THAISA PEREIRA DE FRANÇA

EFFECTS OF SUPPLEMENTATION OF DIFFERENT ENZYMES IN LOW ENERGY DIETS FOR BROILERS

Dissertation submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Leandro Santos Costa

Co-adviser: Luiz Fernando Teixeira Albino

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Leandro Santos Costa
Adviser

To my family and my friends.

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“Mas é preciso ter manha, é preciso ter graça, é preciso ter sonho sempre.
Quem traz na pele essa marca, possui a estranha mania de ter fé na vida” (Milton Nascimento)

ABSTRACT

FRANÇA, Thaisa Pereira de, M.Sc., Universidade Federal de Viçosa, February, 2023. **Effects of supplementation of different enzymes in low energy diets for broilers**. Adviser: Leandro Santos Costa. Co-adviser: Luiz Fernando Teixeira Albino.

Two experiments were conducted to evaluate the effects of different enzymes on the performance and gut morphology of broilers fed reduced energy diets, metabolizable energy values of reduced energy level diets. In experiment I - 1,280 male Cobb broilers, aged 1 to 42 days old, were used to evaluate performance parameters and gut morphology. Animals were distributed into 8 treatments with 8 replicates of 20 broilers each. The treatments consisted of: T1 - positive control (PC) diet with 3050 kcal ME/kg; T2 - negative control (NC) diet with a reduction of 200 kcal/kg; T3 - NC + Carbohydrase I (100 g/ton endo-1,4-beta-mannanase) ; T4 - NC + Carbohydrase II (100 g/ton endo-1,4-beta-xylanase and endo-1,4-beta-glucanase); T5 - NC + phytase (50 g/ton phytase-6); T6 - NC + Carbohydrase II + phytase; T7 - NC + Carbohydrase III (100g/ton endo-1,4-beta-xylanase); T8 - NC + Carbohydrase I + Carbohydrase II + phytase. The evaluated variables were body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), villus height (VH), crypt depth (CD), and villus height: crypt depth ratio (VH: CD). In experiment II – 420 14-day-old broilers were distributed into seven treatments with ten replicates of` six broilers each, to determine the apparent metabolizable energy (AME) and corrected for nitrogen balance (AMEn) of the diets. The same diets were used as treatments, excluding the PC. Comparing the treatments with the NC group, birds fed diets containing enzymes showed better BWG and FCR from 1 to 21 days old ($P \leq 0.05$), and better FCR from 1 to 42 days ($P \leq 0.05$). Additionally, the addition of Carbohydrase II and phytase promoted an increase in AME and AMEn of feed ($P \leq 0.05$). Broilers fed with phytase showed higher VH when compared with the other treatments ($P \leq 0.05$), whereas the treatments with phytase and Carbohydrases I, II, and phytase combination showed a better VH:CD ($P \leq 0.05$). The inclusion of enzymes (carbohydrases and phytase) in broiler diets makes it possible to reduce feed energy levels without affecting performance parameters, AME corrected for nitrogen, and morphometry characteristics of the jejunum.

Keywords: Broiler. Metabolizable Energy. Enzymes.

RESUMO

FRANÇA, Thaisa Pereira de, M.Sc., Universidade Federal de Viçosa, fevereiro de 2023. **Efeitos da suplementação de diferentes enzimas em dietas de baixa energia para frangos de corte.** Orientador: Leandro Santos Costa. Coorientador: Luiz Fernando Teixeira Albino.

Dois experimentos foram conduzidos para avaliar os efeitos de diferentes enzimas no desempenho e na morfologia intestinal de frangos de corte alimentados com dietas com baixo teor de energia e determinar os valores de energia metabolizável. No experimento I - 1.280 frangos de corte Cobb machos, com idade de 1 a 42 dias, foram utilizados para avaliar os parâmetros de desempenho e a morfologia intestinal. Os animais foram distribuídos em 8 tratamentos com 8 repetições de 20 frangos cada. Os tratamentos consistiram em: T1 - dieta controle positivo (PC) com 3050 kcal EM/kg; T2 - dieta controle negativo (NC) com redução de 200 kcal/kg; T3 - NC + Carboidrase I (100 g/tonelada de endo-1,4-beta-mananase); T4 - NC + Carboidrase II (100 g/ton endo-1,4-beta-xilanase e endo-1,4-beta-glucanase);T5 - NC + fitase (50 g/ton fitase-6); T6 - NC + Carboidrase II + fitase; T7 - NC + Carboidrase III (100g/tonelada de endo-1,4-beta-xilanase); T8 - NC + Carboidrase I + Carboidrase II + fitase. As variáveis avaliadas foram ganho de peso (GP), consumo de ração (CR), conversão alimentar (CA), altura de vilosidades (VH), profundidade de cripta (CD) e relação altura de vilosidade:profundidade de cripta (VH:CD). No experimento II – 420 frangos de corte com 14 dias de idade (528.8 ± 13.34 g) foram distribuídos em sete tratamentos com dez repetições de seis frangos cada, para determinar os valores de energia metabolizável aparente (EMA) e corrigida para balanço de nitrogênio (EMAn) das dietas. As mesmas dietas do experimento I foram utilizadas como tratamentos, excluindo o CP. Comparando-se os tratamentos com o grupo CN, as aves alimentadas com dietas contendo enzimas apresentaram melhor GP e melhor CA de 1 a 21 dias de idade ($P \leq 0,05$), e melhor CA de 1 a 42 dias ($P \leq 0,05$). Além disso, a adição de Carboidrase II e de fitase promoveu aumento da EMA e da EMAn da ração ($P \leq 0,05$). Frangos de corte alimentados com fitase apresentaram maior VH quando comparados aos demais tratamentos ($P \leq 0,05$), enquanto os tratamentos com fitase e Carbohidrases I, II e combinação de fitase apresentaram melhor VH:CD ($P \leq 0,05$). A inclusão de enzimas (carboidrases e fitase) em dietas de frangos de corte permite

reduzir os níveis de energia da ração sem afetar os parâmetros de desempenho, EMAn e as características de morfometria do jejuno.

Palavras-chave: Frango de Corte. Energia Metabolizável. Enzimas.

LIST OF TABLES

SUMMARY

1. INTRODUCTION

Poultry diets are mainly composed of plant origin feed ingredients, with corn and soybean meal being the most commonly used ingredients in poultry production. Despite being considered with excellent quality, due to present high digestibility, these ingredients have antinutritional factors that negatively influence animal performance (Bavaresco et al., 2021).

Phytate and non-starch polysaccharides (**NSP**) are two of the main antinutritional factors found in vegetable ingredients (Cowieson, 2005; Babatunde et al., 2020; Wang et al., 2021). Phytate, or phytic acid, is the primary storage form of phosphorus in grains and seeds and reduces the bioavailability of minerals such as calcium, zinc, iron, magnesium, and other nutrients (amino acids, carbohydrates, and lipids), as well as making phosphorus unavailable to non-ruminant species (Broch et al., 2018; Gonzalez Uarquin et al., 2020).

The presence of NSP in the diet influences the increase of intestinal viscosity and consequently, decreases the binding of endogenous enzymes to substrates. Making the nutrients unavailable because they are coated in the insoluble matrix of the cell wall, acting as a barrier between substrates and enzymes, reducing the passage rate, modifying the pH of the intestine, among others (Choct et al., 1997; Raza et al., 2019). In addition to affecting the gut morphology (villus height, crypt depth, and ratio) as the presence of these antinutritional factors can result in the decreased nutrient absorptive capacity due to reduced nutrient digestibility (Gomide Junior et al., 2004; Westbrook and Cherian, 2019).

A solution to minimize the effects of these antinutritional factors is the inclusion of exogenous enzymes in diets of non-ruminant animals, to improve the metabolic performance, nutrient digestibility, and minimize feed costs. These enzymes allow nutritionists to reduce the energy level in diets, in which the combination of exogenous enzymes can improve the performance response of animals and supply the metabolizable energy deficit (Allouche et al., 2015; Bedford and Cowieson, 2020).

Recent studies have shown that the supplementation of enzymes (carbohydrases and phytases), added alone or combined in energy-reduced broiler diets, promoted growth performance, improved nutrient digestibility, intestinal morphometry and consequently, energy utilization, reducing the excretion of these nutrients into the environment and

feed costs (Alagawany et al., 2018; Roofchaei et al., 2019; Wang et al., 2021; Babatunde et al., 2022).

This study hypothesized that supplementation of different enzymes in low energy diets can improve performance, metabolizable energy values, and gut morphology parameters. Thus, the objective of this study was to evaluate the effects of the supplementation of different enzymes in low energy diets on broiler growth performance, gut morphological characteristics, and feed metabolizable energy values.

2. MATERIALS AND METHODS

2.1 Ethics Committee

The Institutional Animal Care and Use Committee approved all animal handling procedures (case number CEUAP/UFV/046/2021), and the experiments were conducted according to the experimental protocol for the use of live birds from the Brazilian College of Animal Experimentation. The experiments were carried out in Viçosa, Minas Gerais State, Brazil (20° 45' 57.19" S, 42° 51' 35.42" W, and 682 m altitude).

2.2 Enzymes used

Four enzymes produced by two different companies operating in the Brazilian market were used. Three carbohydrases (Carbohydrase I (**CHO I**), an endo-1,4-beta-mannanase (800 TMU/kg feed), produced in powder with a recommended dose of 100 g/ton of feed. Carbohydrase II (**CHO II**), which consists of the combination of two enzymes, derived from the microbial fermentation of *Aspergillus niger* (endo-1,4-beta-xylanase and endo-1,4-beta glucanase) in powder (560 TXU/kg and 250 TGU/kg feed), with a recommended dose of 100 g/ton of feed. Carbohydrase III (**CHO III**), the fiber degradation enzyme complex based on 1,4 – beta xylanase (1500 EPU/kg feed) derived from the *Trichoderma citrinoviride* strain (IMI SD 135), with a recommended dose of 100 g/ton of feed) and one phytase (**PHY**) – Hybrid 6-phytase (1000 FTU/kg), derived from bacteria [EC 3.1.3.26] produced by *Aspergillus niger*, in a granulated format), with a recommended dose of 50 g/ton of feed).

2.3 Experimental Design and Diets

2.3.1 Experiment I

A total of 1,280 male Cobb 500 broilers, weighing 42.12 ± 0.44 g, were used to assess growth performance and gut morphology from 1 to 42 days old. The birds were distributed into 8 treatments by following a completely randomized design, with 8 replicates and 20 birds per experimental unit. Broilers were housed in a masonry shed divided into 1.0 × 2.0 m boxes and lined with wood shavings. The animals were subjected to a 24-h light program at 32 ℃ in their first week of life. Afterwards, there was a reduction of 1 h of light daily until reaching 20 h of light and 4 h of dark, which was used until the end of the experiment. Broilers had free access to food and water throughout the experimental period; maximum and minimum temperatures inside the facilities were recorded on daily basis by three thermometers positioned at strategic points, at bird height.

Four diets with different energy levels were formulated (Table 1), which consisted of two positive control (**PC**) diets and two negative control (**NC**) diets (Table 1). The PC diets were formulated to meet metabolizable energy levels in accordance with the recommendations of Rostagno et al. (2017) for two growing phases of birds: 3050 and 3200 ME kcal/kg, in the starter (1–21 days old) and finisher (22–42 days old) phases, respectively. The NC diets were formulated with a reduction of 200 ME kcal/kg, reaching ME values of 2850 and 3000 kcal/kg, in the starter and finisher phases, respectively. In addition, four different enzymes were added “on top” in the NC diets, and two combinations of those enzymes were made, totaling eight treatments: T1) PC diet; T2) NC diet; T3) NC + CHO I; T4) NC + CHO II; T5) NC + PHY; T6) NC + CHO II + PHY; T7) NC + CHO III; T8) NC + CHO I + CHO II + PHY (Table 2).

At 21 and 42 days, the animals and leftover feed were individually weighed in each experimental unit to determine body weight gain (**BWG**), mean feed intake (**FI**), and feed conversion ratio (**FCR**) during experimental periods 1–21 and 1–42 days.

At 21 days, all the birds were weighed and one bird from each experimental unit was chosen according to the average weight and slaughtered by cervical dislocation for collection of 3 cm of the jejunum, using Meckel’s diverticulum as a reference. Then, with the aid of a syringe, the intestinal lumen was washed with saline solution to remove the luminal content. The segments were opened, through a longitudinal

incision, and later stapled to cardboard. Stapling was performed through the mucosa, with the serosa remaining in contact with the cardboard so that it was fixed and distended without harming its structures (Gava, 2012). The samples were identified and placed in plastic pots in their respective treatments in a 10% buffered formalin solution. Samples were dehydrated by increasing the levels of alcohol in the solution, were clarified in xylene, and embedded in liquid paraffin at 60 °C. The sections underwent microtomy to obtain 5µm thick semi-serial cross sections and were stained with hematoxylin and eosin according to Luna (1968). Three 5 µm thick cross sections were placed per slide. To perform the morphological readings, an optical microscope (EVOS® XL Core) with a 10x magnification was used, capturing the slides in photography. Subsequently, villus heights (**VH**), crypt depths (**CD**), and villus height: crypt depth ratio (**VH:CD**) were measured by ImageJ 1.50i software; java1.6.0_20 (National Institutes of Health, USA). Twenty villi and 20 CD were measured in each experimental unit.

2.3.2 Experiment II

A total of 420 14-day-old chicks (528.8 ± 13.34 g) were weighed and transferred to wire floor cages (500 cm^2/bird) in a four-level battery equipped with a trough feeder and a nipple drinker to determine apparent metabolizable energy (**AME**) and nitrogen balance-corrected apparent metabolizable energy (**AMEn**). A completely randomized design was used to assign the birds into 7 treatments with 10 replicates with 6 birds per experimental unit. The treatments used were the same as in experiment I, except for the PC diet which was not included.

The experimental period took place from 14 to 24 days old. Before the experimental period, birds were reared in solid wall houses, equipped with cross ventilation and a stocking density of 38 kg/m^2, according to conditions established by Cobb500™ guideline recommendations and fed with diets formulated according to Rostagno et al. (2017). From 14 d, five days were used for adaptation of the animals to the diets and five days for total collection excreta, twice a day at 8:00 a.m. and 4:00 p.m. Plastic-coated aluminium trays were placed under the cages for excreta collection purposes. Collected excreta were placed in plastic bags, identified based on experimental unit, and kept in a freezer until the end of the collection period. Feed intake was measured during the excreta collection period.

After the end of collection period, the excreta of each experimental unit were weighed, homogenized, and sub samples (300g) were pre-dried at 55 °C for 72 h and milled (Tecnal Equipamentos para Laboratório, TE-350, São Paulo, Brazil) for 5 min, until it turned into a fine mix.

Diets and excreta were analyzed to determine dry matter (**DM**) and crude protein (**CP**) rates (AOAC, 1990). Gross energy (**GE**) values were determined by using a C500 adiabatic calorimetric pump (IKA-Werke GmbH & Co. KG, Staufen, Germany). Values of AME and AMEn were calculated based on GE values recorded for feed and excreta. The Kjeldahl method was used to determine nitrogen levels of the excreta and the diet, based on official analysis methods (AOAC, 1990). Nitrogen excreted (**NE**) was calculated by multiplying the total excretion amount (in DM) by the nitrogen rate found in the excretion (also in DM). The same method was applied to calculate nitrogen intake (**NI**), while nitrogen retained (**NR**) was calculated by subtracting NE from NI. The NR rate (%**NR**) was calculated by considering the amount of nitrogen that was ingested. Nitrogen balance (**NB**) was obtained based on the amount of intake nitrogen minus the excreted nitrogen by using the equations described by Sakomura and Rostagno (2016):

$$AME = (GE_{int} - GE_{exc})/DMI \text{ and}$$

$$AMEn = (GE_{int} - GE_{exc})/ DMI - (8{,}22 \times NB)$$

In which GEint = intake gross energy, GEexc = excreted gross energy, and DMI = intake dry matter.

2.4 Statistical Analysis

The data were subjected to the Shapiro–Wilk test to determine the normality of the residuals; subsequently, an analysis of variance (ANOVA) (*p-value* = 5%) was executed using the ExpDes.pt package of the R statistical program (R Software v. 4.0.4). Dunnett's test was used at a 5% significance level to compare means recorded for the control treatment (PC) to those of other treatments.

The adopted statistical model was:

$$Yi_k = \mu + \tau i + \varepsilon i_k,$$

in which Yi_k = value recorded for the response variable observed in the k-th repetition of the i-th level of the tested factor, μ = mean value recorded for treatments, τi = effect of the i-th level of the tested factor, and εi_k = experimental error associated with the observed Yi_k value.

3. RESULTS

3.1 Experiment I

In the starter phase, birds that received diets with an energy reduction and with enzyme supplementation showed a significant difference for BW and FCR when compared to broilers from the NC treatment ($P \leq 0.05$) and not significantly different from those of the PC treatment (without energy reduction and enzyme addition) ($P > 0.05$) (Table 3). In the finisher phase, no significant differences were observed for any of the analyzed variables.

In the total rearing period (1–42 days), broilers that received the treatments with added enzymes showed a significant difference in FCR, when compared to those from the NC treatment ($P \leq 0.05$) and not statistically different from those of the PC ($P > 0.05$) (Table 3).

Reductions in VH were observed in the jejunum of birds that received the NC diets ($P \leq 0.05$). Broilers subjected to the treatments with added enzymes showed significant differences in PC ($P \leq 0.05$). Furthermore, broilers in the treatment with added phytase had increased VH when compared with other treatments. ($P \leq 0.05$) (Table 5).

The CD of birds was not affected by the treatments ($P > 0.05$). However, for VH:CD, the treatment with the addition of phytase only and the treatment with combinations of three enzymes (NC+CHO I + CHO II + PHY) showed significant difference when compared to the other treatments ($P \leq 0.05$) (Table 5).

3.2 Experiment II

Broilers fed the NC diet (− 200 kcal/kg) had a higher DM intake compared to broilers fed with the treatments NC + CHO I, NC + CHO II + PHY, and NC + CHO I + CHO II + PHY that showed lower DM consumption ($P \leq 0.05$). Compared to the NC, the addition of the enzymes carbohydrases (NC + CHO II) and 6-phytase (NC + PHY) promoted a significantly increased ($P \leq 0.05$) the AME and AMEn of the feed in DM (Table 4).

In addition, it was also observed that the means of NI were significantly lower in the treatments NC + CHO I, NC + CHO II + PHY, and NC + CHO I + CHO II + PHY when compared to the NC ($P \leq 0.05$). Regarding the NE, broilers that received feed with added enzymes had lower values when compared with the NC ($P \leq 0.05$) (Table 4).

The percentages of NR by broilers in the treatments NC + CHO I, NC + CHO II, NC + PHY, and NC + CHO I + CHO II + PHY were higher than the NC ($P \leq 0.05$). On average,

the addition of the different enzymes provided an increase in the percentage of NR by more than 8.1%.

4. DISCUSSION

4.1 Performance

The digestive process in poultry is not 100% efficient. Approximately 15% to 25% of the nutrients that broilers intake is not available due to the presence of antinutritional factors in the feed ingredients, since poultry cannot produce enzymes that can hydrolyze these factors. Therefore, the use of exogenous enzymes in animal nutrition will hydrolyze part of these antinutritional factors, such as phytate and NSP, and consequently will release several nutrients that were unavailable for animals (Bedford and Partridge, 2010; Singh et al., 2019).

The availability of exogenous enzymes can improve the use of energy due to the greater availability of nutrients and consequently, allows the reduction of metabolizable energy levels in feed besides contributing to the improvement of performance parameters (Campestrini et al., 2005). Our findings showed that the broilers that received the treatments with reduced energy levels (NC diets) and were supplied with enzymes, obtained an increase in BWG of 4.0% and an improvement in FCR of 6.5%, showing not significant difference to the PC group.

The results were more expressive in the starter phase, as chicks were favored for exogenous enzyme consumption because they do not have a fully developed gastrointestinal tract (**GIT**) and insufficient endogenous enzyme production for nutrient digestion and absorption (Garcia et al., 2003; Brito et al., 2006; Cowieson et al., 2019). Based on this statement, several studies demonstrated a better effect of exogenous enzymes in the starter phase of broilers. Zou et al. (2013) reported a superior effect of exogenous enzymes (b-mannanase, xylanase, and b-glucanase) on the activity of endogenous enzymes (trypsin and chymotrypsin) in 21-day-old broilers, promoting lower secretion of endogenous enzymes, favoring the digestive process, nutrient digestibility, and broiler performance. Broch et al. (2018) also observed an improvement in performance variables from 1 to 21 days in broilers supplemented with phytase at different inclusion levels.

According to Gonzalez Ortiz et al. (2016) carbohydrase supplementation contributes to animal performance by decreasing the viscosity of the digestate and improving nutrient utilization due to the breakdown of the cell wall matrix and release of encapsulated nutrients, promoting substrate contact with endogenous enzymes, especially in the starter phase.

In the finisher phase (22 – 42 days), no significant differences were observed for any of the analyzed variables. The observed results agree with the study of Gonzalez Ortiz et al., 2017 who found no significant differences in the 21 to 42 days phase.

During the total rearing period (1–42 days), the broilers subjected to the treatments with added enzymes showed a significant improvement in FCR of 5.1% compared to the NC group. Giacobbo et al. (2021) found that, in both phases, the treatment with reduced energy and without enzyme inclusion resulted in lower weight gain when compared to the control diet. In addition, they observed that the inclusion of amylase in the negative treatment, alone or in combination with xylanase and protease improved body weight gain to the same level as the control group in both phases.

The interaction of phytase enzymes and carbohydrases can be seen in two ways. First, when ingredients rich in NSP are used, the cell wall acts as a protective barrier for some nutrients, including phytic acid from enzymatic action. Thus, the inclusion of carbohydrases improves the efficiency of phytase, since the NSP are hydrolyzed, releasing the components that were complexed, including phytic acid. In addition, with the hydrolysis of phytic acid there is the release of other nutrients, such as carbohydrates, making these substrates available for the carbohydrases to act on (Ravidran, 2013; Bedford et al., 2020).

4.2 Metabolizable energy

There are several factors that can impact the digestibility of nutrients in broilers, one of them is the composition of feed ingredients, especially when it contains high levels of NPS (Sethy et al., 2015). Enzymes are added to increase the energy value of feed ingredients and maximize the digestibility, absorption and utilization of carbohydrates, proteins, lipids, and phosphorus, reducing the excretion, especially of phosphorus and nitrogen in the environment (Alabi et al., 2019).

In addition, the use of enzymes in diets with reduced energy or other nutrients, can reduce feed intake and contribute to production costs, because by increasing the

availability of the nutrient, consequently there is a greater availability of energy, reducing the intake by the animal (Ptak et al., 2013; Alabi et al., 2019).

In this study, the FI was influenced by reducing the energy level of the diet, where the broilers that received the NC diet had higher consumption compared to broilers subjected to the treatments NC + CHO I, NC + CHO II + PHY, and NC + CHO I + CHO II + PHY.

According to Richards and Proszkowiec-Weglarz (2007), broilers tend to increase FI in order to compensate for the reduced energy level of the diet. This behavior may result not only from the inclusion of enzymes in the diet, but from the ability of the broilers to regulate intake to maintain growth performance (Leeson et al., 1996). Roofchaei et al. (2019) reported that birds that received a wheat-based diet (NC) had higher FI compared to broilers that consumed diets with carbohydrases associated or not with acidifiers and phytase.

The percentage of NR from treatments with added carbohydrases and phytases was higher than the NC. On average, the addition of the different enzymes increased the percentage of NR by 8.1%. Wang et al. (2021), when evaluating the effects of the combination of phytase (0, 500, 1000, and 1500 FTU/kg) and multi-carbohydrases (500 mg/kg) on performance, bone ash percentage, and nutrient digestibility in broilers from 1 to 18 days observed an increase in NR and AMEn values, equaling the PC, providing improvements in ileal phosphorus digestibility, where values were higher than the other treatments.

Several studies have corroborated that exogenous enzyme supplementation improves energy utilization and performance in broilers by hydrolyzing insoluble NSP (Zhou et al., 2009; Williams et al., 2014; Amerah et al., 2017; Fernandes et al., 2017; Ravn et al., 2019).

According to Cowieson (2010), it is estimated that 400–450 kcal of metabolizable energy per kg of feed are undigested due to the levels of NSP present in soybean meal diets. In contrast, insoluble NSP present in the cell wall trap starch, protein, and other nutrients within the so-called “cage effect”, impairing the access of endogenous enzymes to digestible nutrients (Ravn et al., 2016). In this study, the addition of carbohydrases and phytase (NC+ CHO II + PHY) promoted an increase in AME and AMEn in DM by an average of 3.2% (106 Kcal/kg DM) and 2.9% (92 Kcal/kg DM) compared to the NC, reflecting the best response among the enzymes added. Thus, the inclusion of exogenous enzymes in ingredients with higher values of metabolizable

energy provide better nutrient utilization, and consequently, better performance for birds. In this way, the levels of energy sources in broiler diets can be reduced (Campestrini et al., 2005).

According to Bedford and Cowieson (2020) the inclusion of the enzyme allows the formulator to decrease the energy density of the diet by 100 kcal, which corresponds to replacing 2% fat (a high - cost ingredient) with 2% corn.

4.3 Gut morphology

Gut morphology (villus height, crypt depth and ratio) is directly related to endogenous enzyme production and is used as an indicator of intestinal absorptive capacity and gut health (Santin et al., 2001; Baurhoo et al., 2007; Wang et al., 2020). The presence of exogenous agents, such as the presence or absence of food, food characteristics, presence of antinutritional factors, and pathological conditions also influence these characteristics (Gomide Junior et al., 2004).

The results of this study showed that broilers that received the NC diet had reduced VH ($P \leq 0.05$) by an average of 28.5 % compared to the PC and the broilers in the enzyme treatments. In contrast, the treatment with added phytase increased VH when compared to the other enzymes by 12.6 %, the highest response among the enzymes studied ($P \leq 0.05$).

Roofchaei et al. (2019) reported that combining xylanase with phytase or an acidifier increased villus length in the jejunum of broilers, and no differences were found for the other gut morphometry variables. This increase in VH represents a greater number of enterocytes and enteroendocrine cells and is associated with greater activity of brush border enzymes and improvements in digestive function and absorption (Caspary, 1992; Uni et al., 2000; Westbrook and Cherian, 2019).

According to Broom (2015), the importance of VH is related to determining the functional maturity of enterocytes that migrate to the villus tip. Consequently, in shorter villi, enterocytes reach the apex of the villi early, impairing the development of enzyme secretion capacity, causing reduced digestive and absorptive efficiency.

The VH:CD in the present study showed that the treatment with the addition of phytase alone and the treatment with combinations of three enzymes (CHO I + CHO II + PHY) showed a higher ratio ($P \leq 0.05$). When comparing the other enzyme treatments with the NC, the inclusion of enzymes increased the VH:CD by 25.4%. According to Montagne et al. (2003), increased VH:CD suggests intestinal mucosa with good

differentiation and high digestion and absorption capacity. Liu et al. (2017), when evaluating different levels of xylanase in wheat-based diets, reported that there was an increase in VH and VH:CD for the duodenum, jejunum, and ileum of broiler chickens.

Yaqoob et al. (2022) conducted an experiment with corn and soybean meal-based diets, with different energy reductions, with the inclusion of different levels of b-mannanase and found greater VH and VH:CD in broilers that received the diets with the highest level of b-mannanase inclusion (600 mg/kg feed). According to Alqhtani et al. (2022), the improvement in gut morphology with the use of an enzyme complex or isolated enzymes can be attributed to the decrease in negative impacts of NSP in the diet on intestinal villi.

De Maesschalck et al. (2015) reported that in diets with added carbohydrases, such as xylanase, there is a higher concentration of butyrate-producing bacteria and lactic acid, this lactic acid will be consumed by butyrate-producing bacteria in the distal sections of the GIT, which boosts gut health and consequently performance. The effects on gut histomorphology could be explained by the increased availability of fermentable substrates in the small intestine due to the supplementation of xylanase and other carbohydrases. Consequently, villus height may increase due to the extra source of energy for the enterocytes (Hu and Guo, 2007). In addition, exogenous enzymes improve the balance of the gut by decreasing substrate for pathogenic bacteria, making substrate available for beneficial fermentative organisms, and increasing the ability of the gut to defend against unwanted bacteria (Cowieson and Kluenter, 2019).

In general, deeper crypts and shorter villi reduce the VH:CD, impairing intestinal absorption, increasing secretion by the gastrointestinal tract, and affecting broiler performance (Li et al., 2015; Yaqoob et al., 2022).

5. CONCLUSION

In conclusion, the inclusion of enzymes (carbohydrases and phytase) in broiler diets makes it possible to reduce the energy levels of the feed without affecting performance parameters, AME corrected for NB, and gut morphometry characteristics of the jejunum. In addition, the use of Carbohydrase II with phytase (NC + CHO II + PHY) provided better results for AME in the feed and AMEn, and the combination of Carbohydrases I and II with phytase (NC + CHO I + CHO II + PHY) enabled a better VH:CD ratio

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Table 1. Ingredients and nutrient composition of experimental diets.

Ingredients (%)	Starter (1–21 days)		Finisher (22–42 days)	
	PC	NC	PC	NC
Corn	49.138	49.138	59.956	59.956
Soybean meal	42.306	42.306	31.519	31.519
Soy oil	4.716	2.441	4.963	2.688
Dicalcium phosphate	1.770	1.770	1.431	1.431
Limestone	0.870	0.870	0.838	0.838
Salt	0.503	0.503	0.458	0.458
DL-Methionine, 99%	0.316	0.316	0.308	0.308
BioLys. 54.5%	0.090	0.090	0.258	0.258
L-Threonine, 98%	0.036	0.036	0.014	0.014
Vitamin supplement[1]	0.100	0.100	0.100	0.100
Mineral supplement[2]	0.100	0.100	0.100	0.100
Choline chloride, 60%	0.100	0.100	0.100	0.100
Salinomycin[3] (12%)	0.055	0.055	0.055	0.055
Antioxidant (BHT)[4]	0.001	0.001	0.001	0.001
Inert	0.000	2.275	0.000	2.275
Total	100	100	100	100
Calculated Composition				
ME. Kcal/kg	3050	2850	3200	3000
Crude protein, %	23.3 (23.03) *	23.3 (23.31) *	19.41 (19.39) *	19.41 (19.26) *
Calcium, %	0.878	0.878	0.760	0.760
Available phosphorus, %	0.450	0.450	0.370	0.370
Sodium, %	0.218	0.218	0.200	0.200
Digestible lysine, %	1.250	1.250	1.124	1.124
Digestible met. +Cys., %	0.930	0.930	0.832	0.832
Digestible threonine, %	0.829	0.829	0.669	0.669
Digestible arginine, %	1.508	1.508	1.203	1.203
Digestible valine, %	0.967	0.936	0.827	0.805
Digestible tryptophan	0.270	0.270	0.212	0.212
Chlorine	0.350	0.350	0.324	0.324
P-Fitico	0.240	0.240	0.221	0.221

ME = metabolizable energy; NC = negative control; PC = positive control.

*Analyzed values

[1]Vitamin premix provided per kg of diet: vitamin A, 8250 IU; vitamin D3, 2090 IU; vitamin E, 31.0 IU; vitamin B1, 2.20 mg; vitamin B6, 3.08 mg; pantothenic acid,11.0 mg; biotin, 0.077 mg; vitamin K3, 1.65 mg; folic acid, 0.77 mg; nicotinic acid, 33.0 mg; vitamin B12, 0.013 mg.

[2]Trace mineral premix provided per kg of diet: manganese, 77.0 mg; iron, 55.0 mg; zinc, 71.5 mg; copper, 11.0 mg; iodine, 1.10 mg; selenium, 0.33 mg. [3]Anticoccidia[4]Antioxidant Butylhydroxytoluene

Table 2. Description of the treatments for experiments 1 and 2.

Treatments	ME	Enzyme
PC	3050 kcal ME/kg	-
NC	2850 kcal ME/kg	-
NC + CHO I	2850 kcal ME/kg	100 g/ton endo-1,4-beta-mannanase
NC + CHO II	2850 kcal ME/kg	100 g/ton endo-1,4-beta-xylanase and endo-1,4-beta-glucanase
NC + PHY	2850 kcal ME/kg	50 g/ton phytase-6
NC + CHO III	2850 kcal ME/kg	100g/ton endo-1,4-beta-xylanase
NC + CHO II + PHY	2850 kcal ME/kg	100 g/ton endo-1,4-beta-xylanase, and endo-1,4-beta-glucanase) + 50 g/ton phytase-6
NC + CHO I + CHO II + PHY	2850 kcal ME/kg	100 g/ton endo-1,4-beta-xylanase and endo-1,4-beta-glucanase) + (100g/ton endo-1,4-beta-mannanase) + (50g/ton of phytase-6)

ME: metabolizable energy; CHO I = 800 TMU/kg feed; CHO II = 560 TXU/kg feed and 250 TGU/kg feed; CHO III = 1500 EPU/kg feed; PHY = 1000 FTU/kg),

Table 3. Means for performance broilers: Feed Intake (FI), Body Weight Gain (BWG), and Feed Conversion Rate (FCR).

Treatments	Day 1 – 21			Day 22- 42			Day 1- 42		
	FI (kg)	BWG (kg)	FCR	FI (kg)	BWG (kg)	FCR	FI (kg)	BWG (kg)	FCR
PC	1.353	1.064	1.271	3.857	2.339	1.649	5.210	3.403	1.531
NC	1.370	1.037*	1.320*	3.940	2.310	1.703	5.311	3.350	1.585*
NC+ CHO I	1.339	1.075	1.245	3.823	2.348	1.628	5.162	3.424	1.508
NC+ CHO II	1.327	1.075	1.234	3.826	2.352	1.627	5.153	3.428	1.503
NC+ PHY I	1.341	1.079	1.244	3.853	2.371	1.625	5.195	3.451	1.506
NC+ CHO III	1.338	1.070	1.250	3.838	2.333	1.644	5.177	3.404	1.520
NC+ CHO II + PHY	1.342	1.075	1.249	3.847	2.358	1.631	5.189	3.433	1.511
NC+ CHO I+ CHO II+ PHY	1.337	1.076	1.243	3.826	2.345	1.631	5.164	3.421	1.503
CV	2.51	1.84	1.77	2.22	2.19	2.63	1.77	1.63	2.02
p-value	0.462	0.045	0.036	0.268	0.693	0.740	0.169	0.313	0.005
SEM	0.006	0.004	0.009	0.013	0.005	0.009	0.018	0.009	0.009

Mean followed by * in the same column differs from PC, based on Dunnett's test, at 5% significance level ($P < 0.05$); FI = feed intake; BWG = body weight gain; FCR = feed conversion rate; PC = positive control; NC = negative control; CHO I = 100 g/ton endo-1,4-beta-mannanase; CHO II = 100 g/ton endo-1,4-beta-xylanase and endo-1,4-beta-glucanase; CHO III = 100g/ton endo-1,4-beta-xylanase; PHY = 50 g/ton phytase-6.

SEM = standard error of the mean.

Table 4. Means for Apparent Metabolizable Energy (AME), Metabolizable Energy Corrected for Nitrogen Balance (AMEn), Nitrogen Intake (NI), Nitrogen Excreted (NE), and Nitrogen Retained (NR).

Treatments	Variable					
	DMI (g)	AME (kcal/kg)	AMEn (kcal/kg)	NI (g/bird)	NE (g/Bird)	NR (%)
NC	294.1	3303.7	3131.5	10.8	4.7	56.9
NC+ CHO I	275.8*	3388.7	3201.9	10.1*	3.9*	61.5*
NC+ CHO II	285.2	3424.9*	3234.8*	10.5	3.9*	62.5*
NC+ PHY	281.2	3488.6*	3300.5*	10.4	3.9*	62.0*
NC+ CHO III	285.1	3374.03	3191.8	10.5	4.2	60.2
NC+ CHO II + PHY	276.8*	3393.4*	3209.5	10.1*	4.0*	60.8
NC+ CHO I+ CHO II+ PHY	274.5*	3389.6	3200.7	10.1*	3.8*	62.1*
CV	4.06	2.20	2.07	4.06	10.14	5.72
p-value	0.006	0.001	0.001	0.006	0.001	0.003
SEM	2.610	20.983	19.173	0.092	0.105	0.002

Mean followed by * in the same column differs from PC, based on Dunnett's test, at 5% significance level ($P < 0.05$); DMI = dry matter intake; AME = apparent metabolizable energy; AMEn = nitrogen balance-corrected apparent metabolizable energy; NE = Nitrogen excreted; NI = nitrogen intake; NR = nitrogen retained; NC = negative control; CHO I = 100 g/ton endo-1,4-beta-mannanase; CHO II = 100 g/ton endo-1,4-beta-xylanase and endo-1,4-beta-glucanase; CHO III = 100g/ton endo-1,4-beta-xylanase; PHY I = 50 g/ton phytase-6.
SEM = standard error of the mean.

Table 5. Effect of reduced energy levels and supplementation of different enzymes on jejunum intestinal morphology parameters in broilers.

Treatments	Variables		
	Villi Height (µm)	Crypt Depth (µm)	Villus Hight: Crypt Depth
PC	742.667	209.826	3.541
NC	623.924*	191.048	3.295
NC+ CHO I	812.459	211.712	3.842
NC+ CHO II	780.045	206.358	3.802
NC+ PHY	872.697*	212.591	4.133*
NC+ CHO III	788.354	203.327	3.880
NC+ CHO II + PHY	775.036	200.546	3.864
NC+ CHO I+ CHO II+ PHY	794.597	196.203	4.055*
CV	10.27	9.74	9.12
p-value	0.011	0.276	0.007
SEM	5.342	2.404	2.677

Mean followed by * in the same column differs from PC, based on Dunnett's test, at 5% significance level ($P < 0.05$); PC = positive control; NC = negative control; CHO I = 100 g/ton endo-1,4-beta-mannanase; CHO II = 100 g/ton endo-1,4-beta-xylanase and endo-1,4-beta-glucanase; CHO III = 100g/ton endo-1,4-beta-xylanase; PHY = 50 g/ton phytase-6.
SEM = standard error of the mean.

UNIVERSIDADE FEDERAL DE VIÇOSA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS DE PRODUÇÃO
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Viçosa, 30 de setembro de 2021

CERTIFICADO

Certificamos que o projeto intitulado "**Contribuição de diferentes enzimas nos valores de energia metabolizável da dieta de frangos de corte**", protocolo **nº 046/2021**, sob a responsabilidade de **Luiz Fernando Teixeira Albino** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo chordata, subfilo vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 8 de outubro de 2008, do decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi apreciado pela Comissão de Ética no Uso de Animais de Produção da Universidade Federal de Viçosa (CEUAP-UFV) em reunião de **27 de setembro de 2021.**

Finalidade: **(x)Pesquisa** **( )Ensino** Vigência do Projeto: de **15 de outubro de 2021 a 30 de out. 2022**
Espécie/linhagem: ***Frango de corte (Gallus domesticus)*** Nº de animais: **2920**
Peso: **0,04Kg** Idade: **1 dia** Sexo: **Macho** Origem: **Incubatorio Rivelli CNPJ/CPF: 478.715.616-49**
Endereço: Rua Leão José, 257 Mateus Leme, MG Responsável : Maria Cecilia CRMV: 10595

CERTIFICATE

We certify that the project entitled "**Contribution of different enzymes on metabolizable energy values of diets for broiler chickens**", protocol **nº 046/2021**, under the responsibility of **Luiz Fernando Teixeira Albino** - which involves the production, maintenance and/or use of animals belonging to the phylum chordata, subphylum vertebrata (except man), for scientific research purposes (or education) - is in accordance with the law nº. 11.794, of October 8, 2008, Decree nº. 6899 of July 15, 2009, and the rules issued by the Brazilian National Council for Animal Experimentation Control (CONCEA), and was approved by the Ethics Commission on the use of farm animals of Universidade Federal de Viçosa (CEUAP-UFV) in its meeting on **Sep. 27th of 2021**.

Finality: **(x)Research** **( )Education**
Duration of the Project: from **Oct. 15th of 2021** to **Oct. 30Th of 2022**.
Species / strain: ***Broiler (Gallus domesticus)*** Nº of animals: **2920**
Weight: **0,04Kg** Age: **1 day** Sex: **Male** Source: **Incubatorio Rivelli CNPJ/CPF: 478.715.616-49**
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Luciana Navajas Rennó
Coordenadora da CEUAP/UFV