




Use of exogenous enzymes to improve nutrient digestibility and performance of broilers fed different protein sources

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ABSTRACT - Two experiments were conducted to assess the impact of an enzyme complex (EC) on the metabolizability coefficients of dry matter (MCDM), crude protein (MCCP), and ether extract (MCEE) and on the nitrogen-corrected apparent metabolizable energy (AMEn) of corn gluten meal, soybean protein concentrate, dried bovine plasma, and poultry offal meal. Additionally, the experiments aimed to evaluate how EC affected broiler performance. In experiment I, 720 day-old Cobb 500[®] broilers were assigned to a completely randomized design using a 4 × 2 factorial arrangement. The main factors considered were the feedstuff (corn gluten meal, soybean protein concentrate, dried bovine plasma, and poultry offal meal) and the presence or absence of EC. The broilers were housed in 45 pens, using five replicates, with ten birds in the pre-starter phase and six birds in the starter phase. This design resulted in eight treatment groups, including one reference diet group. In experiment II, 1140 day-old Cobb 500[®] chicks were allotted in a completely randomized design, employing a 4 × 2 factorial arrangement, with the same main factors as in experiment I. Birds were housed in 48 pens, using six replicates/30 birds, yielding eight treatments. The use of the EC did not lead to improvements in digestibility of the feedstuffs. However, the addition of EC resulted in enhanced body weight gain and improved feed conversion ratios across all phases of broiler growth. Enzyme complex inclusion did not affect feed intake. While the EC did not enhance the metabolizability of nutrients in feedstuffs individually, broilers fed diets containing these feedstuffs and supplemented with EC exhibited improved performance at 42 days of age. This suggests that the impact of the EC varies depending on whether it is observed at the feedstuff or diet level.

Keywords: amylase, digestibility, poultry, protease, xylanase

1. Introduction

In poultry production, the nutritional quality of the diet plays a major role in the efficient utilization of nutrients by animals, ultimately influencing broiler performance and economic returns for producers.

Various types of enzymes, including protease, amylase, lipases, xylanase, and phytase, whether used individually or in combination, have been employed in broiler diets. The use of these enzymes has been associated with improved nutrient digestibility and enhanced broiler performance (Aderibigbe et al., 2020; Rao et al., 2021; Attia et al., 2022). The inclusion of enzymes can be particularly advantageous in diets containing unconventional ingredients, so the impact of enzyme supplementation on nutrient metabolism warrants investigation.

The corn gluten meal (CGM) is a byproduct from the manufacturing of corn, with a protein content of around 60% (Longo et al., 2005). It could be used as a substitute of soybean meal, as a protein ingredient in broiler diets. The use of a protease in reduced protein diets for broilers could be an economically

viable dietary strategy to lower feeding cost, improve the environmental impact of farming, affect positively the intestinal microflora of broiler chickens, and retain gut integrity (Giannenas et al., 2017). Soybean protein concentrate (SPC) is a protein feedstuff that has been related to increases in the growth performance of broilers linked to enhanced gut health through reduction of enteric pathogens (Kiarie et al., 2021). However, some investigations have shown that feed efficiency of chicks fed the SPC was lower through three weeks than that of the chicks fed the other protein sources (Batal and Parsons, 2003), indicating that supplementing SPC diets with enzymes could be beneficial.

Poultry offal meal (POM) and dried bovine plasma (DBP) are animal byproducts commonly used in poultry industry. The bovine plasma is obtained from fresh blood treated with an anticoagulant, and centrifugated to the plasma and blood cell fractions separation (Beski et al., 2015). The POM is used to replace part of soybean meal of diets. However, birds fed diets with inclusion of POM are less efficient, even with supplementation of lysine in the diet (Silva et al., 2014). In this context, dietary supplementation of protease may be a potential strategy to improve the digestibility of aminoacids for broilers, a possibility of using animal-origin meals as a protein source of diets (Silva et al., 2021).

The use of nutritional matrices for enzyme products is recommended in feed formulation, enabling the reduction of aminoacid, energy, phosphorus, and protein levels (Campasino et al., 2015). This approach offers several benefits, including cost savings and reduced environmental nutrient excretion (Dersjant-li et al., 2015). Ahiwe et al. (2022) concluded that enzyme supplementation mitigated the negative effects of low-energy and low-protein diets on production parameters, intestinal development, and the digestibility of energy, fiber, and protein in broiler chickens.

The extent of the response in performance variables is dependent upon the enzyme dose (Rao et al., 2021). Furthermore, Aderibigbe et al. (2020) noted that the growth phase of birds could influence their response to exogenous amylase.

In this context, the present study aimed to evaluate the impact of an enzyme complex (EC) composed of xylanase, amylase, and protease on the nutrient metabolism of CGM 60%, SPC, DBP, and POM during the early rearing phases. Additionally, we assessed the performance of broilers up to 42 days of age when fed diets containing CGM, SPC, DBP, and POM, either with or without EC supplementation.

2. Material and Methods

The experimental protocols were granted approval by the institutional Ethics Committee on Animal Use (case no. 066/12). The study was conducted in Goiânia, Goiás, Brazil (16°35'48.3" S and 49°17'08.8" W).

2.1. Experiment I

A total of 720 male day-old Cobb 500[®] broiler chicks were randomly distributed into 45 pens in a 4 × 2 factorial arrangement [protein sources × presence or absence of the EC] with an additional group (reference diet). Each pen contained five replicates, with ten birds in the pre-starter phase and six birds in the starter phase, resulting in eight treatment groups. Treatments consisted of four protein sources, namely, CGM 60%, SPC, DBP, and POM, each of which was either supplemented with EC or left without supplementation, totaling eight treatments.

Control diet was formulated to meet the nutritional requirements of broilers during the early phases, following the guidelines of Rostagno et al. (2011). The experimental diets were created by substituting 40% of the control diet with the evaluated feedstuff (Table 1), with or without the inclusion of EC. The EC (Axtra) contained the following per kilogram: 2,000 U xylanase, 200 U amylase, and 4,000 U protease. The addition of enzymes followed the manufacturer's recommendations. All birds underwent a four-day period of adaptation to the experimental diets and cages. Throughout the experimental period, water and feed were available *ad libitum*, provided in two phases: pre-starter (1 to 7 d) and starter (8 to 21 d).

Table 1 - Composition of the reference diet – experiment I

Ingredient (%)	Pre-starter phase	Starter phase
Corn	53.40	55.90
Soybean meal, 44%	39.82	36.89
Soybean oil	2.42	3.31
Dicalcium phosphate	1.90	1.55
Limestone	0.79	0.84
Common salt	0.51	0.48
Vitamin-mineral supplement ¹	0.40	0.40
DL-Methionine (99%)	0.36	0.31
L-Lysine (78%)	0.28	0.23
L-Threonine (99%)	0.11	0.08
Inert marker ²	0.01	0.01
Total	100	100
Nutritional composition calculated		
Metabolizable energy (kcal/kg)	2,960	3,050
Crude protein (%)	22.40	21.20
Digestible lysine (%)	1.324	1.217
Digestible methionine + cystine (%)	0.953	0.876
Digestible threonine (%)	0.861	0.791
Calcium (%)	0.92	0.841
Available phosphorus (%)	0.47	0.401
Sodium (%)	0.22	0.21

¹ Levels per kg of product: folic acid (min), 200 mg; pantothenic acid (min), 3,120 mg; biotin (min), 10 mg; chloro-hydroxyquinoline (min), 7,500 mg; copper (min), 1,997 mg; choline (min), 78.10 g; iron (min), 11.25 g; iodine (min), 187.47 mg; manganese (min), 18.74 g; monensin (min), 25 g; niacin (min), 8,400 mg; selenium (min), 75 mg; vitamin A (min), 1,680,000 IU; vitamin B1 (min), 436.50 mg; vitamin B12 (min), 2,400 mcg; vitamin B2 (min), 1,200 mg; vitamin B6 (min), 624 mg; vitamin D3 (min), 400,000 IU; vitamin E (min), 3,500 IU; vitamin K3 (min), 360 mg; zinc (min), 17.50 g.

² Kaolin.

The chicks were housed in five broiler battery cages made of galvanized steel, equipped with trough-type feeders and drinkers. Each battery had five floors with divisions measuring 0.33 × 0.50 m. For heating, 40-W incandescent lamps were used on each floor of the battery until the birds reached 14 days of age. Broiler management adhered to standard guidelines for the strain. Daily records of ambient temperature and relative humidity were recorded, and appropriate curtain management was implemented.

To calculate the metabolizability coefficients of dry matter (MCDM), crude protein (MCCP), and ether extract (MCEE) and nitrogen-corrected apparent metabolizable energy (AMEn), total excreta were collected from days 4 to 7 (pre-starter phase) and from days 17 to 21 (starter phase). Excreta collection was conducted twice daily, and collected samples were placed in labeled plastic bags and frozen. At the conclusion of the experiment, the excreta were thawed and homogenized following the method proposed by Sakomura and Rostagno (2016). Feed intake and mortality rates were also determined.

The dry matter (DM), crude protein (CP), ether extract (EE), and gross energy (GE) content of both the diets and excreta were determined. The DM content was assessed through oven drying at 105 °C, CP using the micro-Kjeldahl method (nitrogen distiller Tecnal® TE-0364), EE via the Soxhlet method, and GE by bomb calorimeter. These analyses were performed according to procedures outlined by Silva and Queiroz (2002). The MCDM, MCCP, MCEE, and AMEn values were calculated using equations proposed by Sakomura and Rostagno (2016).

2.2. Experiment II

A total of 1,440 male day-old Cobb 500® broiler chicks were randomly assigned to a 4 × 2 factorial arrangement (protein sources × EC) and housed in 48 boxes with six replicates, each containing 30 birds, resulting in eight treatment groups. These treatments consisted of diets incorporating four protein sources, namely, CGM, SPC, DBP, and POM, with each source either supplemented with EC or

without supplementation. The chicks were acquired from a commercial hatchery, vaccinated against Marek's disease at one day of age and against Gumboro disease at 17 days of age.

The basal diets were iso-nitrogenous, formulated based on corn and soybean meal, and were developed in accordance with the recommendations of Rostagno et al. (2011) (Tables 2, 3, 4, and 5). The treatments were prepared by incorporating the respective feedstuff and EC as follows: basal diet + 6% CGM, basal diet + 6% CGM with EC, basal diet + 6% SPC, basal diet + 6% SPC with EC, basal diet + 6% POM, basal diet + 6% POM with EC, basal diet + 5% DBP, and basal diet + 5% DBP with EC.

Throughout the experiment, the birds had free access to feed and water, provided in four phases: pre-starter (1 to 7 days), starter (8 to 21 days), grower (22 to 35 days), and finisher (36 to 42 days). The EC used (Aextra) contained the following per kg: 2,000 U xylanase, 200 U amylase, and 4,000 U protease. Enzyme addition followed the manufacturer's recommendations.

Broilers were reared in a commercial broiler house equipped with 48 individual boxes, each measuring 1.80 × 1.60 m (2.88 m²). Each box was equipped with a nipple drinker and a tubular feeder during the first seven days, after which a tubular adult feeder was provided. The temperature ranged from a minimum of 25.6 °C to a maximum of 28.6 °C.

Broiler performance was assessed at 7, 21, 35, and 42 days of age by measuring body weight gain (BWG), feed conversion ratio (FCR), and feed intake (FI). Data were corrected for mortality, and the experimental period spanned 42 days.

Table 2 - Composition of the experimental diets in the pre-starter phase (1 to 7 days) – experiment II

Ingredient (%)	CGM		SPC		POM		DBP	
	Without EC	With EC	Without EC	With EC	Without EC	With EC	Without EC	With EC
Corn	59.39	59.37	58.28	58.26	57.13	57.08	59.60	58.78
Soybean meal, 45%	29.86	28.87	30.23	30.25	29.83	29.86	29.32	30.02
Gluten meal, 60%	6.00	6.00	-	-	-	-	-	-
Soybean protein concentrate, 60%	-	-	6.00	6.00	-	-	-	-
Poultry offal meal, 60%	-	-	-	-	6.00	6.00	-	-
Dried bovine plasma, 80%	-	-	-	-	-	-	5.00	5.00
Soybean oil	0.09	0.10	0.96	0.97	3.54	3.55	1.68	1.83
Dicalcium phosphate	1.96	1.96	1.90	1.90	1.14	1.14	1.92	1.91
Limestone	0.83	0.83	0.82	0.82	0.68	0.68	0.83	0.83
Common salt	0.51	0.51	0.51	0.51	0.43	0.43	0.46	0.46
Vitamin-mineral supplement ¹	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
DL-Methionine (99%)	0.32	0.32	0.38	0.38	0.36	0.36	0.40	0.40
L-Lysine (78%)	0.51	0.51	0.37	0.36	0.37	0.36	0.28	0.26
L-Threonine (99%)	0.13	0.13	0.14	0.14	0.13	0.12	0.11	0.10
Aextra®	-	0.01	-	0.01	-	0.01	-	0.01
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Nutritional composition calculated								
Metabolizable energy (kcal/kg)	2,960	2,960	2,960	2,960	2,960	2,960	2,960	2,960
Crude protein (%)	22.40	22.40	22.40	22.40	22.40	22.40	22.40	22.40
Digestible lysine (%)	1.324	1.324	1.324	1.324	1.324	1.324	1.324	1.324
Digestible methionine + cystine (%)	0.953	0.953	0.953	0.953	0.953	0.953	0.953	0.953
Digestible threonine (%)	0.861	0.861	0.861	0.861	0.861	0.861	0.861	0.861
Calcium (%)	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92
Available phosphorus (%)	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Sodium (%)	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22

CGM - corn gluten meal; SPC - soybean protein concentrate; POM - poultry offal meal; DBP - dried bovine plasma; EC - enzyme complex composed of xylanase, amylase, and protease.

¹ Guarantee levels per kg of product: folic acid (min), 200 mg; pantothenic acid (min), 3,120 mg; biotin (min), 10 mg; chloro-hydroxyquinoline (min), 7,500 mg; copper (min), 1,997 mg; choline (min), 78.10 g; iron (min), 11.25 g; iodine (min), 187.47 mg; manganese (min), 18.74 g; monensin (min), 25 g; niacin (min), 8,400 mg; selenium (min), 75 mg; vitamin A (min), 1,680,000 IU; vitamin B1 (min), 436.50 mg; vitamin B12 (min), 2,400 mcg; vitamin B2 (min), 1,200 mg; vitamin B6 (min), 624 mg; vitamin D3 (min), 400,000 IU; vitamin E (min), 3,500 IU; vitamin K3 (min), 360 mg; zinc (min), 17.50 g.

Table 3 - Composition of the experimental diets in the starter phase (8 to 21 days) – experiment II

Ingredient (%)	CGM		SPC		POM		DBP	
	Without EC	With EC	Without EC	With EC	Without EC	With EC	Without EC	With EC
Corn	60.91	60.89	59.98	59.96	59.02	58.99	60.25	59.68
Soybean meal, 45%	28.59	28.60	28.91	28.92	28.57	28.58	29.55	30.03
Gluten meal, 60%	5.00	5.00	-	-	-	-	-	-
Soybean protein concentrate, 60%	-	-	5.00	5.00	-	-	-	-
Poultry offal meal, 60%	-	-	-	-	5.00	5.00	-	-
Dried bovine plasma, 80%	-	-	-	-	-	-	3.50	3.50
Soybean oil	1.36	1.37	2.09	2.09	4.23	4.24	2.79	2.89
Dicalcium phosphate	1.59	1.59	1.55	1.55	0.92	0.92	1.56	1.56
Limestone	0.87	0.87	0.86	0.86	0.74	0.74	0.86	0.86
Common salt	0.48	0.48	0.48	0.48	0.42	0.42	0.45	0.45
Vitamin-mineral supplement ¹	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
DL-Methionine (99%)	0.27	0.27	0.32	0.32	0.31	0.31	0.34	0.33
L-Lysine (78%)	0.43	0.43	0.30	0.30	0.30	0.30	0.23	0.22
L-Threonine (99%)	0.09	0.09	0.10	0.10	0.09	0.09	0.07	0.07
Axtra®	-	0.01	-	0.01	-	0.01	-	0.01
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated nutritional composition								
Metabolizable energy (kcal/kg)	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050
Crude protein (%)	21.20	21.20	21.20	21.20	21.20	21.20	21.20	21.20
Digestible lysine (%)	1.217	1.217	1.217	1.217	1.217	1.217	1.217	1.217
Digestible methionine + cystine (%)	0.876	0.876	0.876	0.876	0.876	0.876	0.876	0.876
Digestible threonine (%)	0.791	0.791	0.791	0.791	0.791	0.791	0.791	0.791
Calcium (%)	0.841	0.841	0.841	0.841	0.841	0.841	0.841	0.841
Available phosphorus (%)	0.401	0.401	0.401	0.401	0.401	0.401	0.401	0.401
Sodium (%)	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210

CGM - corn gluten meal; SPC - soybean protein concentrate; POM - poultry offal meal; DBP - dried bovine plasma; EC - enzyme complex composed of xylanase, amylase, and protease.

¹ Guarantee levels per kg of product: folic acid (min), 200 mg; pantothenic acid (min), 3,120 mg; biotin (min), 10 mg; chloro-hydroxyquinoline (min), 7,500 mg; copper (min), 1,997 mg; choline (min), 78.10 g; iron (min), 11.25 g; iodine (min), 187.47 mg; manganese (min), 18.74 g; monensin (min), 25 g; niacin (min), 8,400 mg; selenium (min), 75 mg; vitamin A (min), 1,680,000 IU; vitamin B1 (min), 436.50 mg; vitamin B12 (min), 2,400 mcg; vitamin B2 (min), 1,200 mg; vitamin B6 (min), 624 mg; vitamin D3 (min), 400,000 IU; vitamin E (min), 3,500 IU; vitamin K3 (min), 360 mg; zinc (min), 17.50 g.

2.3. Statistical analysis

The data underwent analysis of variance, and mean comparisons were conducted using the Scott Knott test at a significance level of 5%. The statistical model encompassed the fixed effects of feedstuffs and EC supplementation, along with their interaction effect, while accounting for the random effects of the experimental units. To explore the interrelationships between variables and treatments, a principal-component multivariate analysis was carried out.

Statistical analyses were conducted using R software, version 3.6.0, with the assistance of the RStudio® platform (version 1.2.1335, 2009-2019, Inc.).

The proposed mathematical model was as follows:

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + \epsilon_{ijk}, \quad (1)$$

in which Y_{ijk} = value observed in feedstuff factor i ($i = 1, 2, 3, 4$), enzyme complex factor j ($j = 1, 2$), and replicate k ($k = 1, 2, 3, \dots, 8$); μ = overall mean of the experiment; a_i = fixed effect of factor i ($i = 1, 2, 3, 4$); b_j = fixed effect of factor j ($j = 1, 2$); $(ab)_{ij}$ = fixed effect of the interaction between factor i ($i = 1, 2, 3, 4$) and factor j ($j = 1, 2$); and ϵ_{ijk} = random error in factor i ($i = 1, 2, 3, 4$), level j ($j = 1, 2$), and replicate k ($k = 1, 2, 3, \dots, 8$).

Table 4 - Composition of the experimental diets in the grower phase (22 to 33 days) – experiment II

Ingredient (%)	CGM		SPC		POM		DBP	
	Without EC	With EC	Without EC	With EC	Without EC	With EC	Without EC	With EC
Corn	63.98	63.96	63.05	63.03	62.09	62.06	63.32	62.75
Soybean meal, 45%	24.91	24.92	25.24	25.24	24.90	24.91	25.88	26.36
Gluten meal, 60%	5.00	5.00	-	-	-	-	-	-
Soybean protein concentrate, 60%	-	-	5.00	5.00	-	-	-	-
Poultry offal meal, 60%	-	-	-	-	5.00	5.00	-	-
Dried bovine plasma, 80%	-	-	-	-	-	-	3.50	3.50
Soybean oil	2.28	2.29	3.01	3.02	5.16	5.17	3.71	3.82
Dicalcium phosphate	1.36	1.36	1.32	1.32	0.69	0.69	1.33	1.33
Limestone	0.83	0.83	0.82	0.82	0.70	0.70	0.82	0.82
Common salt	0.46	0.46	0.46	0.46	0.40	0.40	0.42	0.42
Vitamin-mineral supplement ¹	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
DL-Methionine (99%)	0.25	0.25	0.30	0.30	0.29	0.29	0.32	0.32
L-Lysine (78%)	0.43	0.43	0.30	0.30	0.31	0.30	0.23	0.22
L-Threonine (99%)	0.09	0.09	0.10	0.10	0.08	0.08	0.07	0.06
Axtra®	-	0.01	-	0.01	-	0.01	-	0.01
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated nutritional composition								
Metabolizable energy (kcal/kg)	3,150	3,150	3,150	3,150	3,150	3,150	3,150	3,150
Crude protein (%)	19.80	19.80	19.80	19.80	19.80	19.80	19.80	19.80
Digestible lysine (%)	1.131	1.131	1.131	1.131	1.131	1.131	1.131	1.131
Digestible methionine + cystine (%)	0.826	0.826	0.826	0.826	0.826	0.826	0.826	0.826
Digestible threonine (%)	0.735	0.735	0.735	0.735	0.735	0.735	0.735	0.735
Calcium (%)	0.758	0.758	0.758	0.758	0.758	0.758	0.758	0.758
Available phosphorus (%)	0.354	0.354	0.354	0.354	0.354	0.354	0.354	0.354
Sodium (%)	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200

CGM - corn gluten meal; SPC - soybean protein concentrate; POM - poultry offal meal; DBP - dried bovine plasma; EC - enzyme complex composed of xylanase, amylase, and protease.

¹ Guarantee levels per kg of product: folic acid (min), 162.50 mg; pantothenic acid (min), 2,600.07 mg; chloro-hydroxyquinoline (min), 7,500 mg; copper (min), 1,996.38 mg; choline (min), 71.59 g; ethoxyquin (min), 750 ppm; iron (min), 11.25 g; butylated hydroxyanisole (min), 250 mg; butylated hydroxytoluene (min), 756 mg; iodine (min), 187.47 mg; manganese (min), 18.74 g; niacin (min), 7,000.12 mg; salinomycin, 16.50 g; selenium (min), 75 mg; vitamin A (min), 1,400,062 IU; vitamin B1 (min), 388 mg; vitamin B12 (min), 2,000.05 mcg; vitamin B2 (min), 1,000.02 mg; vitamin B6 (min), 520 mg; vitamin D3 (min), 300,006.87 IU; vitamin E (min), 2,500 IU; vitamin K3 (min), 300 mg; zinc (min), 17.50 g.

3. Results

3.1. Experiment I

There was no interaction effect between feedstuffs and EC on the apparent metabolizability coefficients of nutrients and AMEn ($P > 0.05$; Table 6). The inclusion of EC did not improve the apparent metabolizability coefficients of nutrients or AMEn ($P > 0.05$; Table 6). However, notable differences in apparent metabolizability coefficients of nutrients and AMEn were observed depending on the feedstuff used.

During the pre-starter phase, the highest MCDM ($P = 0.0032$), MCCP ($P < 0.001$), and MCEE ($P < 0.001$) values were obtained with POM, although MCDM was similar to that of CGM. Soybean protein concentrate yielded an AMEn value similar to that obtained with POM ($P < 0.001$). In the starter phase, POM continued to exhibit the highest AMEn value ($P < 0.001$).

According to multivariate analysis of the nutrient metabolizability coefficients in the pre-starter and starter phases (Figures 1 and 2, respectively), EC had a limited influence. There was a positive correlation between MCDM, MCCP, MCEE, and AMEn with both POM and CGM, regardless of the presence of the enzyme complex (Figure 1). In the starter phase, MCDM, MCCP, MCEE, and AMEn displayed a positive correlation with both POM and CGM, whether the enzyme complex was present or not (Figure 2).

Table 5 - Composition of the experimental diets in the finisher phase (34 to 42 days) – experiment II

Ingredient (%)	CGM		SPC		POM		DBP	
	Without EC	With EC	Without EC	With EC	Without EC	With EC	Without EC	With EC
Corn	68.32	68.30	67.39	67.37	66.43	66.40	67.66	67.09
Soybean meal, 45%	20.95	20.95	21.26	21.27	20.92	20.94	21.90	22.39
Gluten meal, 60%	5.00	5.00	-	-	-	-	-	-
Soybean protein concentrate, 60%	-	-	5.00	5.00	-	-	-	-
Poultry offal meal, 60%	-	-	-	-	5.00	5.00	-	-
Dried bovine plasma, 80%	-	-	-	-	-	-	3.50	3.50
Soybean oil	2.21	2.22	2.94	2.95	5.09	5.10	3.64	3.74
Dicalcium phosphate	1.14	1.14	1.10	1.10	0.47	0.47	1.11	1.10
Limestone	0.75	0.75	0.75	0.75	0.63	0.63	0.75	0.75
Common salt	0.44	0.44	0.45	0.45	0.38	0.38	0.41	0.41
Vitamin-mineral supplement ¹	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
DL-Methionine (99%)	0.23	0.23	0.28	0.28	0.27	0.27	0.30	0.29
L-Lysine (78%)	0.46	0.46	0.33	0.33	0.33	0.33	0.26	0.25
L-Threonine (99%)	0.09	0.09	0.10	0.10	0.08	0.08	0.07	0.06
Axtra®	-	0.01	-	0.01	-	0.01	-	0.01
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated nutritional composition								
Metabolizable energy (kcal/kg)	3,200	3,200	3,200	3,200	3,200	3,200	3,200	3,200
Crude protein (%)	18.40	18.40	18.40	18.40	18.40	18.40	18.40	18.40
Digestible lysine (%)	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06
Digestible methionine + cystine (%)	0.774	0.774	0.774	0.774	0.774	0.774	0.774	0.774
Digestible threonine (%)	0.689	0.689	0.689	0.689	0.689	0.689	0.689	0.689
Calcium (%)	0.663	0.663	0.663	0.663	0.663	0.663	0.663	0.663
Available phosphorus (%)	0.309	0.309	0.309	0.309	0.309	0.309	0.309	0.309
Sodium (%)	0.195	0.195	0.195	0.195	0.195	0.195	0.195	0.195

CGM - corn gluten meal; SPC - soybean protein concentrate; POM - poultry offal meal; DBP - dried bovine plasma; EC - enzyme complex composed of xylanase, amylase, and protease.

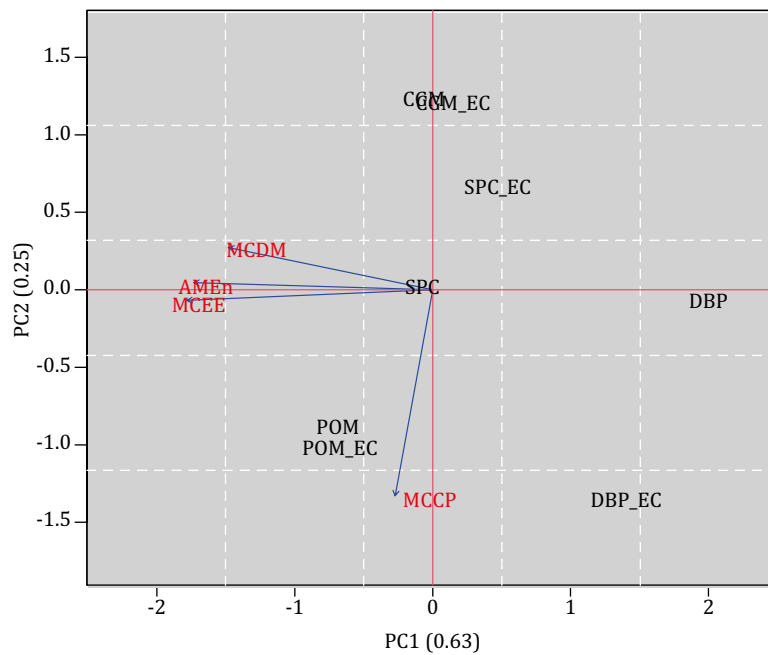
¹ Guarantee levels per kg of product: folic acid (min), 162.50 mg; pantothenic acid (min), 2,600.07 mg; chloro-hydroxyquinoline (min), 7,500 mg; copper (min), 1,996.38 mg; choline (min), 71.59 g; ethoxyquin (min), 750 ppm; iron (min), 11.25 g; butylated hydroxyanisole (min), 250 mg; butylated hydroxytoluene (min), 756 mg; iodine (min), 187.47 mg; manganese (min), 18.74 g; niacin (min), 7,000.12 mg; salinomycin, 16.50 g; selenium (min), 75 mg; vitamin A (min), 1,400,062 IU; vitamin B1 (min), 388 mg; vitamin B12 (min), 2,000.05 mcg; vitamin B2 (min), 1,000.02 mg; vitamin B6 (min), 520 mg; vitamin D3 (min), 300,006.87 IU; vitamin E (min), 2,500 IU; vitamin K3 (min), 300 mg; zinc (min), 17.50 g.

Table 6 - Apparent metabolizability coefficients of dry matter (MCDM), crude protein (MCCP), ether extract (MCEE), and the nitrogen-corrected apparent metabolizable energy (AMEn, in dry matter) of feedstuffs with or without enzyme complex in 7- and 21-day-old broilers

Item	7 days				21 days			
	MCDM	MCCP	MCEE	AMEn	MCDM	MCCP	MCEE	AMEn
Feedstuff (F)								
CGM (60%)	69.03a	38.99b	83.89b	3735.8b	66.98a	66.36a	81.94b	3711.9b
SPC	64.45b	42.84b	85.83b	3965.6a	60.22c	60.04c	81.80b	3381.2d
POM	68.62a	53.15a	90.80a	4068.7a	64.92b	64.45b	85.52a	3908.3a
DBP	63.04b	49.69a	76.32c	3179.8c	64.74b	63.97b	79.50b	3528.4c
SEM	1.2587	2.1205	1.2967	74.3204	0.636	0.6934	1.3276	46.7629
Enzyme complex								
Without EC	66.38	45.57	83.69	3684.3	64.07	63.39	81.43	3609.95
With EC	66.19	46.77	84.73	3790.6	64.36	64.01	82.95	3654.95
SEM	0.89	1.4994	0.9169	52.5525	0.4497	0.4903	0.9388	33.0663
P-value								
F	0.0032	<0.001	<0.001	<0.001	<0.001	<0.001	0.0264	<0.001
EC	0.8806	0.5767	0.4249	0.1625	0.6485	0.3795	0.2598	0.3431
F × EC	0.6772	0.1025	0.8509	0.1431	0.4081	0.6759	0.589	0.2082

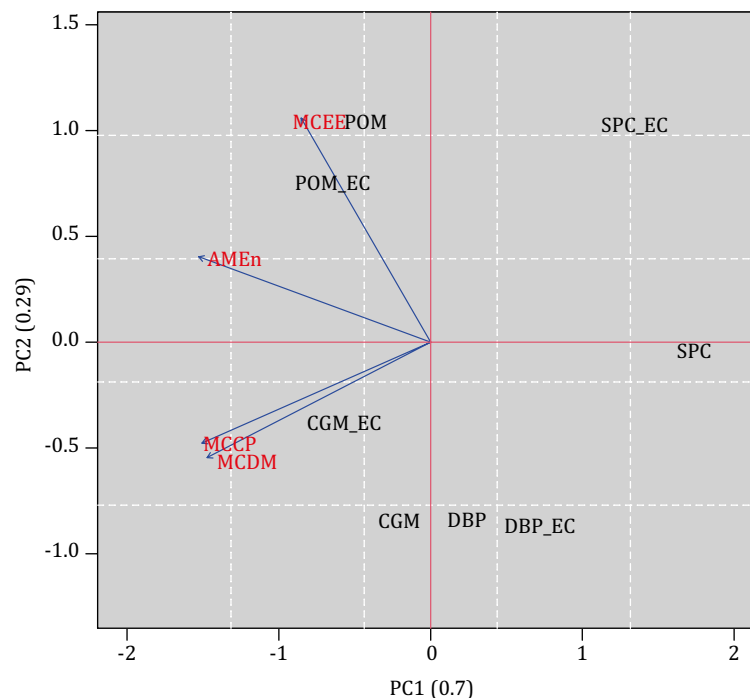
CGM - corn gluten meal; SPC - soybean protein concentrate; POM - poultry offal meal; DBP - dried bovine plasma; EC - enzyme complex composed of xylanase, amylase, and protease; SEM - standard error of the mean.

a-d - Means within a column-subgroup with different letters are significantly different at P<0.05 by Scott Knott test.



CGM - corn gluten meal; CGM_EC - corn gluten meal with enzyme complex; SPC - soybean protein concentrate; SPC_EC - soybean protein concentrate with enzyme complex; POM - poultry offal meal; POM_EC - poultry offal meal with enzyme complex; DBP - dried bovine plasma; DBP_EC - dried bovine plasma with enzyme complex; EC - enzyme complex composed of xylanase, amylase, and protease. The graph shows a separation between the feedstuffs with or without the enzyme complex and their relationship to the metabolizability coefficients of dry matter (MCDM), crude protein (MCCP), and ether extract (MCEE); and to the nitrogen-corrected apparent metabolizable energy (AMEn), based on multivariate analysis in pre-starter phase.

Figure 1 - 2D plot analysis of variables (feedstuffs with or without the enzyme complex and metabolizability coefficients of nutrients) at pre-starter phase.



CGM - corn gluten meal; CGM_EC - corn gluten meal with enzyme complex; SPC - soybean protein concentrate; SPC_EC - soybean protein concentrate with enzyme complex; POM - poultry offal meal; POM_EC - poultry offal meal with enzyme complex; DBP - dried bovine plasma; DBP_EC - dried bovine plasma with enzyme complex; EC - enzyme complex composed of xylanase, amylase, and protease. The graph shows a separation between the feedstuffs with or without the enzyme complex and their relationship to the metabolizability coefficients of dry matter (MCDM), crude protein (MCCP), and ether extract (MCEE); and to the nitrogen-corrected apparent metabolizable energy (AMEn), based on multivariate analysis in starter phase.

Figure 2 - 2D plot analysis of variables (feedstuffs with or without the enzyme complex and metabolizability coefficients of nutrients) at starter phase.

3.2. Experiment II

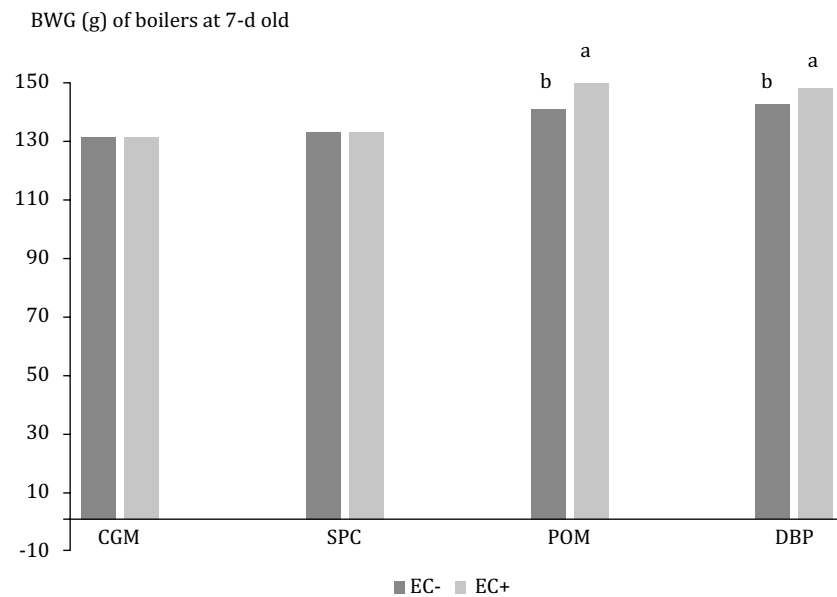
There was an interaction effect between feedstuffs and EC solely on the BWG of broilers during the pre-starter phase ($P = 0.0341$; Table 7; Figure 3). Specifically, the inclusion of EC in diets containing DBP and POM resulted in improved BWG in broilers at seven days of age ($P = 0.006$). No such interaction between feedstuffs and EC was observed for the other variables studied ($P > 0.05$; Tables 7 and 8).

Table 7 - Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) of broilers at pre-starter and starter phases fed different feedstuffs with or without enzyme complex

Item	Pre-starter (7 days)			Starter (21 days)		
	FI (g)	BWG (g)	FCR	FI (kg)	BWG (kg)	FCR
Feedstuff (F)						
CGM (60%)	148.5	130.4b	1.139	1.2862b	0.8536c	1.508a
SPC	155.9	132.1b	1.182	1.3129a	0.8618c	1.526a
POM	155.2	144.5a	1.076	1.2977b	0.9783a	1.327c
DBP	157.8	143.7a	1.100	1.3193a	0.9358b	1.412b
SEM	0.0037	0.0012	0.0287	0.0084	0.007	0.0144
Enzyme complex (EC)						
Without EC	156.3	135.9b	1.152	1.3115	0.8883b	1.483a
With EC	152.3	139.5a	1.097	1.2965	0.9263a	1.404b
SEM	0.0026	0.0094	0.0203	0.006	0.0049	0.0102
P-value						
F	0.3241	<0.001	0.0649	0.035	<0.001	<0.001
EC	0.2854	0.006	0.0633	0.0818	<0.001	<0.001
F × EC	0.9447	0.0341	0.6006	0.9964	0.4853	0.5399

CGM - corn gluten meal; SPC - soybean protein concentrate; POM - poultry offal meal; DBP - dried bovine plasma; EC - enzyme complex composed of xylanase, amylase, and protease; SEM - standard error of the mean.

a-c - Means within a column-subgroup with different letters are significantly different at $P < 0.05$ by Scott Knott test.



CGM - corn gluten meal; SPC - soybean protein concentrate; POM - poultry offal meal; DBP - dried bovine plasma; EC - enzyme complex composed of xylanase, amylase, and protease; EC- without EC; EC+ with EC.

Means within a feedstuff group with different letters are significantly different at $P < 0.05$ by Tukey's test.

Figure 3 - Deployment of interaction effect of feedstuffs with or without enzyme complex in body weight gain (BWG) of seven-day-old broilers.

Table 8 - Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) of broilers at the grower and finisher phases fed different feedstuffs with or without enzyme complex

Item	Grower (35 days)			Finisher (42 days)		
	FI (kg)	BWG (kg)	FCR	FI (kg)	BWG (kg)	FCR
Feedstuff (F)						
CGM (60%)	3.6864	2.1911d	1.683a	5.1749	2.9806c	1.7387a
SPC	3.8003	2.3164c	1.643a	5.1994	3.0907b	1.6852a
POM	3.7368	2.4710a	1.514c	5.1899	3.2676a	1.5930b
DBP	3.7897	2.3896b	1.587b	5.2793	3.1375b	1.6847a
SEM	0.0354	0.023	0.0212	0.0359	0.0297	0.0199
Enzyme complex (EC)						
Without EC	3.7679	2.2915b	1.648a	5.2446	3.0362b	1.7315a
With EC	3.7387	2.3925a	1.566b	5.1772	3.2019a	1.6193b
SEM	0.025	0.0163	0.015	0.0254	0.021	0.0141
P-value						
F	0.1024	<0.001	<0.001	0.1833	<0.001	<0.001
EC	0.4139	<0.001	<0.001	0.0674	<0.001	<0.001
F × EC	0.8612	0.9799	0.9375	0.8939	0.7996	0.9918

CGM - corn gluten meal; SPC - soybean protein concentrate; POM - poultry offal meal; DBP - dried bovine plasma; EC - enzyme complex composed of xylanase, amylase, and protease; SEM - standard error of the mean.

a-b - Means within a column-subgroup with different letters are significantly different at $P < 0.05$ by Scott Knott test.

Both BWG and FCR improved with the addition of EC across all rearing phases ($P < 0.001$; Tables 7 and 8). The inclusion of EC did not have a significant impact on FI for the birds ($P > 0.05$; Tables 7 and 8). At seven days of age, diets containing DBP and POM (animal byproducts) yielded the highest BWG ($P < 0.001$; Table 7). At 21, 35, and 42 days of age, POM consistently led to superior BWG and FCR in the birds ($P < 0.001$; Tables 7 and 8).

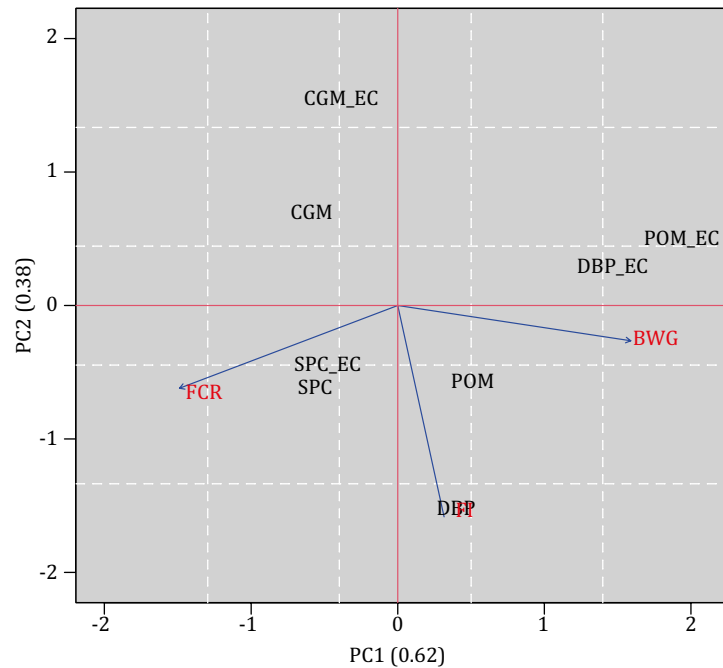
By 42 days of age, the broilers fed diets incorporating animal protein sources exhibited the highest overall performance ($P < 0.001$; Table 8).

Multivariate analysis for broiler performance was conducted across the pre-starter, starter, grower, and finisher phases (Figures 4, 5, 6, and 7, respectively).

4. Discussion

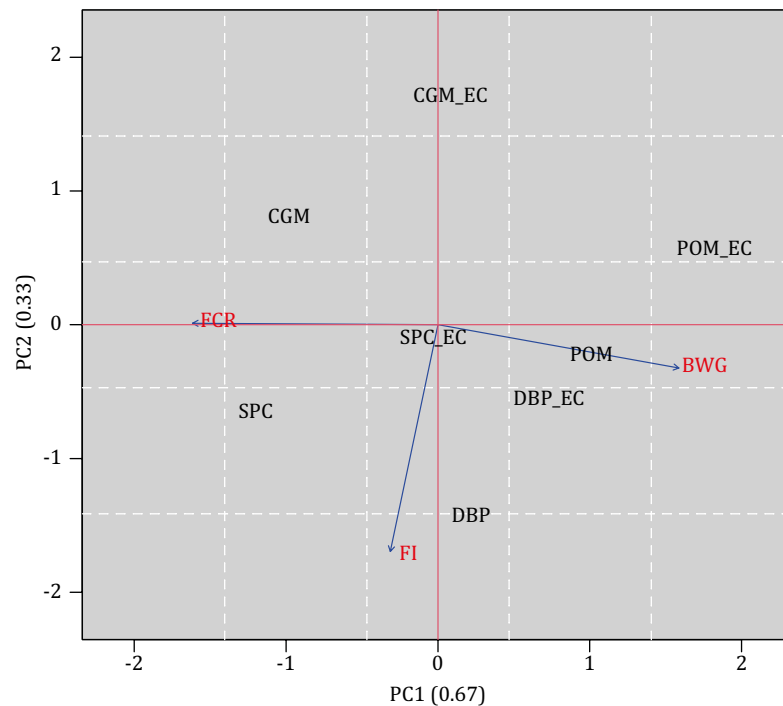
Understanding ingredient digestibility is important when utilizing diverse protein sources in broiler diets. In this study, EC did not enhance the MCDM, MCCP, MCEE, or AMEn of the tested feedstuffs. In contrast, diets supplemented with EC consistently led to higher BWG and improved FCR for broilers across all rearing phases. This improvement in performance was independent of the specific feedstuff used.

The EC under investigation consists of amylase, xylanase, and protease. It was anticipated that the inclusion of these enzymes would lead to an increase in the AMEn of the feedstuffs by increasing the availability of starch and cell wall components for gut and pancreatic enzymes. Xylanase and phytase are efficient to lower the digesta viscosity (Anwar et al., 2023). It is known that the viscous properties of soluble non-starch polysaccharides (NSP) may interfere with the digestion process and thereby reduce the digestibility of other nutrients (Steenfeldt, 2001). Therefore, it was expected that the digestibility and performance of broilers fed with EC—mainly the vegetable byproducts—increased because of the degradation of xylan of wall cell and consequently liberation of starch and protein of cell content. In addition, the EC improved the performance of broilers fed the animal byproducts (POM and DBP). For protein-rich feedstuffs, the greatest effects of exogenous enzymes are to be expected on the NSP present in the cotyledon and germ, whereas the NSP present in the hull layer represent rigid structures that are difficult to degrade (Knudsen, 2014).



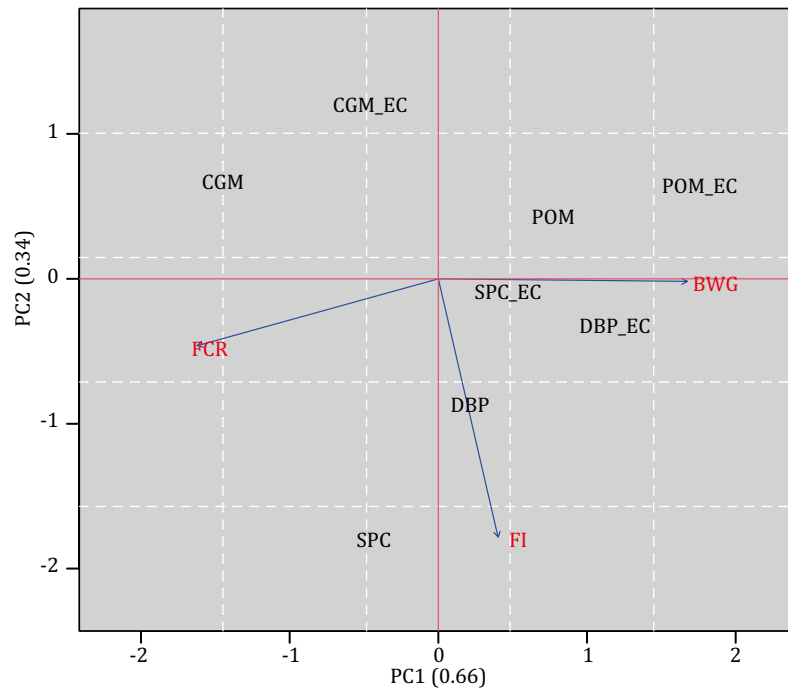
CGM - corn gluten meal; CGM_EC - corn gluten meal with enzyme complex; SPC - soybean protein concentrate; SPC_EC - soybean protein concentrate with enzyme complex; POM - poultry offal meal; POM_EC - poultry offal meal with enzyme complex; DBP - dried bovine plasma; DBP_EC - dried bovine plasma with enzyme complex; EC - enzyme complex composed of xylanase, amylase, and protease. The graph shows a separation between the feedstuffs with or without the enzyme complex and their relationship to the broiler performance, based on multivariate analysis in pre-starter phase.

Figure 4 - 2D plot analysis of variables (feedstuffs with or without the enzyme complex and broiler performance) at pre-starter phase.



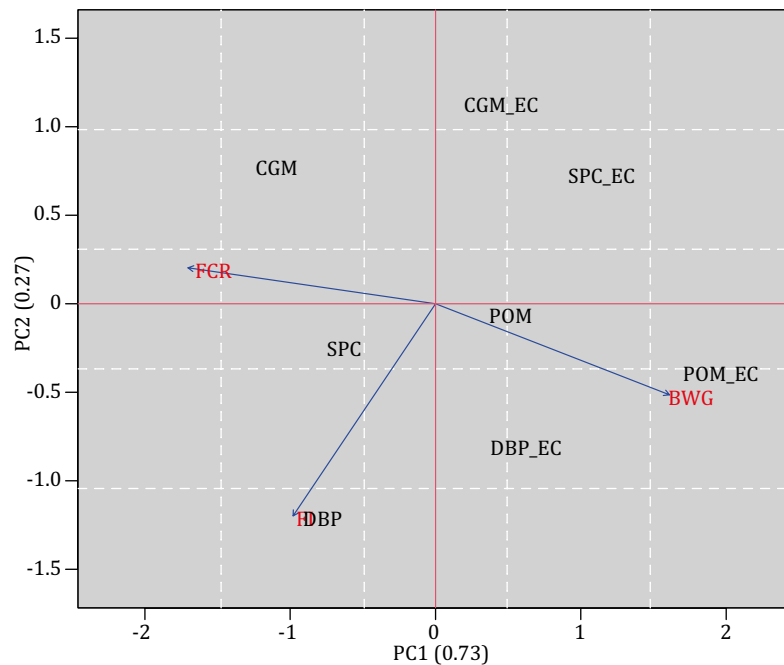
CGM - corn gluten meal; CGM_EC - corn gluten meal with enzyme complex; SPC - soybean protein concentrate; SPC_EC - soybean protein concentrate with enzyme complex; POM - poultry offal meal; POM_EC - poultry offal meal with enzyme complex; DBP - dried bovine plasma; DBP_EC - dried bovine plasma with enzyme complex; EC - enzyme complex composed of xylanase, amylase, and protease. The graph shows a separation between the feedstuffs with or without the EC and their relationship to the broiler performance, based on multivariate analysis in starter phase.

Figure 5 - 2D plot analysis of variables (feedstuffs with or without the enzyme complex and broiler performance) at starter phase.



CGM - corn gluten meal; CGM_EC - corn gluten meal with enzyme complex; SPC - soybean protein concentrate; SPC_EC - soybean protein concentrate with enzyme complex; POM - poultry offal meal; POM_EC - poultry offal meal with enzyme complex; DBP - dried bovine plasma; DBP_EC - dried bovine plasma with enzyme complex; EC - enzyme complex composed of xylanase, amylase, and protease. The graph shows a separation between the feedstuffs with or without the enzyme complex and their relationship to the broiler performance, based on multivariate analysis in growth phase.

Figure 6 - 2D plot analysis of variables (feedstuffs with or without the enzyme complex and broiler performance) at growth phase.



CGM - corn gluten meal; CGM_EC - corn gluten meal with enzyme complex; SPC - soybean protein concentrate; SPC_EC - soybean protein concentrate with enzyme complex; POM - poultry offal meal; POM_EC - poultry offal meal with enzyme complex; DBP_EC - dried bovine plasma with enzyme complex; EC - enzyme complex composed of xylanase, amylase, and protease. The graph shows a separation between the feedstuffs with or without the enzyme complex and their relationship to the broiler performance, based on multivariate analysis in final phase.

Figure 7 - 2D plot analysis of variables (feedstuffs with or without the enzyme complex and broiler performance) at final phase.

Some factors have been identified as influencing the efficacy of exogenous enzymes on nutrient digestibility and broiler performance, including the enzyme dosage (Rao et al., 2021) and growth phase of the birds (Aderibigbe et al., 2020). Moreover, Jabbar et al. (2021) demonstrated an interaction effect between exogenous protease enzyme and dietary crude protein levels on growth and digestibility indices in broiler chickens during the starter phase.

As highlighted by Amerah et al. (2017), broilers possess an immature digestive tract in the initial days of life, characterized by lower production of endogenous enzymes and reduced nutrient digestibility. These authors reported improvements in performance and nutrient digestibility when combining phytase with an enzyme complex comprising amylase, xylanase, and protease in the diets of 21-day-old broilers. The incorporation of xylanase into broiler feed can yield beneficial effects, as it facilitates alterations in the composition of cell walls by hydrolyzing structural arabinoxylans that encapsulate nutrients, thereby increasing nutrient utilization by animals (Kiarie et al., 2014; Munyaka et al., 2016; Amerah et al., 2017). Cowieson et al. (2010) elucidated that the impact of xylanase becomes more apparent when used in conjunction with other exogenous enzymes such as protease, amylase, and phytase.

Kiarie et al. (2014) reported that xylanase supplementation improved growth performance and AMEn in both wheat and corn-based diets, suggesting hydrolysis of both soluble and insoluble NSP. On the other hand, the advantage of enhanced protein digestibility due to enzyme supplementation primarily arises from a reduction in the production of endogenous aminoacids rather than improved digestion of aminoacids in the diet (Bedford, 2000). This benefit, however, reduces the energy expenditure as birds expend less energy on the digestive process, leaving more energy available for productive processes.

Numerous researchers have explored the digestive capacity of birds during the first week of their lives. This phase is of particular interest because birds undergo rapid development during this period, and their nutritional requirements are closely linked to their digestive capabilities (Willemsen et al., 2008; Zavarize et al., 2012; Svihus, 2014; Lilburn and Loeffler, 2015). Furthermore, the limited enzyme activity in young birds contributes to a reduction in digestive processes. Therefore, enhancing nutrient digestibility during this phase is crucial for proper animal development.

Despite these considerations, the addition of EC did not result in increased nutrient metabolizability coefficients in the assessed feedstuffs at two different age periods of birds (4 to 7 and 17 to 21 days old). The average apparent digestibility of protein during the pre-starter phase remained low, at approximately 48.25%. This can be attributed to the nutritional imbalance of the diets, as they consisted of 60% of a control diet and 40% of experimental feedstuff. The intake of diets with aminoacid content disproportional to the actual metabolic requirements of non-ruminant animals leads to physiological changes with metabolic consequences that impede nutrient digestibility in the diet.

The AMEn values directly influence the formulation of broiler diets. High levels of animal meals in broiler feed can potentially lead to adverse effects on the estimation of metabolizable energy values, possibly due to interactions between calcium, fatty acids, and protein. A low protein digestibility can result from the presence of high mineral content, aminoacid imbalances, and reduced intake due to low palatability. Elevated calcium levels (at higher inclusion rates) can interfere with fat absorption, particularly unsaturated fatty acids (Nascimento et al., 2005).

The presence of the EC resulted in improved weight gain and enhanced FCR, indicating its effectiveness. Consistent with these findings, Mohammadigheisar et al. (2018) observed improved broiler chicken performance when fed a multi-enzyme, with no negative effects on health status or meat quality.

Several studies have demonstrated a significant increase in broiler weight gain during the starter phase with the addition of protease to the feed (Wang et al., 2006; Angel et al., 2011). One plausible explanation for the beneficial effects of the enzyme complex, composed of xylanase, amylase, and protease, on weight gain could be the improved utilization of nutrients, as xylanases promote the depolymerization of arabinoxylans into low-molecular-weight components (Ravindran et al., 1999; Olukosi et al., 2007).

Saleh et al. (2018) found that an enzyme complex containing xylanase and protease led to higher expression of the intestinal carnitine acyltransferase I gene (*CPT1*), intestinal peptide transporter 1 gene (*PEPT1*), and intestinal glucose transporter 2 gene (*GLUT2*). The *CPT1* gene is responsible for transporting long-chain fatty acids into the mitochondria, thereby enhancing beta-oxidation, whereas *PEPT1* and *GLUT2* play vital roles in the absorption of peptides and glucose in the intestinal mucosa.

This increased absorption and beta-oxidation of nutrients ultimately result in greater availability of energy and aminoacids for protein synthesis, leading to improved BWG in broilers. Building on this context, Head et al. (2019) reached a similar conclusion in their study, in which broilers fed an enzyme mixture containing carbohydrase and protease exhibited increased expression of genes associated with fatty acid catabolism (*ACOX1* and *CPT1*) and inhibited expression of genes related to *de novo* fatty acid synthesis.

5. Conclusions

The enzyme complex does not enhance the metabolizability of nutrients in corn gluten meal, soybean protein concentrate, dried bovine plasma, or poultry offal meal. However, broilers fed diets comprising these ingredients as well as enzyme complex display improved performance by 42 days of age. This suggests that the impact of the enzyme complex varies depending on whether its effects are observed at the feedstuff or diet level.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Stringhini, J. H. and Café, M. B. **Data curation:** Arnhold, E. **Formal analysis:** Arnhold, E. **Funding acquisition:** Café, M. B. **Investigation:** Fortes, B. D. A. **Methodology:** Fortes, B. D. A.; Stringhini, J. H.; Café, M. B. and Mello, H. H. C. **Project administration:** Fortes, B. D. A. and Café, M. B. **Resources:** Café, M. B. **Supervision:** Stringhini, J. H. and Café, M. B. **Writing – original draft:** Fortes, B. D. A. **Writing – review & editing:** Café, M. B. and Mello, H. H. C.

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References

- Aderibigbe, A.; Cowieson, A. J.; Sorbara, J. O. and Adeola, O. 2020. Growth phase and dietary α -amylase supplementation effects on nutrient digestibility and feedback enzyme secretion in broiler chickens. *Poultry Science* 99:6867-6876. <https://doi.org/10.1016/j.psj.2020.09.007>
- Angel, C. R.; Saylor, W.; Vieira, S. L. and Ward, N. 2011. Effects of a monocomponent protease on performance and protein utilization in 7 - to 22-day-old broiler chickens. *Poultry Science* 90:2281-2286. <https://doi.org/10.3382/ps.2011-01482>
- Amerah, A. M.; Romero L. F.; Awati, A. and Ravindran, V. 2017. Effect of exogenous xylanase, amylase, and protease as single or combined activities on nutrient digestibility and growth performance of broilers fed corn/soy diets. *Poultry Science* 96:807-816. <https://doi.org/10.3382/ps/pew297>
- Anwar, U.; Riaz, M.; Khalid, M. F.; Mustafa, R.; Farooq, U.; Ashraf, M.; Munir, H.; Auon, M.; Hussain, M.; Hussain, M.; Chisti, M. F. A.; Bilal, M. Q.; Rehman, A. and Rahman, M. A. U. 2023. Impact of exogenous xylanase and phytase, individually or in combination, on performance, digesta viscosity and carcass characteristics in broiler birds fed wheat-based diets. *Animals* 13:278. <https://doi.org/10.3390/ani13020278>

- Attia, Y. A.; Al-Khalaifah, H. S.; Alqhtani, A. H.; Abd El-Hamid, H. S.; Alyileili, S. R.; Abd El-Hamid, A. E.; Bovera, F. and El-Shafey, A. A. 2022. The impact of multi-enzyme fortification on growth performance, intestinal morphology, nutrient digestibility, and meat quality of broiler chickens fed a standard or low-density diet. *Frontiers in Veterinary Science* 9:1012462. <https://doi.org/10.3389/fvets.2022.1012462>
- Ahiwe, E. U.; Ejiofor, I.; Oladipupo, O. A.; Ogbuewu, I. P.; Aladi, N. O.; Obikaonu, H. O. and Emenalom, O. O. 2022. Effect of composite enzyme supplementation on production parameters, intestinal segment measurements, and apparent nutrient digestibility of broiler chickens fed low energy and protein diets. *Tropical Animal Health and Production* 54:399. <https://doi.org/10.1007/s11250-022-03402-5>
- Batal, A. B. and Parsons, C. M. 2003. Utilization of different soy products as affected by age in chicks. *Poultry Science* 82:454-462. <https://doi.org/10.1093/ps/82.3.454>
- Bedford, M. R. 2000. Exogenous enzymes in monogastric nutrition: their current value and future benefits. *Animal Feed Science and Technology* 86:1-13. [https://doi.org/10.1016/S0377-8401\(00\)00155-3](https://doi.org/10.1016/S0377-8401(00)00155-3)
- Beski, S. S. M.; Swick, R. A. and Iji, P. A. 2015. Specialized protein products in broiler chicken nutrition: a review. *Animal Nutrition* 1:47-53. <https://doi.org/10.1016/j.aninu.2015.05.005>
- Camposino, A.; Williams, M.; Latham, R.; Bailey, C. A.; Brown, B. and Lee, J. T. 2015 Effects of increasing dried distillers' grains with solubles and non-starch polysaccharide degrading enzyme inclusion on growth performance and energy digestibility in broilers. *Journal of Applied Poultry Research* 24:135-144. <https://doi.org/10.3382/japr/pfv018>
- Cowieson, A. J.; Bedford, M. R. and Ravindran, V. 2010. Interactions between xylanase and glucanase in maize-soy-based diets for broilers. *British Poultry Science* 51:246-257. <https://doi.org/10.1080/00071661003789347>
- Dersjant-li, Y.; van de Belt, K.; van der Klis, J. D.; Kettunen, H.; Rinttilä, T. and Awati, A. 2015. Effect of multi-enzymes in combination with a direct-fed microbial on performance and welfare parameters in broilers under commercial production settings. *Journal of Applied Poultry Research* 24:80-90. <https://doi.org/10.3382/japr/pfv003>
- Giannenas, I.; Bonos, E.; Anestis, V.; Filioussis, G.; Papanastasiou, D. K.; Bartzanas, T.; Papaioannou, N.; Tzora, A. and Skoufos, I. 2017. Effects of protease addition and replacement of soybean meal by corn gluten meal on the growth of broilers and on the environmental performances of a broiler production system in Greece. *PLoS One* 12:e0169511. <https://doi.org/10.1371/journal.pone.0169511>
- Head, B.; Biozaz, M. and Cherian, G. 2019. Flaxseed and carbohydrase enzyme supplementation alters hepatic n-3 polyunsaturated fatty acid molecular species and expression of genes associated with lipid metabolism in broiler chickens. *Veterinary Sciences* 6:25. <https://doi.org/10.3390/vetsci6010025>
- Jabbar, A.; Tahir, M.; Khan, R. U. and Ahmad, N. 2021. Interactive effect of exogenous protease enzyme and dietary crude protein levels on growth and digestibility indices in broiler chickens during the starter phase. *Tropical Animal Health and Production* 53:23. <https://doi.org/10.1007/s11250-020-02466-5>
- Knudsen, K. E. B. 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. *Poultry Science* 93:2380-2393. <https://doi.org/10.3382/ps.2014-03902>
- Kiarie, E.; Romero, L. F. and Ravindran, V. 2014. Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. *Poultry Science* 93:1186-1196. <https://doi.org/10.3382/ps.2013-03715>
- Kiarie, E. G.; Mohammadigheisar, M.; Kakhki, R. A. M. and Madsen, M. H. 2021. Impact of feeding modified soy protein concentrate in the starter phase on growth performance and gastrointestinal responses in broiler chickens through to day 42 of age. *Poultry Science* 100:101147. <https://doi.org/10.1016/j.psj.2021.101147>
- Lilburn, M. S. and Loeffler, S. 2015. Early intestinal growth and development in poultry. *Poultry Science* 94:1569-1576. <https://doi.org/10.3382/ps/pev104>
- Longo, F. A.; Menten, J. F. M.; Pedroso, A. A.; Figueiredo, A. N.; Racanicci, A. M. C.; Gaiotto, J. B. and Sorbara, J. O. B. 2005. Diferentes fontes de proteína na dieta pré-inicial de frangos de corte. *Revista Brasileira de Zootecnia* 34:112-122. <https://doi.org/10.1590/S1516-35982005000100015>
- Mohammadigheisar, M.; Kim, H. S. and Kim, I. H. 2018. Effect of inclusion of lysolecithin or multi-enzyme in low energy diet of broiler chickens. *Journal of Applied Animal Research* 46:1198-1201. <https://doi.org/10.1080/09712119.2018.1484358>
- Munyaka, P. M.; Nandha, N. K.; Kiarie, E.; Nyachoti, C. M. and Khafipour, E. 2016. Impact of combined β -glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. *Poultry Science* 95:528-540. <https://doi.org/10.3382/ps/pev333>
- Nascimento, A. H.; Gomes, P. C.; Rostagno, H. S.; Albino, L. F. T. and Donzele, J. L. 2005. Valores de energia metabolizável de farinhas de penas e de vísceras determinados com diferentes níveis de inclusão e duas idades das aves. *Revista Brasileira de Zootecnia* 34:877-881. <https://doi.org/10.1590/S1516-35982005000300020>
- Olukosi, O. A.; Cowieson, A. J. and Adeola, O. 2007. Age-related influence of a cocktail of xylanase, amylase, and protease or phytase individually or in combination in broilers. *Poultry Science* 86:77-86. <https://doi.org/10.1093/ps/86.1.77>

- Ravindran, V.; Hew, L. I.; Ravindran, G.; Gill, R. J.; Pittolo, P. H. and Bryden, W. L. 1999. Influence of xylanase supplementation on the apparent metabolisable energy and ileal amino acid digestibility in a diet containing wheat and oats, and on the performance of three strains of broiler chickens. *Australian Journal of Agriculture Research* 50:1159-1163. <https://doi.org/10.1071/AR98208>
- Rao, S. V. R.; Raju, M. V. L. N.; Nagalakshmi, D.; Prakash, B. and Paul, S. S. 2021. Effect of supplementation of graded concentrations of xylanase and α -amylase on performance, slaughter variables, and energy digestibility in broiler chickens fed corn-soybean meal-based diet. *Journal of Applied Poultry Research* 30:100139. <https://doi.org/10.1016/j.japr.2021.100139>
- Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T. and Euclides, R. F. 2011. *Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais*. 3.ed. Universidade Federal de Viçosa, Departamento de Zootecnia, Viçosa, MG.
- Saleh, A. A.; El-Far, A. H.; Abdel-Latif, M. A.; Emam, M. A.; Ghanem, R. and Abd El-Hamid, H. S. 2018. Exogenous dietary enzyme formulations improve growth performance of broiler chickens fed a low-energy diet targeting the intestinal nutrient transporter genes. *PLoS One* 13:e0198085. <https://doi.org/10.1371/journal.pone.0198085>
- Sakomura, N. K. and Rostagno, H. S. 2016. *Métodos de pesquisa em nutrição de monogástricos*. 2.ed. Funep, Jaboticabal.
- Silva, J. M. S.; Oliveira, N. R.; Gouveia, A. B. V. S.; Vieira, R. A.; Santos, R. O. F.; Minafra, C. S. and Santos, F. R. 2021. Effect of protease supplementation on the digestibility of amino acids in animal-origin meals for broiler diets. *Czech Journal of Animal Science* 66:29-37. <https://doi.org/10.17221/134/2020-CJAS>
- Silva, D. J. and Queiroz, A. C. 2002. *Análises de alimentos (métodos químicos e biológicos)*. 3.ed. UFV, Viçosa, MG.
- Silva, E. P.; Rabello, C. B. V.; Lima, M. B.; Ludke, J. V.; Arruda, E. M. F. and Albino, L. F. T. 2014. Poultry offal meal in broiler chicken feed. *Scientia Agricola* 71:188-194. <https://doi.org/10.1590/S0103-90162014000300003>
- Steenfeldt, S. 2001. The dietary effect of different wheat cultivars for broiler chickens. *British Poultry Science* 42:595-609. <https://doi.org/10.1080/00071660120088416>
- Svihus, B. 2014. Function of the digestive system. *Journal of Applied Poultry Research* 23:306-314. <https://doi.org/10.3382/japr.2014-00937>
- Wang, J. J.; Garlich, J. D. and Shih, J. C. H. 2006. Beneficial effects of versazyme, a keratinase feed additive, on body weight, feed conversion, and breast yield of broilers chickens. *Journal of Applied Poultry Research* 15:544-550. <https://doi.org/10.1093/japr/15.4.544>
- Willemsen, H.; Everaert, N.; Witters, A.; De Smit, L.; Debonne, M.; Verschuere, F.; Garain, P.; Berckmans, D.; Decuyper, E. and Bruggeman, V. 2008. Critical assessment of chick quality measurements as an indicator of posthatch performance. *Poultry Science* 87:2358-2366. <https://doi.org/10.3382/ps.2008-00095>
- Zavarize, K. C.; Sartori, J. R.; Gonzales, E. and Pezzato, A. C. 2012. Morphological changes of the intestinal mucosa of broilers and layers as affected by fasting before sample collection. *Brazilian Journal of Poultry Science* 14:21-25. <https://doi.org/10.1590/S1516-635X2012000100004>