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Energy utilization, nutrient digestibility and bone quality of broiler chickens fed Tanzania-type diets in different forms with enzymes

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Abstract

A study was conducted to determine the influence of feed form and microbial enzyme supplementation on energy utilization, bone quality, and amino acid and mineral digestibility of broiler chickens. Four hundred and eighty Ross 308, day-old broiler chickens were randomly assigned to eight diets formulated from commonly used ingredients in Tanzania. A 2 (pellet or mash) × 4 (control, Axtra XB, Quantum Blue (QB) and Axtra XB + QB enzyme) factorial array in a completely randomized design having six replicates per treatment (10 birds per replicate) was used. Birds were raised in climate-controlled rooms in a 3-phase; starter (0–10 days), grower (11–24 days) and finisher (25–35 days). Apparent metabolizable energy (AME), metabolizable energy intake, net energy of production, energy retained as protein (REp), and efficiency of metabolizable energy use for energy and protein retention were higher ($p < 0.05$) in birds fed pelleted diets. The AME and REp was higher ($p < 0.05$) with enzyme supplementation. Ash content, weight, length, width and breaking strength of tibia bones were highest ($p < 0.05$) in birds on pelleted diets. Tibia bone traits were improved ($p < 0.05$) when enzymes were included, particularly in a combination of QB and Axtra XB. However, potassium, magnesium, and zinc contents were highest ($p < 0.05$) when QB was supplemented. Digestibility of all amino acids was higher ($p < 0.05$) in birds supplied with pellets and with enzyme supplementation for most amino acids, except for serine. There was a positive interaction ($p < 0.05$) between feed form and enzymes on lysine and phenylalanine digestibility. Digestibility of Ca, P, K, S, Zn, and Fe was higher ($p < 0.05$) in birds fed pelleted diets, while those on mashed diets had higher ($p < 0.05$) digestibility of Cu and B. The digestibility of P, K, and Zn was highest ($p < 0.001$) when QB was added, while Ca, P, S, and B digestibility was highest when a combination of Axtra XB + QB was applied. Pelleted diets with or without enzymes improved energy utilization, digestibility of amino acids, and minerals, and increased bone strength in broiler chickens.

Keywords: Feed efficiency, Mash, Meat chickens, Enzymes, Pellets

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Background

Feed is the most expensive input in poultry production, and can account for about 70% of total variable costs [1,2]. Thus, when formulating diets, at least a proper combination between feed costs and bird nutrient requirements is essential to optimize the efficiency of nutrient utilization [3]. Although broilers are highly efficient in converting feed to high quality meat, this is not always the case because much of the nutrients ingested are not adequately digested and utilized but are lost through excretion. According to Ravindran [4], broiler chickens excrete a significant amount of nutrients in droppings, including 30% dry matter (DM), 25% gross energy, 50% nitrogen, and 55% phosphorus, which could have been absorbed and utilized by the birds for growth. So, offering birds with adequate nutrients will lead to improved feed efficiency. Worldwide, the broiler industry has adopted different nutritional strategies to improve nutrient utilization and bird productivity. Physical processing of feed and the use of additives, particularly microbial enzymes, have been used to improve quality of feeds and nutrient utilization by poultry. The performance of broiler chickens is strongly affected by the physical form of feed, nutrient density, and presence of anti-nutrients in feed ingredients, among other factors [5]. Compared to pelleted diets, feeds in mash form have been reported to be inferior in quality and utilization as well as performance of birds, because of its dusty form, which may reduce feed palatability and intake [6]. The high fibre content and presence of antinutritional factors (ANF) in vegetable protein ingredients result in reduced energy and digestibility due to the low capacity of broiler chickens to digest fibre [7]. Therefore, feeding diets of vegetable origin can lead to poor utilization, and negative impact on the performance of birds [8]. Feed processing methods such as grinding, pelleting, and the use of microbial enzymes solubilize some cellulose and hemicellulose fractions in feed ingredients, which results to improved nutrient availability [7]. Application of microbial enzymes to broiler diets tend to reduce the impact of ANF, and increase nutrient digestibility and utilization [9]. Feed ingredients, especially diets based on grains like maize, contain phytate and other anti-nutrients, which limit the bioavailability of bound phosphorus and other minerals, amino acids, and starch. Phytase increases the release of organic phosphorus present in plant cells through hydrolysis of myo-inositol or phytic acid [10]. Birds lack endogenous phytase to hydrolyze the phytate present in grains, which results in poor performance and bone health [11]. According to Bhuiyan [12], high levels of microbial phytase in diets based on cereal grain, such as maize, wheat, and sorghum improve their nutritive value and efficiency of utilizations in broiler chickens.

Maize-soybean-based diets contain considerable amounts of oligosaccharides and non-starch polysaccharides (NSP) such as arabinoxylans, β-glucans and cellulose, which affect the digestibility and utilization of their nutrients [13]. Therefore, inclusion of NSP-degrading enzymes such as xylanase, β-glucanase, β-mannases, α-galactosidase and pectinase has been reported to decrease intestinal viscosity in poultry, and hence increase digestibility and performance [14]. Xylanase and beta-glucanase have been effectively applied in non-ruminant diets rich in cereals such as wheat, barley, rye, oats and maize to improve nutrient availability and hence improve performance in broiler production [10].

To be competitive, the poultry industry in Tanzania, as in other developing countries, needs to adopt modern technologies, such as the use of microbial enzymes, and the pelleting of diets to improve bird performance. Application of these technologies in Tanzania has not been widely adopted due to lack of knowledge, cost-benefit analysis and availability of these supplements. Therefore, the current study aimed at evaluating how feed form and exogenous enzyme supplementation can improve the amino acid digestibility, bone quality, and energy utilization of broiler chickens fed on replica Tanzanian diets.

Materials and Methods

Experimental design and dietary management

Four hundred and eighty Ross 308 day-old broiler chickens were randomly assigned to eight dietary treatments. Each diet was replicated six times with 10 birds per replicate in a 2 × 4 factorial arrangement. Diets were based on maize, soybean, cottonseed, and fish meal, and were supplied to birds in two feed forms (mash or pellets) in a four-enzyme supplementation plan (control, Axtra XB, QB, and Axtra XB + QB). Both enzymes were provided by AB Vista (UK) and were applied to top-up at the rate of 100 mg/kg of Axtra XB and 100 g/kg of QB. Birds were reared in climate-controlled deep litter floor pens from 0 to 35 d with *ad libitum* access to feed and water. Titanium dioxide (TiO_2) at a concentration of 5 g/kg was incorporated into the grower diets as an indigestible marker to calculate the ileal digestibility of amino acids and minerals. Birds were raised in three phases - starter (1–10 d), grower (11–24 d) and finisher (25–35 d). Dietary ingredient components and their nutrient composition are presented in Tables 1 and 2, while the amino acid composition of the diets is presented in Table 3.

Laboratory analyses

Twelve day-old chicks were electrically stunned, killed by cervical dislocation, minced and later analysed to obtain the baseline data of energy, fat and protein contents. Excreta was collected from 19–21 d using aluminium foil sheets. The excreta samples were pooled by replicate (per pen), mixed and stored at –20℃. Two birds per cage were killed at 24 d and immediately stored intact at

Table 1. Feed ingredient composition of the test diets[1] (g/kg)

Ingredients	Starter				Grower				Finisher			
	Control	AXB	QB	AXB + QB	Control	AXB	QB	AXB + QB	Control	AXB	QB	AXB + QB
Maize	623.6	624.2	623.6	623.6	659.4	659.3	665.2	665.2	670.7	670.6	676.3	676.3
SBM	179.4	177.5	180.0	180.0	162.2	162.2	163.6	163.6	174.3	174.3	175.8	175.8
Cottonseed meal	90.4	91.1	91.0	91.0	109.4	109.4	110.4	110.4	79.5	79.5	80.2	80.2
Fish meal	61.5	62.1	60.5	60.5	17.4	17.4	17.5	17.5	8.1	8.1	8.2	8.2
Canola oil	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.2	18.1	18.3	18.3
Mineral premix[2]	8.6	8.6	8.6	8.6	7.6	7.6	7.7	7.7	8.7	8.7	8.8	8.8
Dicalcium phosphate	8.2	8.2	8.4	8.3	12.6	12.6	4.8	4.8	12.1	12.1	4.8	4.8
Vitamin premix[3]	7.0	7.0	7.0	7.0	7.2	7.2	7.2	7.2	6.8	6.8	6.8	6.8
Limestone	6.7	6.6	6.9	6.9	10.5	10.5	10.6	10.6	9.3	9.3	9.3	9.3
L-Lysine	5.1	5.1	5.2	5.2	4.8	4.8	4.8	4.8	4.3	4.3	4.3	4.3
DL-Methionine	3.7	3.7	3.7	3.7	1.8	1.8	1.8	1.8	1.6	1.6	1.6	1.6
L-Threonine	2.5	2.5	2.5	2.5	2.4	2.4	2.4	2.4	1.8	1.8	1.8	1.8
Salt	2.2	2.2	2.0	2.0	1.2	1.2	1.2	1.2	1.3	1.3	1.3	1.3
Choline Cl 70%	1.0	1.0	0.4	0.4	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Sodium bicarbonate	0.1	0.1	0.2	0.2	2.4	2.4	1.4	1.4	2.6	2.6	1.5	1.5
AXB	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1
Quantum blue	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.1
TiO_2	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.0	0.0	0.0	0.0

[1]Half of each diet was pelleted and the other half was fed as mash. AXB = axtra XB (xylanase + β-glucanase), QB = quantum blue (phytase), SBM = soyabean meal, Dical phosphate = dicalcium phosphate.

[2]Mineral premix (DM basis) contains (g/kg): P = 0.1, K= 0.08, Cl = 0.27, S = 3.05, Se = 0.80, Zn = 266, Fe = 106, Cu = 42.67, Mn = 240.

[3]Vitamin premix (95.8%) contains vitamin A (IU) 24,000,000, vitamin E (IU) = 150,000, vitamin K (g/kg) = 6, thiamine (g/kg) = 6, riboflavin (g/kg) 16, niacin (g/kg) = 110,000, pantothenic acid (g/kg) = 26, pyridoxine (g/kg) = 10, folacin (g/kg) = 4, and biotin (g/kg) = 0.5.

Table 2. Nutrient composition of dietary treatments

Nutrients	Starter	Grower	Finisher
ME P (kcal/kg)	3,000	3,100	3,200
ME (MJ/kg)	12.6	13.0	13.4
CP (%)	23.0	21.5	19.5
Crude fat (%)	5.1	5.0	6.8
Crude fibre (%)	3.5	3.8	3.5
Arginine (%)	1.4	1.3	1.2
Lysine (%)	1.3	1.1	1.0
Methionine (%)	0.7	0.5	0.4
Methionine + cystine (%)	1.0	0.7	0.7
Tryptophan (%)	0.2	0.2	0.2
Leucine (%)	1.5	1.4	1.4
Isoleucine (%)	0.8	0.7	0.6
Threonine (%)	0.9	0.8	0.7
Valine (%)	0.9	0.8	0.7
Calcium (%)	1.0	0.9	0.8
Phos. avail (%)	0.5	0.4	0.4
Sodium (%)	0.2	0.2	0.2
Chloride (%)	0.3	0.2	0.2
Choline (mg/kg)	1,700	1,600	1,500

AXB, axtra XB (xylanase + β-glucanase); QB, quantum blue (phytase).

The four diets in three phases had similar nutritional composition.

Table 3. Analyzed amino acid contents of grower diets

Amino acid	Control	AXB	QB	AXB + QB
Histidine	5.2	5.3	5.6	5.6
Serine	8.6	8.7	9.4	9.5
Arginine	13.6	13.8	14.3	14.6
Glycine	7.4	7.5	8.4	8.5
Aspartic acid	16.8	16.8	17.6	18.3
Glutamic acid	33.8	34.5	36.4	36.6
Threonine	8.8	8.9	9.6	10.7
Alanine	8.9	9.1	10.1	10.0
Proline	10.6	10.7	11.7	11.5
Lysine	12.4	12.3	12.8	14.0
Tyrosine	4.3	4.5	4.4	4.6
Methionine	3.9	4.1	4.3	4.5
Valine	8.6	8.7	9.4	9.4
Isoleucine	7.2	7.4	7.9	7.8
Leucine	15.8	16.2	17.6	17.1
Phenylalanine	9.4	9.7	10.2	10.1

AXB, axtra XB (xylanase + β-glucanase); QB, quantum blue (phytase).

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–20℃. The intact day-old and adult carcasses (24 d) were minced and freeze-dried. Ileal digesta samples were collected from another two birds at 24 d of age. They were then ground (0.5 mm) and analysed for DM, CP(crude protein), GE (gross energy) and total fat. Excreta samples were freeze-dried, ground and analysed for GE and TiO_2 contents.

Dry matter

The DM content of diets was determined by drying samples at 105℃ in a forced air convection oven (Qualtex Universal Series 2000, Watson Victor Ltd, Perth, Australia) for 24 hours, while the DM contents of digesta, whole carcasses and excreta were determined by freeze drying (Martin Christ freeze dryer, Osterode am Harz, German) at –50℃ for 72 hours.

Gross energy and crude protein

Approximately 0.5 g of finely ground (0.5 mm) diet and ileal digesta samples were weighed into metallic crucibles to determine the GE content, using an IKA®-WERKE bomb calorimeter (C7000, GMBH & Co., Staufen, Germany).

The nitrogen content of diets, ileal digesta and whole carcasses was determined according to the Dumas combustion using technique of a LECO FP-2000 automatic nitrogen analyser (LECO Corp., St Joseph, Mich., USA) following the method described by Sweeney [15]. Samples of diets, digesta and carcase were weighed and kept in aluminium foil crucibles before setting in a LECO nitrogen analyser. Nitrogen was released by combustion at high temperature in pure oxygen and measured by thermal conductivity detection and converted to equivalent CP by the numerical factor of 6.25. The furnace temperature was maintained at 105℃ for hydrolysis of the sample in ultra-pure oxygen. To interpret the detector response as percentage nitrogen (w/w), calibration was conducted using a pure primary standard of ethylenediaminetetraacetic acid (EDTA).

Crude fat content

The ether extract (EE) of whole carcasses at hatch and 24 d was determined using a Soxhlet extraction system. All samples were weighed and pressed in pre-weighed 185 mm filter paper that was later placed in the Soxhlet tubes. The thimbles containing the samples were then placed in the sample cylinder for 48–50 h after filling the solvent flask with chloroform and turning on the water to the condenser. During this time, reflux occurred every 2 h (24–25 refluxes). The following day, samples were removed from the thimbles and placed in an oven (80℃) for 72 h for total drying before weighing.

Intramuscular fat (IMF) percentage was calculated as:

IMF% = Weight of extracted fat /
(pre-extraction sample weight / sample dry matter) × 100

Weight of extracted fat = Post-extraction sample weight
– Pre-extraction sample weight

Bone morphology and mineral contents

On 35 d, the left drumstick of two randomly chosen birds were taken from each replicate and frozen at –20℃ overnight. After thawing, all the tissue adherent to the bones was removed. The bones were then weighed, while the length and width were measured using digital calipers. Tibia bone length was measured from the proximal end to the distal end, while width was measured at the medial diaphysis. The breaking strength of the tibia bone was measured by positioning a 10 mm diameter compression rod against the bones and applying pressure (Lloyd, Hampshire, UK). Breaking strength was recorded as the force required to break the bone and was measured in the range of 0 to 500 N. The entire bones were then dried for 12 h at 105℃ in a forced-air convection oven (Qualtex Universal Series 2000, Watson Victor Ltd, Perth, Australia) and ashed (550℃ for 4 h) in a Carbolite CWF 1200 chamber furnace (Carbolite, Sheffield, UK). The ashed bone samples were ground and stored at 4℃ in an airtight plastic container to be analysed for DM and mineral contents.

Analysis of titanium dioxide

Titanium dioxide concentration in the diet and ileal digesta samples was measured in accordance with methods described by Short et al. [16]. About 0.2 g of diet or 0.1 g of freeze-dried digesta samples were ashed in a porcelain crucible for 13 h at 580℃ and dissolved in 5 mL of 7.4 M H_2SO_4. The samples were gently boiled for about 30 min at 200℃ and then for a further 40 min at 250℃ until completely dissolved. After cooling, the solution was transferred quantitatively into a 50 mL volumetric flask through filter paper (Whatman 541, hardened ash-less, 90 mm Ø, Cat No. 1541 090, Whatman PLC, Maidstone, United Kingdom). Subsequently, 10 mL of H_2O_2 (30% v/v) were added to each flask and the contents were diluted up to 50 mL with Milli-Q water and mixed thoroughly through inversion to avoid bubbles. The absorbance of aliquots and the prepared standard solutions was read on a Hitachi 150-20 UV spectrophotometer (Hitachi Science Systems Ltd., Ibaraki, Japan) at 410 nm. The concentration of TiO_2 (mg/mL) was determined from the standard curve and converted to mg/g of the sample.

$$\text{Digestibility coefficient} = 1 - \frac{\text{digesta nutrient (g/kg DM)} / \text{digesta } TiO_2 \text{ (g/kg DM)}}{\text{diet nutrient (g/kg DM)} / \text{diet } TiO_2 \text{ (g/kg DM)}}$$

Amino acid contents and ileal digestibility

The amino acid contents of grower diets and digesta samples were analysed at the Australia Proteome Analysis Facility (APAF), Macquarie University, Sydney, NSW, Australia. The amino acid contents were determined using pre-column derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) followed by separation of the derivatives and quantification by reversed-phase high-performance liquid chromatography (RP-HPLC). Amino acids were detected by UV absorbance. About 100 mg of sample was hydrolyzed in 6 M HCl for 24 hours at 110℃. After hydrolysis, an internal standard (α -aminobutyric acid [AABA]) was added to each sample. After that, 10 μL of the solution was derivatized using an AccQ-Tag Ultra Derivatization Kit (Waters Corp. Milford, Massachusetts. USA; 70 μL borate buffer and 20 μL AccQ-Tag solution) and incubated for 10 min at 50℃. The HPLC analysis was based on the method of Cohen [17] but adapted for use with an ACQUITY Ultra Performance Liquid Chromatography (Waters Corp., USA) system. An ACQUITY UPLC BEH C18 1.7 μm column was employed with detection at 260 nm and a flow rate of 0.7 mL/min. All samples were analyzed in duplicates and averages computed. The ileal digestibility of amino acid ileal was calculated using the following equation:

$$\text{Digestibility coefficient} = 1 - \frac{\text{digesta nutrient (g/kg DM)} / \text{digesta } TiO_2 \text{ (g/kg DM)}}{\text{diet nutrient (g/kg DM)} / \text{diet } TiO_2 \text{ (g/kg DM)}}$$

Determination of ileal mineral contents and digestibility

On 24 d, two birds per replicate were randomly selected, electrically stunned and killed by cervical dislocation, to collect ileal digesta. The dried diet and ileal digesta samples collected on 24 d were analyzed for minerals by the inductively coupled plasma (ICP) method (Vista MPX-radial, Burladingen, German) as described by Anderson and Henderson [18]. The sealed chamber digestion (SCD) method was also used for macro elements (Ca, P, Mg, K, and S), and trace minerals (Mn, Cu, Al, B, Zn, and Fe). This digest method is the most appropriate for ICP analyses in which final oxidation occurs in the high temperature plasma of the ICP. About 0.2 g of diet and ileal digesta samples was weighed and transferred into appropriate 50 mL borosilicate Schott reagent bottles. Then, 2 mL of a 7:3 (v/v) mixture of perchloric acid ($HClO_4$–70%) and hydrogen peroxide (H_2O_2–30%) were added to each digestion bottle and bottles were capped tightly. After pre-digestion for 2 h at room temperature, another one mL of H_2O_2 was added, and the bottles sealed tightly and placed into a warming oven at 80℃ for 30 min. After cooling slightly, a further one mL of H_2O_2 was added and the samples were then digested in the oven at 80℃ for a further 60 min. After that, the final volume was adjusted to 25 mL of total volume using distilled water and mixed thoroughly. To reduce adsorption onto the plastic and inhibit the growth of micro-organisms, the samples were briefly stored at 4℃ before taking the absorbance reading at 785 nm against a blank. Ileal mineral digestibility of the diets and digesta was thereafter calculated according to the method developed by Moughan et al. [19] and revised by Kong and Adeola [20] using TiO_2 as an indigestible maker as described in the following equation:

$$\text{Digestibility coefficient} = 1 - \frac{\text{digesta nutrient (g/kg DM)} / \text{digesta } TiO_2 \text{ (g/kg DM)}}{\text{diet nutrient (g/kg DM)} / \text{diet } TiO_2 \text{ (g/kg DM)}}$$

Energy utilization calculation

The methodology as explained by Olukosi et al. [5] was used to calculate the nutrient retention and utilization parameters as follows:

$$AME\ (MJ/kg) = GE_i - [GE_o \times (T_i / T_o)]$$

where GE_i is gross energy (MJ/kg) in feeds; GE_o is the gross energy (MJ/kg) in excreta, T_i is the titanium concentration in the diets; and T_o is the titanium concentration in the excreta.

The net energy of production (NE_p) was computed using the following equations:

$$\text{Initial GE of carcase (kJ)} = \text{carcase GE (kJ / g)} \times \text{body weight of bird (g)} \quad \text{-----(1)}$$

$$\text{Final contents of carcase (kJ)} = \text{carcase GE (kJ/g)} \times \text{body weight of bird (g)} \quad \text{-----(2)}$$

$$NE_p\ (kJ) = (2) - (1)$$

Heat of production (HP), which consists of the heat increment of feeding and fasting HP was calculated as the difference between NE_p and ME intake:

$$HP\ (kJ) = MEI - NE_p$$

where ME intake (MEI) was calculated using the following formula:

$$MEI\ (kJ) = ME\ (kJ/g) \times \text{feed intake (g)}$$

Energy retention:

Energy retained as fat (RE_f) and as protein (RE_p) were calculated as follows:

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RE_f (kJ) = carcase fat (g) × 38.2 kJ/g
RE_p (kJ) = carcase crude protein content (g) × 23.6 kJ/g

The values 38.2 and 23.6 kJ/g are energy values per gram of fat and protein, respectively, as derived by Larbier and Leclercq [21].

Metabolizable energy efficiencies:

Efficiency of ME use for energy retention (k_{RE}) = NE_p / MEI
Efficiency of ME use for lipid retention (k_{REf}) = RE_f / MEI
Efficiency of ME use for protein retention (k_{REp}) = RE_p / MEI

Statistical analysis

Complete randomization was applied in this study, and the data obtained were analysed in a two-way analysis of variance using the general linear model (GLM) procedure of Minitab statistical software, version 17 [22]. Tukey's pairwise comparison test was used to separate differences between mean values at the $p \leq 0.05$ level of probability.

Animal ethics

The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No. AEC17-079). Health and animal husbandry practices complied with the Code of Practice for the Care and Use of Animals for Scientific Purposes issued by the Australia Bureau of Animal Health [23].

Results

Energy utilization

The results of AME and energy utilization due to feed form and microbial enzyme supplementation are illustrated in Table 4. There was no interaction ($p > 0.05$) between feed form and enzyme supplementation on either AME or the energy utilization parameters. Results showed that the pelleted diet had higher ($p < 0.001$) AME than the mash diets. Enzyme supplementation resulted to higher ($p < 0.005$) AME, with the best result was observed when the test enzymes Axtra XB and QB were combined in the diet. Metabolizable energy intake, NE_p and the energy retained as protein were better ($p < 0.002$) when pelleted diets were fed than mash diets. Including microbial enzymes to the diets resulted to more ($p < 0.01$) energy retained as protein, with a slight increase in MEI and efficiencies of ME use. Neither feed form nor microbial enzyme supplementation had a significant effect ($p > 0.05$) on the heat production (HP), energy retained as fat, or efficiency of ME use for lipid retention.

Bone morphology and mineral content

There was no interaction ($p > 0.05$) between the two factors on the bone morphological traits (Table 5). The tibia bone weight, length, width, and breaking strength were higher ($p < 0.002$) in the group of birds supplied with pelleted diets than those raised on mash. Heavier, longer, wider and stronger bones were recorded in birds on diets supplemented with exogenous enzymes ($p < 0.038$), with

Table 4. Effect of feed form and microbial enzymes supplementation on energy utilization of broiler chickens

Feed form	Enzyme	AME	MEI (KJ/d)	Energy utilization (KJ/d)		Energy retained (KJ/d) as		Efficiency of ME use for		
				NE_p	HP	Protein	Fat	Energy	Protein	Lipid
Mash	No enzyme	11.8^{b}	747.5^{b}	424.1^{ab}	323.4	226.2^{bc}	240.7	0.50^{c}	0.27^{d}	0.28
	AXB	12.4^{ab}	777.7^{ab}	461.7^{ab}	316.0	242.0^{abc}	242.6	0.53^{bc}	0.28^{cd}	0.28
	QB	12.4^{ab}	812.1^{ab}	413.6^{b}	398.4	217.1^{c}	235.0	0.54^{bc}	0.28^{bcd}	0.31
	AXB + QB	12.6^{ab}	823.6^{ab}	479.1^{ab}	344.5	246.5^{ab}	271.5	0.60^{ab}	0.31^{abc}	0.34
Pellet	No enzyme	12.5^{ab}	847.7^{ab}	487.8^{ab}	359.9	257.5^{a}	255.1	0.61^{ab}	0.32^{a}	0.32
	AXB	12.9^{a}	857.8^{ab}	506.3^{ab}	351.5	259.4^{a}	269.8	0.63^{a}	0.32^{a}	0.34
	QB	13.1^{a}	824.9^{ab}	501.0^{ab}	323.8	247.3^{ab}	259.5	0.63^{a}	0.31^{abc}	0.33
	AXB + QB	13.4^{a}	876.0^{a}	504.0^{a}	372.0	259.9^{a}	267.3	0.62^{ab}	0.32^{a}	0.33
SEM		0.101	10.4	8.18	11.1	3.02	5.11	0.012	0.005	0.007
Significance										
Feed form		0.001	0.002	0.001	0.783	0.001	0.138	0.001	0.001	0.065
Enzyme		0.005	0.285	0.191	0.795	0.010	0.397	0.391	0.523	0.429
Feed form × enzyme		0.947	0.399	0.455	0.245	0.419	0.698	0.446	0.348	0.348

[a–d]Means with different superscripts within the columns are different ($p < 0.05$).

Values are means of 6 replicates (10 birds per replicate).

AXB, axtra XB (xylanase and beta-glucanase composite); QB, quantum blue (phytase); SEM, standard error of the mean; AME, apparent metabolizable energy; MEI, metabolizable energy intake; NE_p, net energy for production; HP, heat production.

Table 5. Effect of feed form and enzyme supplementation on the morphology of tibia bone of broilers

Feed form	Enzyme	WT (g)	L (mm)	D (mm)	Strength (N)
Mash	No enzyme	5.7^{d}	86.6^{c}	14.5^{c}	199.4^{c}
	AXB	6.4^{cd}	88.2^{bc}	15.5^{abc}	213.5^{bc}
	QB	7.5^{ab}	88.5^{abc}	15.1^{bc}	240.1^{abc}
	AXB + QB	7.5^{ab}	88.3^{bc}	15.0^{bc}	218.6^{bc}
Pellet	No enzyme	7.1^{bc}	89.5^{abc}	15.4^{abc}	225.5^{bc}
	AXB	7.3^{abc}	89.2^{abc}	15.5^{abc}	234.7^{abc}
	QB	7.8^{ab}	91.3^{ab}	15.9^{ab}	250.7^{ab}
	AXB + QB	8.3^{a}	91.7^{a}	16.3^{a}	274.8^{a}
SEM		0.14	0.33	0.10	4.67
Significance					
Feed form		0.001	0.001	0.001	0.001
Enzyme		0.001	0.022	0.038	0.004
Feed form × Enzyme		0.158	0.368	0.063	0.168

$^{a-d}$Means with different superscripts within the columns are significantly different ($p < 0.05$).

Values are means of 6 replicates (10 birds per replicate).

AXB, axtra XB (xylanase and beta-glucanase composite); QB, quantum blue (phytase); SEM, standard error of the mean; WT= bone weight; L= bone length; D, bone diameter; N, newton (SI unit of breaking strength).

the highest value observed when a combination of QB (phytase) and Axtra XB was added.

Tibia bone ash and mineral contents for the broiler chickens fed different forms of feed and enzyme supplementation are shown in Table 6. There was no significant interaction ($p > 0.05$) observed between the feed form and microbial enzymes on the ash and mineral contents of the broiler chickens. Tibia ash content was higher ($p < 0.002$) in birds fed pelleted diets, and this was further increased ($p < 0.001$) by enzyme supplementation. The mineral content of tibia bones was not affected ($p > 0.05$) by feed form. Only potassium and zinc contents were higher ($p < 0.005$) in birds fed on diets supplemented with enzymes, with the highest value observed when phytase and a combination of the enzymes was used.

Ileal amino acid digestibility

There was a positive interaction effect ($p > 0.04$) between feed form and enzyme supplementation on the digestibility of lysine and phenylalanine. Broiler chickens offered pelleted diets had signifi-

Table 6. Effect of feed form and enzyme supplementation on mineral contents (mg/kg) of tibia bones

Feed form	Enzyme	Ash (%)	Ca	P	K	Mg	S	Zn
Mash	No enzyme	43.0^{c}	402.3	183.1	9.0^{abc}	7.7	2.4	366.8^{bc}
	AXB	43.5^{bc}	403.1	183.3	8.9^{bc}	7.6	2.3	349.7^{bc}
	QB	43.8^{bc}	402.9	183.5	9.5^{a}	7.4	2.4	459.1^{a}
	AXB + QB	45.3^{ab}	403.3	184.0	9.4^{ab}	7.6	2.3	464.6^{a}
Pellet	No enzyme	43.9^{bc}	404.1	183.2	8.8^{c}	7.6	2.3	321.9^{c}
	AXB	44.2^{abc}	406.5	183.5	8.9^{bc}	7.6	2.4	349.8^{bc}
	QB	45.7^{a}	403.1	182.9	9.3^{abc}	7.3	2.4	475.8^{a}
	AXB + QB	445.9^{a}	403.9	182.7	9.5^{a}	7.4	2.4	413.4^{ab}
SEM		0.20	0.67	0.23	0.07	0.03	0.03	10.5
Significance								
Feed form		0.002	0.295	0.357	0.478	0.265	0.972	0.162
Enzyme		0.001	0.816	0.976	0.005	0.112	0.751	0.001
Feed form x enzyme		0.392	0.854	0.671	0.684	0.446	0.585	0.246

$^{a-c}$Means with different superscripts within the columns are significantly different ($p < 0.05$).

Values are means of 6 replicates (10 birds per replicate).

AXB, axtra XB (xylanase and beta-glucanase composite); QB, quantum blue (phytase); SEM, standard error of the mean.

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Table 7. Effect of feed form and enzyme supplementation to broiler chickens on digestibility of indispensable amino acids

Feed form	Enzyme	Arg	His	Ile	Leu	Ly	Met	Thr	Phe	Val
Mash	No enzyme	0.88^{d}	0.80^{d}	0.78^{d}	0.67^{d}	0.83^{c}	0.91^{c}	0.76^{d}	0.83^{d}	0.79^{bc}
	AXB	0.92^{abc}	0.82^{cd}	0.84^{abc}	0.75^{abc}	0.88^{ab}	0.94^{ab}	0.83^{abc}	0.89^{abc}	0.83^{ab}
	QB	0.91^{c}	0.83^{bcd}	0.82^{bcd}	0.68^{cd}	0.86^{bc}	0.92^{bc}	0.80^{cd}	0.86^{c}	0.78^{c}
	AXB + QB	0.92^{abc}	0.81^{d}	0.81^{cd}	0.72^{bcd}	0.87^{ab}	0.93^{ab}	0.83^{abc}	0.87^{bc}	0.80^{bc}
Pellet	No enzyme	0.91^{bc}	0.86^{abc}	0.83^{abc}	0.74^{abc}	0.90^{a}	0.93^{ab}	0.81^{bc}	0.88^{abc}	0.82^{abc}
	Axtra XB	0.92^{abc}	0.87^{ab}	0.88^{a}	0.79^{a}	0.98^{ab}	0.95^{a}	0.87^{a}	0.90^{a}	0.86^{a}
	QB	0.93^{ab}	0.86^{abc}	0.87^{a}	0.78^{ab}	0.89^{ab}	0.93^{b}	0.84^{ab}	0.89^{ab}	0.86^{a}
	AXB + QB	0.93^{a}	0.88^{a}	0.86^{ab}	0.77^{ab}	0.90^{a}	0.94^{ab}	0.86^{a}	0.90^{a}	0.87^{a}
SEM		0.003	0.005	0.006	0.008	0.004	0.002	0.005	0.004	0.006
Significance										
Feed form		0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001
Enzyme		0.001	0.040	0.001	0.001	0.044	0.001	0.001	0.001	0.008
Feed form x enzyme		0.189	0.141	0.665	0.182	0.007	0.153	0.395	0.040	0.062

[a–d]Means that do not share a letter superscript within the columns are significantly different ($p < 0.05$).

Values are means of 6 replicates (10 birds per replicate).

AXB, axtra XB (xylanase and beta-glucanase composite); QB, quantum blue (phytase); SEM, standard error of the mean.

Table 8. Effect of feed form and enzyme supplementation to broiler chickens on digestibility of dispensable amino acids

Feed form	Enzyme	Ala	Asp	Glu	Gly	Pro	Ser	Tyr
Mash	No enzyme	0.78^{e}	0.79^{c}	0.86^{c}	0.72^{d}	0.77^{c}	0.77^{b}	0.79^{d}
	AXB	0.85^{bcd}	0.83^{bc}	0.90^{ab}	0.76^{cd}	0.83^{a}	0.81^{ab}	0.85^{ab}
	QB	0.82^{d}	0.82^{bc}	0.88^{b}	0.78^{bc}	0.85^{a}	0.81^{a}	0.80^{cd}
	AXB + QB	0.83^{cd}	0.82^{bc}	0.90^{ab}	0.78^{bc}	0.82^{ab}	0.80^{ab}	0.84^{bc}
Pellet	No enzyme	0.84^{bcd}	0.85^{ab}	0.89^{ab}	0.80^{abc}	0.83^{a}	0.83^{a}	0.83^{bc}
	AXB	0.89^{a}	0.87^{ab}	0.91^{ab}	0.82^{ab}	0.86^{a}	0.84^{a}	0.88^{a}
	QB	0.87^{ab}	0.87^{ab}	0.91^{a}	0.84^{a}	0.85^{a}	0.84^{a}	0.85^{ab}
	AXB + QB	0.86^{abc}	0.88^{a}	0.92^{a}	0.82^{ab}	0.86^{a}	0.86^{a}	0.86^{ab}
SEM		0.005	0.005	0.003	0.007	0.006	0.006	0.005
Significance								
Feed form		0.001	0.001	0.001	0.001	0.001	0.001	0.001
Enzyme		0.001	0.005	0.001	0.001	0.001	0.074	0.001
Feed form x enzyme		0.458	0.725	0.064	0.384	0.121	0.360	0.382

[a–e]Means that do not share a letter superscript within the columns are significantly different ($p < 0.05$).

Values are means of 6 replicates (10 birds per replicate).

AXB, axtra XB (xylanase and beta-glucanase composite); QB, quantum blue (phytase); SEM, standard error of the mean.

cantly higher ($p < 0.05$) apparent digestibility of both indispensable and dispensable amino acids than those fed mash diets (Table 7 and Table 8). Inclusion of microbial enzymes increased ($p < 0.04$) the digestibility of most amino acids (except serine), with the highest coefficients observed when the combination of Axtra XB + QB was used. Inclusion of Axtra XB resulted in higher digestibility for isoleucine and leucine.

Ileal mineral digestibility

The ileal digestibility of macro and trace minerals is shown in Table 9. The interaction between feed form and enzyme supplementation increased ($p < 0.05$) the digestibility of P, K, S, Cu, and Zn. The digestibility coefficients of Ca, P, K, S, Zn, and Fe was highest ($p < 0.05$) in birds supplied with pellets, while mash-fed birds recorded improvement ($p < 0.05$) in the digestibility of Cu and B only. However, the digestibility of Mg and Al was lower with mash diets than in pelleted diets, but these differences were not significant ($p > 0.05$). Enzyme inclusion improved ($p < 0.001$) the digestibility of all macro minerals, except for Mg, while of the micro minerals only the digestibility of Zn and Fe was improved ($p < 0.009$). The

Table 9. Effect of feed form and enzyme supplementation on ileal mineral digestibility

Treatments		Macro elements					Trace elements					
Feed form	Enzyme	Ca	P	Mg	K	S	Mn	Cu	Al	B	Zn	Fe
Mash	No enzyme	0.51^{e}	0.48^{e}	0.33	0.86^{ab}	0.65^{c}	0.30^{ab}	0.25^{ab}	0.34	0.69^{a}	0.24^{b}	0.20^{c}
	AXB	0.59^{de}	0.55^{d}	0.30	0.87^{ab}	0.73^{abc}	0.30^{ab}	0.37^{ab}	0.39	0.70^{a}	0.32^{ab}	0.34^{b}
	QB	0.63^{cd}	0.61^{cd}	0.37	0.91^{a}	0.67^{bc}	0.34^{b}	0.44^{a}	0.33	0.70^{a}	0.32^{ab}	0.36^{ab}
	AXB + QB	0.73^{ab}	0.66^{bc}	0.32	0.83^{b}	0.77^{a}	0.38^{a}	0.41^{ab}	0.40	0.74^{a}	0.35^{a}	0.36^{b}
Pellet	No enzyme	0.65^{bcd}	0.61^{cd}	0.27	0.88^{ab}	0.74^{ab}	0.26^{ab}	0.32^{ab}	0.29	0.64^{ab}	0.35^{a}	0.36^{b}
	AXB	0.70^{abc}	0.64^{bc}	0.32	0.90^{a}	0.76^{a}	0.27^{ab}	0.31^{ab}	0.31	0.68^{ab}	0.30^{ab}	0.41^{ab}
	QB	0.69^{abc}	0.75^{a}	0.24	0.92^{a}	0.71^{abc}	0.25^{ab}	0.23^{b}	0.31	0.59^{b}	0.40^{a}	0.40^{ab}
	AXB + QB	0.78^{a}	0.70^{ab}	0.30	0.90^{a}	0.75^{ab}	0.25^{ab}	0.26^{ab}	0.42	0.65^{ab}	0.35^{a}	0.49^{a}
SEM		0.014	0.012	0.118	0.039	0.008	0.017	0.017	0.015	0.013	0.010	0.015
Significance												
Feed form		0.001	0.001	0.169	0.001	0.011	0.030	0.006	0.288	0.007	0.009	0.001
Enzyme		0.001	0.001	0.999	0.001	0.001	0.894	0.508	0.139	0.468	0.025	0.001
Feed form x enzyme		0.202	0.003	0.544	0.048	0.030	0.705	0.014	0.663	0.645	0.026	0.153

$^{a-e}$Means with different superscripts within the columns are significantly different ($p < 0.05$).

Values are means of 6 replicates (10 birds per replicate).

AXB, axtra XB (xylanase and beta-glucanase composite); QB, quantum blue (phytase); SEM, standard error of the mean.

digestibility of K and Zn was better when QB was supplemented, while Ca, P, S, and Fe digestibility was improved when a combination of Axtra XB and QB was added.

Discussion

Energy utilization

In the present study, AME, MEI, NE_p, and the energy retained as protein were better when pelleted diets were provided. These improvements in pellet-fed birds could be due to the fact that pelleting diets reduces feed wastage and particle selection during consumption. Furthermore, birds use less energy when eating pelleted diets, thus conserving more energy, some of which could be lost when fed mash diets [24]. Pellet processing conditions, including pressing, heating, and addition of moisture, might deactivate anti-nutritive factors (ANF) and improve the palatability of diets, leading to an improvement in nutrient availability, particularly energy, for the birds [25]. These results support the findings of Greenwood et al. [26], who reported that pelleting diets proportionally increases dietary energy and hence makes more energy available to birds, thereby achieving maximum utilization and retention. It was also observed in the current study that microbial enzymes improved the amount of energy retained as protein, with a slight increase in MEI and efficiencies of ME usage, particularly when a combination of Axtra XB and QB was supplemented. Similar enzymes have been shown to reduce the negative effects of ANF and improve endogenous enzyme activity [27]. The current results also partially agree with those reported by Olukosi et al. [5], who found an improvement in NE_p, RE_p, and RE_p when diets were supplemented with a combination of phytase and carbohydrases. According to Khattak et al. [28], inclusion of exogenous enzymes in poultry diets enhances the nutrient availability, which then increases the efficiency of nutrient utilization by the birds.

Bone quality

Feeding pelleted diets increased the length, width, and breaking strength of tibia bones of broiler chickens in the current study. This could be explained by the increased availability of some minerals, particularly Ca, P, and Mg from the pelleted diets. Pelleting reduces the wastage of these important minerals, leading to improved performance and bone characteristics. Further improvement of these bone physical traits was then observed when the microbial enzymes were supplemented, with better results shown when a combination of QB (phytase) and Axtra XB was added to the diets. This could be due to, especially the effect of phytase, in degrading the phytate-nutrient complex, which could increase utilization of minerals. The improved bone physical characteristics could also be related to the increased body weight for birds on pelleted diets. These results are in accord with the findings of Hossain et al. [29] who found that tibia length and strength were increased when the birds were offered diets with phytase and carbohydrases. Tibia ash content was increased in the group of birds supplied with pelleted diets, and this was also improved by enzymes, especially when QB was added in combination with Axtra XB. These results are in agreement with previous studies by De Sousa et al. [8] and Brenes et al. [30], who found an increase in ash content of tibial bone

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when phytase and carbohydrase were added to maize-soybean-based diets. In the current study, feed form did not influence the mineral contents of tibia bone; however, microbial enzyme inclusion increased the content of some minerals: QB supplementation specifically increased the potassium and zinc contents. A previous study by Brenes et al. [30] showed that phytase supplementation increased Zn, P, and Ca of broiler tibia bones in a maize-soybean-based diets. Furthermore, phytase inclusion in maize-based diets has been reported to increase the bioavailability of bound phosphorus and other minerals such as Ca, Zn, Fe, and Cu [31].

Ileal nutrient digestibility

In the current study, pelleted diets increased the digestibility of both indispensable and dispensable amino acids. This could be related to the process of pelleting which increases the surface area of the ingredients to the action of digestive enzymes [7]. Furthermore, the heat applied during pelleting inactivates proteinaceous enzyme inhibitors, and breaks the cell wall of ingredients, providing greater access for the enzymes to the nutrients, hence increasing the digestibility [25]. Pelleting increases protein digestibility by deactivating anti-nutrients, which may improve amino acid digestibility.

Broiler chickens, like other young animals, have undeveloped digestive enzyme capacity, and thus require exogenous enzymes to enhance the digestibility of amino acid for maintenance and growth [9]. The results of the current study are in line with the findings of Cowieson and Ravindran [32] who observed improvement in the ileal digestibility of most amino acids when an enzyme cocktail of phytase and carbohydrase was added to broiler diets. A study by Rutherfurd et al. [33] revealed improved broiler ileal digestibility for most measured amino acids ecept methionine, tyrosine, histidine, and threonine when microbial phytase was added to maize–soybean-based diet. He et al. [34] also demonstrated an improvement in apparent ileal digestibility of histidine, threonine, aspartic acid, cystine, proline, and serine when diets were supplemented with phytase.

The increased digestibility of some minerals in pelleted diets is explained by the fact that pelleting tends to conserve more nutrients compared to mash diets. However, this response was not observed for all minerals, for reasons that are not clear. The digestibility of Ca, P, K, S, Zn, and Fe was also increased by enzyme supplementation, when QB (phytase) was added singly or combined with Axtra XB. It has been reported that the addition of phytase to maize-based diets increases the availability of phytate-phosphorus and other minerals, including Ca, Zn, Cu, and Fe [31,35]. Similarly, Bradbury et al. [36] found that phytase addition to broiler diets improved the digestibility of both Ca and P.

Conclusion

The results of the present study suggest that both pelleting diets and enzyme supplementation improved the AME of the feed supplied, and enhanced the energy utilized by the broiler chickens, improved nutrient digestibility and bone quality. Despite the high nutrient density in maize–soybean-based diets, it is important to supplement broiler chicken diets with microbial enzymes in order to optimize the nutrient availability.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and material

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Ethics approval and consent to participate

This article does not require IRB/IACUC approval but was approved by the University of New England Animal Ethics Committee (AEC17-079).

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