



Carbohydrase inclusion in a corn-soybean diet improves broiler growth performance

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ABSTRACT. An experiment was conducted to evaluate the effect of diets with reduced energy level content, supplemented with carbohydrase, on broiler performance and the coefficient of metabolizability of nutrients. A total of 720 one-day-old male Cobb-500 chicks were distributed in a completely randomized design, with six treatments, eight repetitions of 15 birds each. The treatments were: (1) a positive control, basal diet to meet the requirements of broiler chickens (PC); (2) a negative control, basal diet with a reduction of 80 kcal kg⁻¹ (NC); (3) NC + alphagalactosydase; (4) NC + xylanase; (5) NC + xylanase and alphagalactosydase, and (6) NC + enzymatic blend (alphagalactosydase, xylanase, pectinase and amylase). The nutrient digestibility was not improved by use of enzymes. At 7 days of age, the broilers which were fed diets supplemented with enzymes showed a lower feed intake (FI) and better feed conversion ratio (FCR) than the broilers fed on PC. Both the NC and enzymatic blend resulted in a worse performance of the broilers at 21, 35 and 42 days old. The use of alphagalactosydase and xylanase, isolated or in combination, in a corn-soybean meal-based diet is effective in improving the growth performance of broilers fed energy-reduced diets.

Keywords: poultry; carbohydrate; enzymes; non-starch polysaccharides.

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Introduction

The broiler diet is composed, for the most part, of corn and soybean meal acting as energy and amino acids sources, respectively. These feeds contain soluble and insoluble non-starch polysaccharides (NSP) that are indigestible to poultry. The soybean meal and the corn contain 136.7 and 76.3 g kg⁻¹ of NSP, respectively (Meng & Slominski, 2005a).

Exogenous enzymes have been suggested for poultry diets because of the limited endogenous production of these enzymes in the gastrointestinal tract (Khattak, Pasha, Hayat, & Mahmud, 2006). The most studied exogenous enzymes are phytase (Cowieson et al., 2017; Scholey, Morgan, Riemensperger, Hardy, & Burton, 2018; Gonzalez-Uarquin, Kenéz, Rodehutschord, & Huber, 2020), proteases (Xu et al., 2017; Srilatha, Reddy, Preetam, Rao, & Reddy, 2019; Jabbar, Tahir, Khan, & Ahmad, 2020), carbohydrases (Musigwa, Cozannet, Morgan, Swick, & Wu, 2020; Llamas-Moya et al., 2020) and their associations (Cowieson & Adeola, 2005; Yuan, Wang, Zhang, & Wang, 2017; Sousa et al., 2019).

Cellulose, hemicellulose and pectin comprise the three major categories of NSP that make up nearly 90% of plant cell walls (Ward, 2020). Carbohydrase enzymes are used in broiler corn-soybean-based diets to increase nutrient digestibility and improve the birds' growth performance. Carbohydrase improves the digestibility of dry matter, organic matter, protein, starch, fat, insoluble and soluble fibers, and energy utilization (Cozannet, Kidd, Neto, & Geraert, 2017). This enhancement of digestibility can be associated with improvements in growth performance regardless of dietary energy levels (Wickramasuriya et al., 2019). The use of carbohydrase allows the reduction of energy in broiler diets. It also leads to lower production costs (Bavaresco et al., 2020).

Interaction between the enzymes had been verified (Woyengo, Bogota, Noll, & Wilson, 2019). According to Ward (2020), synergies between the main chain and debranching enzymes have been documented for a wide range of indigestible carbohydrates in corn, wheat, and high protein feed ingredients. In addition, many studies have reported that the association between phytase and carbohydrase is beneficial in improving

nutrient utilization for broiler chickens (Gallardo, Dadalt, & Neto, 2020), the performance of broilers (Ennis, Jackson, Gutierrez, Cantley, & Wamsley, 2020) and bone mineralization (Francesch & Geraert, 2009).

The objective was to evaluate the effect of corn-soybean diets with reduced energy level content, supplemented with carbohydrase, on broiler growth performance and the coefficient of the metabolizability of nutrients.

Material and methods

The experiment was carried out in Goiania, Goias, Brazil (17°27'49" S latitude, 48°12'06" W longitude, 807 m altitude). The animal research was conducted in accordance with the requirements of the institutional committee on animal use (CEUA; case number. 029/19).

To assess the growth performance, a total of 720 1-d old male Cobb500® broiler chicks were randomly housed in 48 pens where six treatments were provided, resulting in eight replicates with 15 birds each. The treatments were:

- (1) Positive control - a basal diet without enzyme (PC)
- (2) Negative control - a basal diet with a reduction of 80 kcal kg⁻¹ without enzyme (NC)
- (3) NC supplemented with alphagalactosydase
- (4) NC supplemented with xylanase
- (5) NC supplemented with alphagalactosydase + xylanase
- (6) NC supplemented with an enzymatic blend (alphagalactosydase + xylanase + pectinase + amylase)

The alphagalactosydase was provided by AlphaGal 140 – Kerry Inc., from *Saccharomyces cerevisiae*; the xylanase was provided by Econase XT – AB Vista – from *Pichia pastoris*, and the complex alphagalactosydase + xylanase + pectinase + amylase was provided by AlphaGal 280p – Kerry Inc. – from *Saccharomyces cerevisiae* and *Trichoderma longibrachiatrum*. The levels of inclusion of enzymes in diets were preconized by the manufacturer (AlphaGal 140p = 1.5 kg ton of soybean meal⁻¹; AlphaGal 280p = 200 g ton of diet⁻¹; Econase XT = 100 g ton of diet⁻¹).

The diets were iso-nitrogenous, based on corn and soybean meal, and formulated following the recommendations of Rostagno et al. (2017). The birds had free access to feed and water, given in three periods: pre- initial (1 to 7d), initial (8 to 21d) and growth (22 to 42d) (Table 1).

The broilers were housed on a litter of rice straw in boxes located in the central area of the shed with a negative pressure system, nebulisation system and evaporative cooling. Each box contained a line with five nipple drinkers and a tubular chicken feeder. The box represented the experimental unit.

The broiler performance was measured at 7, 21, 35 and 42 days old. The final body weight (FBW), daily body weight gain (BWG), feed conversion ratio (FCR) and feed intake (FI) were calculated. Data were corrected for mortality. The experimental period lasted 42 days.

To assess the coefficient of the metabolizability of diets, 210 chicks were randomly housed in metabolic cages, distributed into seven repetitions and five birds each. The battery cages were equipped with feeders and linear drinkers and metal trays to remove excreta. Each battery contained five floors, with divisions of 0.33 x 0.50 m, and 40 experimental units. The management of the broilers was done according to breeder manual management. The ambient temperature and relative humidity were recorded daily. The total excreta were collected from the 17 to 21 day-old chicks to calculate the coefficient of the metabolizability of dry matter (CMDM), of crude protein (CMCP), of calcium (CMCa) and of phosphorus (CMP). The birds were allowed to adapt to the cages for five days before the excreta were collected. The broilers were fed the experimental diets of the initial phase and had free access to the diets and water. The light program adopted was 24h daily of light. The excreta collection was performed twice a day. After collection, the excreta were conditioned in identified plastic bags and then frozen. At the end of the experiment, the excreta were defrosted and homogenized according to the method proposed by Sakomura and Rostagno (2016). The feed intake and mortality were assessed.

The content of dry matter, crude protein, calcium and phosphorus of the diets and excreta were assessed. The dry matter content was determined by drying in an oven (105°C), the crude protein by the micro-Kjeldahl method (nitrogen distiller Tecnal® TE-0364); the calcium was assessed by absorption atomic spectrophotometry (spectrophotometer Shimadzu® AA-7000) and phosphorus by spectrophotometry (spectrophotometer Metash® UV-5100). The analysis was determined according to the procedures described by Silva and Queiroz (2006).

Table 1. Composition of basal experimental diets (g kg⁻¹).

| Ingredients | Pre-Initial | Initial | Growth |
|---|-------------|---------|---------|
| Corn | 573.787 | 611.407 | 700.013 |
| Soybean Meal | 332.667 | 288.667 | 220.000 |
| Meat meal | 34.000 | 28.667 | 28.000 |
| Viscera meal | 32.000 | 34.667 | 10.000 |
| Feather meal | 0.000 | 0.000 | 10.000 |
| Poultry fat | 2.000 | 12.667 | 8.667 |
| Calcarium | 5.333 | 6.333 | 6.333 |
| Salt | 3.000 | 2.267 | 2.533 |
| Sodium bicarbonate | 2.133 | 2.000 | 1.400 |
| DL-Methionine | 4.007 | 3.480 | 2.900 |
| Choline Cl | 0.573 | 0.627 | 0.873 |
| Acidifiant | 0.000 | 0.120 | 0.180 |
| Vehicle (soybean meal) | 2.007 | 0.700 | 1.713 |
| Copper sulphate | 0.300 | 0.300 | 0.300 |
| L-Treonine | 1.747 | 1.520 | 1.433 |
| L-Valine | 0.200 | 0.000 | 0.000 |
| Prebiotic | 0.400 | 0.400 | 0.000 |
| Nicarbazin +Senduramicin | 0.000 | 0.600 | 0.000 |
| Salinomycin 12% | 0.000 | 0.000 | 0.600 |
| Antioxidant | 0.040 | 0.040 | 0.040 |
| Adsorvent | 1.000 | 1.000 | 0.000 |
| Phytase (500 FTU*) | 0.100 | 0.100 | 0.100 |
| Vitamin premix | 0.500 | 0.500 | 0.500 |
| Mineral premix | 0.600 | 0.500 | 0.500 |
| L-Lysine | 3.107 | 2.940 | 3.413 |
| Bacitracin | 0.500 | 0.500 | 0.500 |
| Total | 1000.0 | 1000.0 | 1000.0 |
| Energy Metabolizable (Kcal) | 2900 | 3006 | 3100 |
| Crude Protein (g kg ⁻¹) | 240.4 | 220.0 | 191.8 |
| Digestible Arginine (g kg ⁻¹) | 14.0 | 12.7 | 10.5 |
| Lysine Digestible (g kg ⁻¹) | 13.4 | 12.2 | 10.6 |
| Methionine+ Cysteine Digestible (g kg ⁻¹) | 9.9 | 9.0 | 8.0 |
| Treonine Digestible (g kg ⁻¹) | 8.8 | 8.0 | 6.9 |
| Tryptophan Digestible (g kg ⁻¹) | 2.3 | 2.1 | 1.7 |
| Calcium (g kg ⁻¹) | 9.6 | 9.2 | 7.9 |
| Avaible phosphorus (g kg ⁻¹) | 4.8 | 4.5 | 3.8 |
| Sodium (g kg ⁻¹) | 2.3 | 1.9 | 1.8 |
| Chlorine(g kg ⁻¹) | 3.0 | 2.5 | 2.7 |
| Potassium (g kg ⁻¹) | 8.7 | 7.9 | 6.7 |
| Elctrolitic Balance(mEq kg ⁻¹) | 2445.0 | 2200.0 | 1750.0 |
| Choline (ppm) | 18000.0 | 17500.0 | 16500.0 |

The coefficient of metabolizability of dry matter (CMDM), of crude protein (CMCP), of calcium (CMCa) and phosphorus (CMP) were determined according to equations proposed by Sakomura and Rostagno (2016).

Data were subjected to analysis of variance, and means were compared by the Scott-Knott test, at $\alpha = 0.05$ significance level. Statistical analyses were performed by the "R" version 3.6.0 (Plataforma RStudio - 1.2.1335, © 2009-2019, Inc.) software.

The proposed mathematical model was as follows:

$$Y_{ij} = \mu + a_i + \epsilon_{ij}$$

in which y_{ij} = value observed in treatment ($i = 1, 2, \dots, 6$) and repetition ($j = 1, 2, 3, \dots, 8$); μ = overall mean of the experiment; a_i = fixed effect of treatment i ($i = 1, 6$); and ϵ_{ij} = random error in the treatment ($i = 1, 2, \dots, 6$), and repetition ($j = 1, 2, 3, \dots, 8$).

Results

At 7 days of age, broilers fed diets supplemented with enzymes showed lower FI and better FCR than broilers fed on the basal diet (Table 2).

Broilers at 21 days old fed on diets with reduced energy level content and an added enzymatic blend (alphagalactosydase + xylanase + pectinase + amylase) presented lower BWG and FBW, lower FI and better FCR (Table 2).

At 35 days old, the BWG and the FBW increased in broilers fed the basal diet, fed alphagalactosydase, and fed xylanase separately. The FCR was better in broilers fed a basal diet and alphagalactosydase, xylanase and alphagalactosydase+ xylanase (Table 2). The FI was similar among the treatments.

It was confirmed that the diet with reduced energy level content and the diet supplemented with an enzymatic blend resulted in worse FCR at 42 days. The FI was not affected by the experimental diets. The basal diet and the use of alphagalactosydase and xylanase separately resulted in better FBW (Table 2).

The mortality was not significantly affected by the treatments.

Table 2. Productive performance of broilers at seven, 21, 35 and 42 days old fed experimental diets.

| Parameters | PC | NC | NC + AlphaGal | NC + xylanase | NC + AlphaGal + xylanase | NC + blend | P value | CV (%) |
|--------------------------|---------|---------|---------------|---------------|--------------------------|------------|---------|--------|
| 1 to 7 days | | | | | | | | |
| FBW (g) | 189 | 187 | 190 | 186 | 188 | 189 | 0.849 | 3.53 |
| BWG (g d ⁻¹) | 20.7 | 20.2 | 20.5 | 19.9 | 20.4 | 20.4 | 0.694 | 4.72 |
| FI (g) | 184.5 a | 148.6 b | 160.8 b | 155.8 b | 154.9 b | 150.8 b | 0.001 | 7.11 |
| FCR | 1.26 a | 1.04 b | 1.10 b | 1.06 b | 1.11 b | 1.06 b | 0.001 | 7.91 |
| Viab (%) | 100 | 99.1 | 100 | 99.1 | 100 | 100 | 0.556 | 1.37 |
| 1 to 21 days | | | | | | | | |
| FBW (g) | 1050 a | 884 b | 1017 a | 1017 a | 1023 a | 909 b | 0.001 | 3.91 |
| BWG (g d ⁻¹) | 47.8 a | 39.9 b | 46.3 a | 46.2 a | 46.5 a | 41.1 b | 0.001 | 4.08 |
| FI (g) | 1335 a | 1244 b | 1258 b | 1292 a | 1324 a | 1252 b | 0.001 | 3.06 |
| FCR | 1.355 b | 1.480 a | 1.310 b | 1.350 b | 1.347 b | 1.449 a | 0.001 | 4.66 |
| Viab (%) | 98.3 | 99.1 | 100 | 98.3 | 98.0 | 99.1 | 0.802 | 3.01 |
| 1 to 35 days | | | | | | | | |
| FBW (g) | 2525 a | 2365 b | 2521 a | 2470 a | 2403 b | 2397 b | 0.001 | 3.39 |
| BWG (g d ⁻¹) | 70.8 a | 66.2 b | 70.7 a | 69.3 a | 67.3 b | 67.2 b | 0.001 | 3.46 |
| FI (g) | 3744 | 3724 | 3775 | 3720 | 3698 | 3728 | 0.714 | 4.35 |
| FCR | 1.512 b | 1.602 a | 1.516 b | 1.550 b | 1.499 b | 1.588 a | 0.008 | 4.02 |
| Viab (%) | 98.3 | 97.4 | 98.3 | 98.3 | 98.3 | 95.8 | 0.729 | 3.90 |
| 1 to 42 days | | | | | | | | |
| FBW (g) | 3342 a | 3184 b | 3325 a | 3321 a | 3194 b | 3247 b | 0.01 | 3.08 |
| BWG (g d ⁻¹) | 78.5 a | 74.7 b | 78.1 a | 77.9 a | 74.9 b | 76.2 b | 0.01 | 3.12 |
| FI (g) | 5266 | 5222 | 5283 | 5314 | 5241 | 5277 | 0.821 | 2.54 |
| FCR | 1.589 b | 1.661 a | 1.603 b | 1.603 b | 1.597 b | 1.650 a | 0.006 | 2.69 |
| Viab (%) | 97.4 | 97.4 | 98.3 | 98.3 | 97.4 | 95.8 | 0.858 | 4.29 |

Means followed by different letters in line differ from each other by the Scott-Knott test ($p < 0.05$); CV = coefficient of variation; FBW = final body weight; BWG = daily body weight gain; FI = feed intake; FCR = feed conversion ratio; Viab = viability. PC = positive control (basal diet without enzyme); NC = negative control (basal diet with a reduction of 80 kcal kg⁻¹ without enzyme); AlphaGal = alphagalactosydase; Blend = alphagalactosydase + xylanase + pectinase + amylase.

The CMDM, CMCP, CMCa and CMP were not affected by reducing energy and use of enzymes in the diet (Table 3).

Table 3. Coefficient of metabolizability of nutrients of broilers fed experimental diets.

| Parameters | PC | NC | NC + AlphaGal | NC + xylanase | NC + AlphaGal + xylanase | NC + blend | P value | CV (%) |
|------------|-------|-------|---------------|---------------|--------------------------|------------|---------|--------|
| CMMS (%) | 74.66 | 75.95 | 75.22 | 73.71 | 73.77 | 74.99 | 0.241 | 2.58 |
| CMCP (%) | 75.96 | 76.99 | 75.93 | 74.82 | 74.54 | 75.76 | 0.192 | 2.46 |
| CMCa (%) | 59.28 | 59.52 | 60.40 | 57.04 | 58.00 | 59.75 | 0.471 | 5.77 |
| CMP (%) | 58.27 | 60.10 | 59.37 | 55.61 | 56.60 | 58.52 | 0.122 | 5.61 |

Means followed by different letters in column differ from each other by the Scott-Knott test ($p < 0.05$); CV = coefficient of variation. Coefficient of metabolizability dry matter (CMDM), of crude protein (CMCP), of calcium (CMCa), of phosphorus (CMP). PC = positive control (basal diet without enzyme); NC = negative control (basal diet with a reduction of 80 kcal kg⁻¹ without enzyme); AlphaGal = alphagalactosydase; Blend = alphagalactosydase + xylanase + pectinase + amylase.

Discussion

The purpose of the experiment was to determine whether alphagalactosydase, xylanase, used individually and in combination, and an enzymatic blend (alphagalactosydase + xylanase + pectinase + amylase) could improve the growth performance and the digestibility of nutrients of broilers fed a reduced energy diet. The present trial showed an improvement in the growth performance of broilers, but not in the nutrient digestibility.

It was observed that at 7 days of age, the use of enzymes, individually and in combination, reduced the FI and improved the FCR compared with the PC diet. The explanation for the improved FCR of birds fed diets containing the enzymes would seem to be enhanced digestibility of nutrients; however, the improvement in digestibility did not occur as expected. According to Meng, Slominski, Nyachoti, Campbell, and Guenter (2005b), the enzyme addition should increase NSP digestibility from 11.1 to 30.1% and the addition of an appropriate combination of carbohydrase enzymes to target cell wall polysaccharide structures could further improve enzyme efficacy in practical broiler diets. The benefits of enzymes on broiler performance without effect on dry matter digestibility has also been demonstrated by Yu, Wu, Liu, Gauthier, and Chiou (2007). Carbohydrase supplementation causes a reduction in the feed intake of the birds under 14 days of age (Abellera & Tadeo Jr., 2019). According to Yu et al. (2007), less feed intake decreases the heat increment in the hot season and enhances the feed efficiency in an enzyme supplemented diet.

Both the NC and enzymatic blend resulted in a worse performance by the broilers at 21, 35 and 42 days old. Although some studies have reported a positive effect on growth performance in maize-based diets supplemented with enzymatic blends, others have shown no improvement.

The FI was affected by treatment until 21 days old but not in the older broilers. It is known that the benefits of endoxylanase supplementation of broiler feeds depend on the interaction of the intestinal microbiota and xylanase present in the gastrointestinal tract at specific broiler ages (Bautil et al., 2019).

At 35 and 42 days old, the BWG of broilers fed alphanagalactosydase and xylanase isolated were similar to the PC, indicating that the energy reduction was compensated by the use of both enzymes. The use of alphanagalactosydase and xylanase, isolated or in combination, were efficient in enhancing the FCR. The beneficial effects of such combinations observed in the broiler study may have resulted from eliminating the nutrient encapsulating effect of the cell wall polysaccharides and, to some extent, from the reduction of intestinal viscosity (Meng et al., 2005b). Reduced viscosity leads to improvements in protein digestibility, apparent metabolizable energy, feed consumption, body weight gain, and feed conversion (Raza, Bashir, & Tabassum, 2019).

Although the broiler performance increased with enzymes in the diets, the CMDM, CMCP, CMCa and CMP were not affected by the treatment. In the present trial, there was no relationship between nutrient digestibility and growth performance. Similar results had been reported in previous studies. Tahir, Saleh, Ohtsuka, and Hayashi (2006) confirmed that pectinase combined with cellulase and hemicellulose led to an improvement in digestibility of a corn-soybean meal diet without improvements in broiler performance. Measures based on alternative responses are useful in developing a wider understanding of the phenomenon but performance data is always the ultimate judge of the efficacy of a feed enzyme (Aftab & Bedford, 2018).

Conclusion

Alphanagalactosydase and xylanase, isolated or in combination, are effective in improving the growth performance of broilers fed reduced energy diets. The enzymes did not increase the digestibility of nutrients.

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