This result is in agreement with the report of Moftakharzadeh *et al*. (2019) who concurred that carbohydrase enzyme supplementation improved ME in broiler chickens fed low-energy wheat-soybean-based diets.

Conclusion

Feeding low energy and protein diet to broiler chickens depressed flock uniformity, protein, fiber, and energy retention. However, supplementation of low energy and protein diet with 0.50 g/kg multi-enzyme improved the flock uniformity, energy, fiber, and protein retention of broiler chickens without adverse effects on haematological indices and serum metabolites.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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