Effect of a multicarbohydrase supplementation to diets varying in metabolisable energy level on the performance, carcass traits, caecal microbiota, intestinal morphology, and nutrient digestibility in broiler chickens

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ABSTRACT
This trial was conducted to investigate the effects of multi-carbohydrase supplementation on the performance, carcass traits, intestinal histomorphology, caecal microbiota and nutrient digestibility of broiler chickens fed diets with varying energy levels. A total of 400-day-old male chicks were allocated into eight dietary treatments in a 4\times2 factorial arrangement with 5 replicates per treatment. There were 4 dietary energy levels (the standard breed recommendation and reductions of 50, 100 and 150 kcal/kg from the standard) with or without multi-carbohydrase supplementation. Reducing the energy level by 50 kcal/kg did not significantly impact the measured parameters compared to the standard recommendation while it is reducing by 100 and 150 kcal/kg resulted in significant decreases in the body weight (BW) by levels up to 4.41% and a significant increase in the feed conversion ratio (FCR) by levels up to 4.67%. Enzyme supplementation significantly improved the BW and FCR by 3.24% and 2.95%, respectively. Dietary energy lowered by 100–150 kcal/kg resulted in a significant decrease in the dressing (up to 2.42%), breast yield (up to 2.82%), fat pads (up to 8.72%), liver (up to 6.30%) percentages and coliform count (up to 28.5%). The digestibility of dry matter, crude protein, ether extract and gross energy was increased due to enzyme supplementation by 4.15, 3.50, 3.35 and 3.72%, respectively. In conclusion, broiler performance and carcass traits can be negatively impacted if the energy density is reduced by 100–150 kcal/kg diet. Enzyme supplementation can improve the performance and nutrient digestibility regardless of the dietary energy level.

HIGHLIGHTS
- The metabolisable energy level can be reduced by 50 kcal/kg diets compared to the breed recommendation.
- Decreasing the energy level by 100 or 150 kcal/kg diet compared to breed recommendations resulted in a negative impact on the performance.
- Multicarbohydrase blend supplementation resulted in significant improvements in the performance and nutrient digestibility.

Introduction
Feed cost is a major contributor to poultry farming expenses as it represents approximately 60–70% of the total production costs (Attia et al. 2019). Modern broiler chicken feed is mostly composed of raw materials of plant origin. Cereal grains and their co-/by-products (corn, wheat, barley, corn gluten meal, DDGS, etc.) have a major share in the feed formulations (representing approximately 55–70%), while oil-bearing seed meals such as soybean meal, canola meal, and sunflower meal represent approximately 20–35%. These plant-based ingredients contain significant quantities of non-starch polysaccharides (NSPs) such as arabinoxylans, \(\beta\)-glucans, cellulose, \(\beta\)-mannans, pectin, and \(\alpha\)-galactosides. NSPs possess several anti-nutritive properties; soluble NSPs can increase digesta viscosity, subsequently decreasing nutrient utilisation and disturbing the microbial ecosystem in the gut, while non-soluble NSPs can encapsulate nutrients and render them unavailable for digestion (Chocht 2015; Wickramasuriya et al. 2019). It was estimated that
approximately 400–450 kcal/kg of the energy in corn/soy diets of broiler chickens remains undigested because of the NSPs (Cowieson 2010).

Due to the limited endogenous secretion of NSP-degrading enzymes, broiler chickens are unable to hydrolyse the NSP components in the feed. Therefore, supplementing their diets with exogenous enzymes could increase energy availability, improve gut health, and boost broiler performance (Slominski 2011). Exogenous NSPases can be incorporated into broiler feed either via the top addition to the formulation without changing the dietary energy density to achieve better nutrient utilisation and performance, or via energy-sparing addition by decreasing the energy density of the feed formulation then restore this reduced energy through enzyme supplementation based on the expected improvement in energy availability which subsequently reduces feed cost while maintaining the feed performance of the original formulation (Perazzo Costa et al. 2008).

The most widely used NSPases are \( \beta \)-xylanase and \( \beta \)-glucanase which target arabinoxylan and \( \beta \)-glucans substrates that predominate in cereal grains (Adeola and Cowieson 2011). Dietary xylanase supplementation has been reported to improve the energy utilisation (Campasino et al. 2015; Williams et al. 2018) and performance (Coppedge et al. 2012; Latham et al. 2016) of broiler chickens. Likewise, \( \beta \)-glucanase supplementation led to an increase in the broiler’s performance (Józefiak et al. 2006). Other NSPases are used on a lesser scale, such as \( \alpha \)-galactosidase which targets the \( \alpha \)-galactosides in soybean meal and positively impacted nutrient utilisation and birds performance (Ghazi et al. 2003; Pettersson and Pontoppidan 2013), and \( \beta \)-mannanase which targets \( \beta \)-mannans in vegetable proteins and improves energy utilisation and broilers performance (Kidd et al. 2001; Shastak et al. 2015).

While most of the earlier studies focussed on utilising enzymes that act on the NSPs of the grains (arabinoxylans and \( \beta \)-glucans) under sufficient or deficient dietary energy levels, there is limited studies on the enzymes that target the NSP of soybean meal (\( \alpha \)-galactosides and \( \beta \)-mannans). Therefore, the current study aimed to use an enzyme blend that can work on the NSPs of both corn and soybean meal under varying dietary energy levels. The study hypothesised that supplementation of broiler chicken diets of varying energy levels with a multi-carbohydrase blend containing \( \beta \)-xylanase, \( \beta \)-glucanase, \( \alpha \)-galactosidase and \( \beta \)-mannanase could additionally or synergistically impact the nutrient digestibility, gut microbiota balance, and intestinal histomorphology, subsequently improving the performance parameters and carcass traits.

Materials and methods

Experimental birds and management

This trial was conducted at the Poultry Nutrition Research Facility, College of Veterinary Medicine, Zagazig University, Egypt. All the experimental procedures were conducted in accordance with the standard protocols of the Zagazig University Institutional Animal Care and Use Committee. A total of 400-day-old male broiler chicks (Cobb500) were purchased from a local hatchery. The chicks were individually weighed, leg banded, and distributed into 40 experimental floor pens (10 chicks/pen in 5 replicate blocks). The size of the individual pen was 1.0 m\(^2\), and the bedding materials were fresh wood shavings. The study was executed for 42 days. The ambient temperature was 33 °C at the start of the trial and lowered by 2 °C each week. The average relative humidity was 65%. The lighting program was 23 h light and 1 h of darkness.

Vaccination program

The chicks were vaccinated against Newcastle disease virus (Hitchner B1 strain on day 7 and LaSota strain on days 17 and 27), infectious bursal disease virus (D78 strain on days 12 and 22), and infectious bronchitis virus (H 120 strain on day 7) via ocular route. They were also vaccinated against avian influenza virus (reassortant inactivated H5N1 subtype, Re-1 strain on day 9) via s/c injection of 0.3 mL per chick.

Experimental diets and treatments

The chicks were fed diets varying in the metabolisable energy level (standard level (STD) based on the breed recommendation or a reduction of the standard level by 50, 100 and 150 kcal/kg (R50, R100, and R150, respectively) with or without the addition of a multi-enzyme blend, making 8 dietary treatments as follows: treatment 1, standard without enzyme (STD –ve); treatment 2, standard with an enzyme (STD +ve); treatment 3, STD reduced by 50 kcal/kg without enzyme (R50 –ve); treatment 4, STD reduced by 50 kcal/kg with enzyme (R50 +ve); treatment 5, STD reduced by 100 kcal/kg without enzyme (R100 –ve); treatment 6, STD reduced by 100 kcal/kg with enzyme (R100 +ve); treatment 7, STD reduced by 150 kcal/kg
without enzyme (R150 –ve); and treatment 8, STD reduced by 150 kcal/kg with an enzyme (R150 þ ve). The standard diet was formulated to meet Cobb500 requirements (Anonymous, 2012). The multi-enzyme blend used was ZYMPEX VR 008, a product of Impextraco NV, Belgium. The blend was included in the appropriate diets at a level of 0.05% according to the manufacturer’s recommendation. It provided 1250 \( \beta \)-xylanase units, 750 \( \beta \)-glucanase units, 1500 \( \alpha \)-galactosidase units, and 2500 \( \beta \)-mannanase units per kg of feed. The birds had free access to feed and water, and the feed was presented as a mash. The formulated diets and their chemical specification are shown in Table 1. The feeding program consisted of a starter period (0–14 days of age), grower period (15–28 days of age), and finisher period (29–42 days of age). Feed ingredients and diets were analysed according to the methods published by the AOAC International (1990) for dry matter (method 967.03), crude protein (method 984.13), ether extract (method 920.29), crude fibre (method 962.09), and ash (method 942.05). The analysed values were in agreement with the calculated values.

### Table 1. Diet composition and nutrient content (g/kg as fed).

| Ingredient                  | Starter STD | R50 | R100 | R150 | Grower STD | R50 | R100 | R150 | Finisher STD | R50 | R100 | R150 |
|-----------------------------|-------------|-----|------|------|------------|-----|------|------|--------------|-----|------|------|
| Corn, ground yellow         | 534.1       | 546.2| 558.1| 570.0| 582.1      | 594.1| 606.0| 618.0| 629.5        | 641.3| 653.2| 665.2|
| Soybean meal, 470 CP        | 336.5       | 335.0| 334.0| 333.1| 295.2      | 294.2| 293.3| 292.4| 259.9        | 259.1| 258.1| 257.2|
| Corn gluten meal, 600 CP    | 50.0        | 49.4| 48.6 | 47.7 | 41.0       | 39.9| 39.1 | 38.2 | 27.5         | 26.6| 25.7 | 24.8 |
| Limestone                   | 13.2        | 13.2| 13.3 | 13.3 | 12.4       | 12.4| 12.4 | 12.4 | 11.2         | 11.2| 11.2 | 11.2 |
| Monocalcium phosphate       | 16.5        | 16.5| 16.5 | 16.5 | 15.4       | 15.3| 15.3 | 15.3 | 13.6         | 13.6| 13.6 | 13.6 |
| Premix \( \alpha \)         | 3.0         | 3.0 | 3.0  | 3.0  | 3.0        | 3.0| 3.0 | 3.0  | 3.0          | 3.0| 3.0 | 3.0  |
| Sodium carbonate            | 3.0         | 3.0 | 3.0  | 3.0  | 3.0        | 3.0| 3.0 | 3.0  | 3.0          | 3.0| 3.0 | 3.0  |
| Sodium bicarbonate          | 1.5         | 1.5 | 1.5  | 1.5  | 1.5        | 1.5| 1.5 | 1.5  | 1.5          | 1.5| 1.5 | 1.5  |
| DL Methionine               | 2.3         | 2.2 | 2.2  | 2.2  | 2.0        | 2.0| 2.0 | 2.0  | 1.9          | 1.9| 1.9 | 1.9  |
| L. Lysine HCl               | 2.9         | 2.9 | 2.9  | 2.9  | 2.6        | 2.6| 2.6 | 2.6  | 2.1          | 2.1| 2.1 | 2.1  |
| L. Threonine                | 0.3         | 0.3 | 0.3  | 0.3  | 0.3        | 0.3| 0.3 | 0.3  | 0.3          | 0.3| 0.3 | 0.3  |
| Vegetable oil               | 37.0        | 26.8| 16.8 | 6.7  | 41.6       | 31.6| 21.5 | 11.5 | 46.5         | 36.4| 26.4 | 16.3 |

| Nutrient composition        |             |     |      |      |             |     |      |      |             |     |      |      |
|----------------------------|-------------|-----|------|------|-------------|-----|------|------|-------------|-----|------|------|
| Crude protein (analysed)    | 22.0        | 22.0| 22.0 | 22.0 | 20.0       | 20.0| 20.0 | 20.0 | 18.0        | 18.0| 18.0 | 18.0 |
| AIME, kcal/kg               | 3035        | 2985| 2935 | 2885 | 3110       | 3060| 3010 | 2960 | 3180        | 3130| 3080 | 3030 |
| AIME, Kcal/kg (determined)  | 3077        | 3020| 2974 | 2919 | 3130       | 3091| 3045 | 2982 | 3219        | 3155| 3101 | 3065 |

STD: Standard ME; R50: 50 kcal/kg reduced than standard; R100: 100 kcal/kg reduced than standard; R150: 150 kcal/kg reduced than standard; \( \alpha \) ME: Apparent metabolisable energy
\( \beta \) Supplied the following per kg of diet: Vit. A (12000 IU), Vit. D3 (3000 IU), Vit. E (10 mg), Vit. K3 (1 mg), Vit. B1 (1 mg), Vit. B2 (5 mg), Vit. B6 (1.5 mg), Pantothenic acid (10 mg), Vit. B12 (0.01 mg), Niacin (30 mg), Folic acid (1 mg), Biotin (0.05 mg), choline (540 mg), Zn (60 mg), Mn (60 mg), Fe (30 mg), Cu (4 mg), I (0.3 mg), Co (0.1 mg), and Se (0.1 mg).
\( \beta \) Determined from the analysed gross energy value according to Ravindran et al. (2008).

The birds had free access to feed and water, and the feed was presented as a mash. The formulated diets and their chemical specification are shown in Table 1. The feeding program consisted of a starter period (0–14 days of age), grower period (15–28 days of age), and finisher period (29–42 days of age). Feed ingredients and diets were analysed according to the methods published by the AOAC International (1990) for dry matter (method 967.03), crude protein (method 984.13), ether extract (method 920.29), crude fibre (method 962.09), and ash (method 942.05). The analysed values were in agreement with the calculated values.

### Measurements

#### Growth performance indices

At the end of each feeding period, the birds were individually weighed and the feed consumed per pen was measured and used to calculate the growth performance parameters (average body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio corrected for mortality (FCR)). Dead birds were recorded daily to estimate the percent mortality. Dead birds were weighed before removal. At the end of the trial, the European production efficiency factor (EPEF) was calculated as follows:

\[
\text{EPEF} = \left( \frac{\text{BW (kg)} \times \text{surviving percentage}}{\text{PP} \times \text{FCR}} \right) \times 100
\]

where PP is the production period length (days) (Marcu et al. 2013).

### Carcase traits

At the end of the trial, five birds per treatment (one bird from each replicate pen) of similar weight were selected and fastened overnight with free access to drinking water. They were individually weighed, slaughtered to complete bleeding, defeathered and eviscerated to evaluate the carcase traits. Hot carcase weight was determined as the weight of the carcase after bleeding, defeathering and removal of the head, shanks, and viscera. Dressing percentage was determined as the hot carcase weight/live weight \( \times 100 \). The carcase was dissected, and the weights of the breast, thigh plus drumstick, wings, back, abdominal fat, liver, heart and gizzard were determined and expressed as a percentage of the carcase weight (Ricard et al. 1983).
**Caecal microbiota**
At 42 days of age, the left caecum of each of the sacrificed birds (five birds per treatment, one bird from each replicate pen) were excised, bagged, labelled and stored at 4°C for 12 h then the content of each caecum was squeezed into a sterile tubes to determine the counts of coliforms and lactobacilli bacteria. Each sample was serially diluted from initial 10⁻¹ to 10⁻⁹, then 100 μL of the diluted samples were plated on agar media (MacConkey for coliforms and MRS for lactobacillus) and incubated at 37°C for 24 and 48 h under aerobic and anaerobic conditions, respectively. The results are expressed as log CFU/g of the caecal digesta (Mountzouris et al. 2011).

**Intestinal histomorphology**
On day 42, the eviscerated small intestines of five birds per treatment (one bird per replicate pen) were collected and processed for histomorphological analysis. Approximately 2-cm segments from the middle part of the duodenum, jejunum and ileum were excised and flushed with physiological saline. The collected segments were fixed in 10% neutralised formalin solution, dehydrated with increasing concentrations of ethyl alcohol, cleared in xylene and embedded in paraffin. Five micrometre sections of each sample were cut, placed onto a glass slide and stained with haematoxylin and eosin (H&E). The villus height, crypt depth, and their ratio were measured in each section using a light microscope. The villus height was measured as the distance from the apex of the villus to the junction of the villus and crypt. The crypt depth was measured from the base of the crypt upward to the region of transition between the crypt and villus (Murugesan et al. 2015).

**Ileal nutrient digestibility coefficient**
At 36 days of age, TiO₂ was included in the finisher diets at 0.3% and fed to all the treated birds during the last week of the trial. At 42 days of age, three broiler birds from each pen making a total of 15 birds per treatment, were randomly selected and sacrificed by severing the jugular vein, and the intestine was exposed. The ileum was separated from the rest of the intestine. The ileum was defined as the segment of the small intestine that extended from Meckel’s diverticulum to the ileocecal junction. The digesta within each ileal segment of the selected birds per pen were gently collected by finger stripping and pooled together into a labelled plastic container, frozen immediately after collection and subsequently freeze-dried. The dried digesta and the experimental diet samples were milled on a 0.50 mm mesh screen and analysed for dry matter, starch, crude protein, ether extract, and gross energy according to the standard methods cited by the AOAC International (1990). The concentration of TiO₂ in the feed and excreta was measured as described by Short et al. (1999). The digestibility coefficient of different nutrients was calculated using the equation described by Huang et al. (2005). Digestibility coefficient % = 100 – [(ID × AF)/(IF × AD) × 100], where ID is the concentration of the indigestible marker in the diet, IF is the concentration of the indigestible marker in the ileal digesta, AF is the nutrient concentration in the ileal digesta, and AD is the nutrient concentration in the diet.

**Experimental design and statistical analysis**
The study design was a completely randomised block design, with two factors (energy level and enzyme addition) and their interaction making eight dietary treatments (4 × 2 factorial design). There were five replicate pens per treatment. The pen was the experimental unit for the performance indices, while the bird was the experimental unit for all other measurements. The model was as follows: Xij,k = μ + Ri + βj + (αβ)ij + eij,k, where μ is the overall (grand) mean; Ri is the replicates effect; βi is the main effect of Factor A; βj is the main effect of Factor B; (αβ)ij is the interaction effect between A and B; eij,k is the experimental error; Xi,j,k is the dependent variable. The obtained results were analysed by two-way analysis of variance (ANOVA). LSD was used to determine if significant differences exist among treatments. All statements of statistical significance were based on p < .05. The statistical analysis was conducted using Statistix® 9 (Analytical Software 2008).

**Results**

**Performance indices**

**Average body weight**
A significant decrease (p < .01) in the average BW was observed due to lowering dietary energy content by 100 and 150 kcal/kg during different age periods. At 42 days of age, broilers fed the R100 and R150 diets were 3.16 and 4.41% lighter than those fed the STD diet. Reducing the energy level by 50 kcal/kg (R50) did not result in a significant difference when compared to the standard diet. The enzyme effect was significant (p < .01) at 28 and 42 days of age, in which an increase in the average BW was observed due to the inclusion
of the enzyme blend. At 42 days of age, the average BW increased by 3.24% in broilers fed enzyme-supplemented diets compared to those fed un-supplemented diets. No significant energy by enzyme interaction \((p > .05)\) was observed during different feeding periods (Table 2).

**Average daily gain**
The ADG significantly decreased by lowering the energy density of the diets during different feeding periods. Broilers fed the R100 and R150 diets showed significantly \((p < .01)\) lower overall (0–42 days of age) average daily gain compared to those fed the STD diet by 3.21 and 4.52%, respectively. The addition of the exogenous enzyme blend had a positive influence on the ADG during the feeding periods of 0–28 and 0–42 days of age \((p < .01)\). No significant energy by enzyme interaction \((p > .05)\) was observed during the different feeding periods (Table 2).

**Average daily feed intake**
The effect of energy was not significant during different feeding periods \((p > .05)\), except during the period of 0–14 days of age, in which broilers fed the R150 diet consumed more feed than those fed the STD or R100 diets \((p < .05)\). The effects of the enzyme and energy by the enzyme were not significant \((p > .05)\) during the different feeding periods (Table 3).

**Feed conversion ratio**
Broilers fed the R100 and R150 diets showed a poor FCR compared to those fed the STD or R50 diets during different feeding periods \((p < .01)\). The best overall FCR was found in the broilers fed the STD and R50 diets.

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**Table 2.** Effect of dietary energy, enzyme and their interaction on the average BW and ADG of broiler chickens (g).

| Effect of energy | Average BW (g) | ADG (g) |
|-----------------|----------------|---------|
|                 | Day 14 | Day 28 | Day 42 | 0–14 days | 0–28 days | 0–42 days |
| STD             | 462.7a | 1510a  | 2720a  | 30.10a    | 52.45a    | 63.78a    |
| R50             | 464.4a | 1495a  | 2709a  | 30.22a    | 51.91a    | 63.53a    |
| R100            | 446.4b | 1450b  | 2634b  | 28.91b    | 50.30b    | 61.73b    |
| R150            | 445.8b | 1421b  | 2600b  | 28.89b    | 49.28b    | 60.90b    |

**Effect of enzyme**
- ve
  - Energy: <0.01
  - Enzyme x Enzyme: ns

**p-Value**
- Energy: <0.01
- Enzyme: <0.05
- Energy x Enzyme: ns

**Pooled SEM**
- 3.71
- 14.13
- 26.93
- 0.27
- 0.51
- 0.64

STD: Standard ME; R50: 50 kcal/kg reduced than standard; R100: 100 kcal/kg reduced than standard; R150: 150 kcal/kg reduced than standard; BW: body weight; ADG: average daily gain; ns: no significant effect \((p > .05)\)

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**Table 3.** Effect of dietary energy, enzyme and their interaction on the ADFI, FCR and EPEF of broiler chickens.

| Effect of energy | ADFI (g) |
|-----------------|---------|
|                 | 0–14 days | 0–28 days | 0–42 days |
| STD             | 31.50b  | 71.02 | 104.1 |
| R50             | 31.61ab | 71.02 | 103.3 |
| R100            | 31.39b  | 70.91 | 103.9 |
| R150            | 31.94a  | 71.28 | 104.3 |

**Effect of enzyme**
- ve
  - Energy: <0.05
  - Enzyme x Enzyme: ns

**p-Value**
- Energy: <0.01
- Enzyme: <0.05
- Energy x Enzyme: ns

**Pooled SEM**
- 0.19
- 0.54
- 0.89
- 0.01
- 0.02
- 0.02
- 8.15

STD: Standard ME; R50: 50 kcal/kg reduced than standard; R100: 100 kcal/kg reduced than standard; R150: 150 kcal/kg reduced than standard; ADFI: average daily feed intake; FCR: feed conversion ratio; EPEF: European production efficiency factor; ns: no significant effect \((p > .05)\)

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AThe average initial body weight of day-old chicks was 41.4 ± 1.6.

- ve means without enzyme addition; +ve means with enzyme addition at 0.05%.
diets, which were significantly \((p < .01)\) lower than those fed the R100 and R150 diets by 2.97 and 4.67\%, respectively. Except for the 0–14 days of age period, the impact of enzyme supplementation was significant during the other feeding periods; broilers fed an enzyme supplemented diet showed a 2.95\% improvement in the overall FCR compared to those fed diets without enzymes \((p < .05)\). The interaction between energy and enzyme was not significant \((p > .05)\) during different feeding periods (Table 3).

### Percent mortality and European production efficiency factor

The overall percent mortality during the trial period was 2.25\% which is in the normal range of mortality in broiler farms, with no significant impact from the energy level, enzyme supplementation or their interaction (data not shown). The EPEF was significantly influenced by reducing the feed energy and by enzyme supplementation \((p < .01)\); the best EPEF was found in broilers fed the STD and R50 diets compared to those fed the R100 and R150 diets. Broilers fed an enzyme-supplemented diet showed a 2.95\% improvement in the EPEF compared to those fed the un-supplemented diets. The impact on energy by enzyme interaction was not significant \((p > .05)\) during different feeding periods (Table 3).

### Carcase traits

Reducing the energy level resulted in a decrease in the percentages of dressing by up to 2.42\% \((p < .05)\), breast by up to 2.82\% \((p < .01)\), abdominal fat by up to 8.72\% \((p < .05)\), and liver by up to 6.3\% \((p < .05)\). The energy level had no significant impact on the percentages of thigh and drumstick, back, wing, gizzard and heart \((p > .05)\). The effects from enzyme supplementation and energy by enzyme were not significant for the measured carcarse traits \((p > .05)\;\text{Table 4}).

### Caecal microbiota

As shown in Table 5, the caecal coliform count was significantly decreased by up to 28.5\% when the dietary energy was lowered from the standard level to the reduced energy levels \((p < .05)\). The lactobacilli count was not significantly influenced by changing the energy level. Total coliform or lactobacilli counts were not significantly modified by the effects of enzyme or energy by enzyme \((p > .05)\).

### Intestinal histomorphology

The villi length, crypt depth and villi length to crypt depth ratio of the different small intestine segments (duodenum, jejunum and ileum) were not impacted \((p > .05)\) by the energy level, enzyme supplementation or their interaction (Table 6).

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**Table 4. Effect of dietary energy, enzyme and their interaction on the carcase traits (%) of broiler chickens at 42 days of age.**

| Carcase Traits | Dressing | Breast | Thigh + drumstick | Back | Wing | Abdominal fat | Liver | Gizzard | Heart |
|---------------|----------|--------|-------------------|------|------|---------------|-------|---------|-------|
| **Effect of energy** |          |        |                   |      |      |               |       |         |       |
| STD           | 72.94\(^a\) | 26.58\(^a\) | 31.43            | 14.99 | 11.17 | 1.72\(^a\)    | 3.00\(^a\) | 2.14    | 0.85  |
| R50           | 72.44\(^ab\) | 26.58\(^a\) | 31.40            | 14.98 | 11.08 | 1.61\(^ab\)   | 3.02\(^a\) | 2.18    | 0.87  |
| R100          | 71.58\(^bc\) | 25.83\(^b\) | 31.63            | 14.90 | 11.38 | 1.57\(^b\)    | 2.84\(^b\) | 2.26    | 0.91  |
| R150          | 71.17\(^c\)  | 25.86\(^b\) | 31.73            | 14.83 | 11.22 | 1.57\(^c\)    | 2.83\(^b\) | 2.16    | 0.86  |
| **Effect of enzyme** |          |        |                   |      |      |               |       |         |       |
| - ve          | 72.24     | 26.22  | 31.50             | 14.93 | 11.19 | 1.63          | 2.94  | 2.19    | 0.88  |
| + ve          | 71.82     | 26.20  | 31.59             | 14.91 | 11.24 | 1.61          | 2.91  | 2.17    | 0.87  |
| **p-Value**   |          |        |                   |      |      |               |       |         |       |
| Energy        | <0.05     | <0.01  | ns                | ns   | ns   | <0.05         | <0.05 | ns      | ns    |
| Enzyme        | ns        | ns     | ns                | ns   | ns   | ns            | ns    | ns      | ns    |
| Energy x Enzyme | ns    | ns     | ns                | ns   | ns   | ns            | ns    | ns      | ns    |
| Pooled SEM    | 0.59      | 0.23   | 0.27              | 0.10 | 0.18 | 0.05          | 0.08  | 0.12    | 0.05  |

STD: Standard ME; R50: 50 kcal/kg reduced than standard; R100: 100 kcal/kg reduced than standard; R150: 150 kcal/kg reduced than standard; ns: no significant effect \((p > .05)\).

\(^{a,b,c}\)Means in a column with no common superscript letter differ significantly \((p < .05)\).

\(-ve\) means without enzyme addition; \(+ve\) means with enzyme addition at 0.05%.

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**Table 5. Effect of dietary energy, enzyme and their interaction on the caecal microflora (log CFU/g) of broiler chickens at 42 days of age.**

| Caecal Microflora | Coliform Count | Lactobacilli Count |
|-------------------|----------------|-------------------|
| **Effect of energy** |                |                   |
| STD               | 2.45\(^a\)    | 4.87              |
| R50               | 1.90\(^b\)    | 5.48              |
| R100              | 1.90\(^b\)    | 5.60              |
| R150              | 1.75\(^b\)    | 5.61              |
| **Effect of enzyme** |                |                   |
| - ve              | 2.10           | 5.30              |
| + ve              | 1.91           | 5.49              |
| **p-Value**       |                |                   |
| Energy            | <0.05          | ns                |
| Enzyme            | ns             | ns                |
| Energy x Enzyme   | ns             | ns                |
| Pooled SEM        | 0.05           | 0.07              |

STD: Standard ME; R50: 50 kcal/kg reduced than standard; R100: 100 kcal/kg reduced than standard; R150: 150 kcal/kg reduced than standard; ns: no significant effect \((p > .05)\).

\(^{a,b}\)Means in a column with no common superscript letter differ significantly \((p < .05)\).

\(-ve\) means without enzyme addition; \(+ve\) means with enzyme addition at 0.05%.
Enzyme supplementation resulted in a significant increase \((p < .05)\) in the digestibility coefficient of dry matter by 4.15%, starch by 3.54%, crude protein by 3.5%, ether extract by 3.35% and gross energy by 3.72%. The effect of energy or energy by enzyme was not significant \((p > .05)\) on the digestibility coefficient of the measured nutrients (Table 7).

### Discussion

The present study tested four dietary energy levels representing the standard level (breed recommendations) and three reduced levels. Reducing the energy level by 50 kcal/kg did not result in significant differences in the performance parameters, while its reduction by 100 and 150 kcal/kg resulted in negative effects on body weight gain and FCR. Similarly, previous research findings revealed that lowering the energy density of the diets resulted in poor weight gain and feed conversion (Nahashon et al. 2005; Mirshekar et al. 2013). Unexpectedly, the overall feed intake in the current study was not impacted by reducing dietary energy. It is known that feed intake is inversely related to dietary energy level, so birds will tend to consume more feed when fed a low energy diet to satisfy their energy requirements. It seems that modern broiler chickens have been primarily selected to consume feed at almost full capacity regardless of the dietary energy level (Barbato, 1994).

Multi-carbohydrase supplementation in the current study resulted in significant improvement in the average BW, ADG, and FCR without a significant impact on the ADFI. The enzymes act in low viscosity corn-soy diets mainly by degrading plant cell wall constituents that encapsulate feed nutrients which in turn results in an increase in protein, starch, and energy digestibility (Kaczmarek et al. 2014) and by releasing

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**Table 6.** Effect of dietary energy, enzyme and their interaction on the intestinal histomorphology of broiler chickens at 42 days of age.

| Nutrient | Duodenum | Jejunum | Ileum |
|----------|----------|---------|-------|
| | VL(μm) | CD (μm) | VL:CD | VL(μm) | CD (μm) | VL:CD | VL(μm) | CD (μm) | VL:CD |
| Effect of energy | | | | | | | | | |
| STD | 1230 | 223.0 | 5.54 | 991.2 | 194.4 | 5.13 | 574.2 | 161.1 | 3.59 |
| R50 | 1286 | 237.0 | 5.44 | 987.0 | 196.3 | 5.20 | 549.1 | 142.0 | 3.89 |
| R100 | 1295 | 225.2 | 5.78 | 1028 | 202.0 | 5.14 | 571.2 | 147.1 | 3.92 |
| R150 | 1257 | 223.5 | 5.64 | 1033 | 204.0 | 5.14 | 575.0 | 146.0 | 4.03 |
| Effect of enzyme | | | | | | | | | |
| - ve | 1257 | 229.4 | 5.51 | 1017 | 193.0 | 5.33 | 558.0 | 144.0 | 3.94 |
| + ve | 1277 | 225.0 | 5.69 | 1002 | 205.4 | 4.96 | 577.0 | 154.0 | 3.78 |
| p Value | | | | | | | | | |
| Energy ns | ns | ns | ns | ns | | ns | ns | ns |
| Enzyme ns | ns | ns | ns | ns | | ns | ns | ns |
| Energy x Enzyme ns | ns | ns | ns | ns | | ns | ns | ns |
| Pooled SEM 43.85 | 8.11 | 0.18 | 22.55 | 11.38 | 0.28 | 24.58 | 7.10 | 0.21 |

STD: Standard ME; R50: 50 kcal/kg reduced than standard; R100: 100 kcal/kg reduced than standard; R150: 150 kcal/kg reduced than standard; VL: villi length; CD: crypt depth; VL:CD : villi length to crypt depth ratio; ns: no significant effect \((p > .05)\).

**Table 7.** Effect of dietary energy, enzymes and their interaction on the nutrient digestibility coefficient \(\%\) of broiler chickens at 42 days of age.

| Nutrient | Dry matter | Starch | Crude protein | Ether extract | Gross energy |
|----------|------------|--------|--------------|---------------|--------------|
| Effect of energy | | | | | |
| STD | 82.64 | 93.80 | 79.88 | 83.27 | 73.73 |
| R50 | 85.26 | 96.18 | 81.88 | 85.42 | 75.65 |
| R100 | 83.60 | 94.42 | 81.06 | 83.75 | 74.16 |
| R150 | 83.70 | 95.36 | 81.72 | 84.71 | 75.60 |
| Effect of enzyme | | | | | |
| - ve | 82.10<sup>a</sup> | 93.30<sup>b</sup> | 80.00<sup>b</sup> | 82.90<sup>b</sup> | 73.4<sup>2b</sup> |
| + ve | 85.51<sup>a</sup> | 96.60<sup>a</sup> | 82.80<sup>a</sup> | 85.67<sup>a</sup> | 76.15<sup>a</sup> |
| p-Value | | | | | |
| Energy ns | | | | | |
| Enzyme p < .05 | | | p < .05 | | |
| Energy x Enzyme ns | | | ns | | |
| Pooled SEM 1.82 | 1.88 | 1.67 | 1.78 | 1.51 |

STD: Standard ME; R50: 50 kcal/kg reduced than standard; R100: 100 kcal/kg reduced than standard; R150: 150 kcal/kg reduced than standard; ns: no significant effect \((p > .05)\).

**Nutrient digestibility**

Enzyme supplementation resulted in a significant increase \((p < .05)\) in the digestibility coefficient of dry matter by 4.15%, starchy by 3.54%, crude protein by 3.5%, ether extract by 3.35% and gross energy by 3.72%. The effect of energy or energy by enzyme was not significant \((p > .05)\) on the digestibility coefficient of the measured nutrients (Table 7).
The positive impact of enzyme supplementation on broiler performance appeared during the grower and finisher stages but not in the starter stage. It seems that the response to carbohydrate supplementation occurs primarily during the later stages of broiler growth due to increased microbial population and fermentation challenges in the gut of older birds that can be modulated by exogenous enzymes (Choct et al. 1996). Enzyme supplementation improved the FCR without altering the feed intake, which indicates that this improvement occurred due to increased nutrient utilisation. This was in agreement with the findings of Govil et al. (2017) and Wickramasuriya et al. (2019).

Although the broiler EPEF was significantly decreased by reducing the energy level by 100 and 150 kcal/kg, this parameter increased when the energy level was reduced by 50 kcal/kg. This gives an indication that it could be economical to lower the dietary energy level by 50 kcal/kg compared with the level mentioned in the breed manual. The EPEF increased when the multi-carbohydrase blend was included in the diets. Likewise, Rehman et al. (2016) reported a significant increase in the EPEF due to 8-mannanase enzyme supplementation. In contrast, Saleh et al. (2018) did not observe a significant impact on the EPEF due to dietary enzyme blend supplementation in broiler chickens.

Reducing the energy level led to a significant decrease in the percentages of dressing, breast, fat pad and liver. This might be attributed to less energy retention by the birds. Similarly, Zhao et al. (2008) reported a significant reduction in the weights of the carcass, breast, thigh and abdominal fat while decreasing the dietary energy density and lysine. Likewise, Wang et al. (2014) reported a significant decrease in abdominal fat due to the decreasing dietary energy density of broiler chickens. In contrast, other researchers did not observe significant differences in carcass and breast yield, heart or liver due to the use of different dietary energy levels (Infante-Rodriguez et al. 2016; Abouelezz et al. 2019). This variation in response could be attributed to differences in the dietary energy level or broiler breed used. The inclusion of exogenous enzymes in the current study did not significantly impact any of the measured carcass traits. It seems that the increased nutrient and energy availability due to enzyme blend supplementation did not reflect on the carcass traits. Similarly, Rehman et al. (2016) reported no impact from 8-mannanase supplementation on the dressing, carcase, thigh, abdominal fat and gizzard percentages of broiler chickens. Lei et al. (2017) didn’t observe significant differences in the relative weights of the breast muscle, liver, abdominal fat, gizzard and spleen due to dietary supplementation of a multi-carbohydrase blend in broiler chickens.

Gut microbiota can be modified (controlling the growth of pathogenic bacteria and/or enhancing the growth of beneficial bacteria) by decreasing the amount of nutrients available for bacterial fermentation (Silva and Smithard 2002) with subsequent improvement in broiler growth performance. Lowering the dietary energy in the current study resulted in a significant decrease in coliform bacterial growth. This might be attributed to reducing the amount of nutrients available for bacterial fermentation that came from energetic feed ingredients (Apajalahti et al. 2004). Unexpectedly, dietary supplementation of the multi-enzyme blend did not result in significant changes in the coliform or lactobacilli count. This result disagrees with Jozefiak et al. (2010), who observed a significant decrease in the coliform count in the broiler chicken gut due to dietary supplementation with xylanase and /-glucanase and Sharmila et al. (2015), who reported a significant decrease in the caecal pathogenic bacteria (enterobacteria, E. coli and Salmonella spp.) of broiler chickens due to supplementation of their diets by xylanase and cellulase enzymes.

Intestinal histomorphology (villi length, crypt depth and their ratio) can be used as an indicator for nutrient absorption. Taller villi and reduced crypt depth are indicators for better nutrient absorption and lower mucosal tissue turnover rate (Apperson and Cherian, 2017). Our findings revealed that neither the energy level nor enzyme supplementation affected the intestinal histomorphological parameters. Our results agree with Wang et al. (2015), who did not observe a significant difference in the histomorphological characteristics of broiler chickens due to lowering dietary energy by 100 kcal/kg, and Wickramasuriya et al. (2019), who did not observe a significant impact on intestinal histomorphology by lowering dietary energy or adding enzymes. On the other hand, Karimi and Zhandi (2015) reported significant improvements in the intestinal histomorphological parameters of broiler chickens due to /-glucanase and/or 8-mannanase supplementations. The reported improvement in body weight gain and FCR due to enzyme supplementation in the
The present study may not be related to any improvement in intestinal morphology.

Multi-enzyme blend supplementation in the present study significantly increased the digestibility coefficient of the dry matter, starch, crude protein, ether extract and gross energy regardless of the energy level. This may be attributed to the degradation of NSPs with subsequent disruption of the cell wall, which allows endogenous digestive enzymes to access cell-bound nutrients (Wickramasuriya et al. 2019). Similar reports have revealed an increase in the digestibility coefficient of dry matter, crude protein, starch, energy and/or NSPs in broiler chickens due to dietary enzyme supplementation such as xylanase, glucanase, mannanase, α-galactosidase and/or pectinase (Ghazi et al. 1997a, b; Meng et al. 2005; Pourreza et al. 2007; Jasek et al. 2018). On the other hand, other researchers did not find a significant impact from enzyme supplementation on nutrient digestibility (Onderci et al. 2006; Yu et al. 2007). This variation in response could be attributed to differences in diet ingredients (different NSP fractions) and the composition of the enzyme blend and its dosage.

Conclusions

Lowering the metabolisable energy level by 50 kcal/kg diet against the breed recommendations did not impact the broiler performance, while its lowering by 100–150 kcal/kg diet resulted in a negative impact on the performance and some carcase traits. Multi-carbohydrase supplementation resulted in better performance and nutrient digestibility regardless of the dietary energy level.

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Ethical approval

This study follows the principles of the declaration of Helsinki.

Disclosure statement

The authors declare no potential conflict of interest.

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