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Protease inclusion in plant- and animal-based broiler diets: Performance, digestibility and biometry of digestive organs

D.P. Carvalho[1], N.S.M. Leandro[1], M.A. Andrade[1], H.F. de Oliveira[1#], M.F. Pires[1], K.A. Teixeira[1], P.S. Assunção[1] & J.H. Stringhini[1]
[1]Federal University of Goiás, Department of Animal Science, Goiânia, Goiás, Brazil

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Abstract

The addition of protease to broiler diets may complement the action of endogenous enzymes and improve protein digestibility. Here, the authors evaluated the effect of adding protease to broiler diets that contained animal-based meal on bird performance, digestibility, and biometry of digestive tract organs. Four treatments, which contained animal- or plant-based meals with or without supplementary protease were compared, namely basal vegetable feed (BVF), BVF + protease (BFP), basal vegetable feed + animal by-product meal (BFA), and BFA + protease. In the first experiment, 320 one-day-old Cobb 500® chicks were allocated to eight replicates with 10 birds per replicate. The experimental period was seven days, and nutrient metabolizability was evaluated. In the second experiment, 720 one-day-old Cobb 500® chicks were assigned to treatments in a similar manner, with six replicates and 30 birds per replicate, but the experimental period was 42 days. Significant differences (P <0.05) were observed between treatments for nutrient digestibility, weight gain, feed consumption, average final weight, food conversion, viability, and biometry of the pancreas. Treatments with animal-based meals had the highest digestibility. Birds fed these meals grew faster in the pre-starter phase and consumed less feed between 1 and 21 days. However, between 1 and 42 days old, broilers fed plant-based diets had better feed conversion, and the group that did not receive protease supplementation had a better liveability rate.

Keywords: additive, exogenous enzyme, nutrition, organ biometry, poultry
[#]Corresponding author: helder@zootecnista.com.br

Introduction

Broiler production in Brazil is growing fast, and the country has become the world's second largest producer and the greatest exporter. In 2017, production of the poultry sector was approximately 13.05 million tons (BAAP, 2018).

Broiler production provides quality animal protein at low cost and is accessible to most social classes (Oliveira *et al.*, 2018). Because nearly 70% of all costs in broiler production are related to feeding, there is a need to promote the use of alternative ingredients in their diets (Wachholz *et al.*, 2017). The increase in chicken production and resultant increase in residues produced by the sector have led to higher production of animal by-product meals, which can be considered an alternative source of nutrients to reduce broiler production costs by partly replacing soybean bran. The inclusion of such by-products as animal meals has the potential to improve efficiency in the use of traditional foods.

However, besides corn and soybean bran, the majority of foods used in diets contain anti-nutritional factors such as protease inhibitors and phytates, which reduce food quality and inhibit nutrient digestibility for chickens. In this context, Matias *et al.* (2015) suggested the use of exogenous enzymes as an alternative way to reduce production costs, by improving efficiency in the use of traditional foods and enabling the use of animal by-product meals. Among the exogenous enzymes, proteases have been prominent, as they promote higher protein digestibility of the ingredients in feeds by hydrolysing them into peptides and amino acids, thus favouring their absorption (Ribeiro *et al.*, 2015).

Protease supplementation is of particular interest for very young animals, for which the relative activity of endogenous proteases may not be optimal. Endogenous proteases catalyse the hydrolysis of dietary

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proteins, thereby complementing the animals' digestive enzymes, such as pepsin and pancreatic proteases, but they also destroy anti-nutrients, such as lectins and trypsin inhibitors. Exogenous proteases are thought not to have this effect (Ghazi *et al.*, 2002; Cowieson *et al.*, 2016).

The present study evaluated the effects of adding protease enzyme (0.05%) to broiler diets containing animal meal. The authors measured nutrient digestibility, performance, and the biometry of digestive tract organs for chicks aged between 1 and 42 days.

Material and Methods

The authors conducted two experiments at the Poultry Farming Sector of the Zootechny Department of the Federal University of Goiás. All procedures in this study were conducted according to protocol registration No. 055/15, and were approved by the Ethics Committee for Animal Use of the Federal University of Goiás.

For the first experiment, 320 one-day-old male Cobb 500® chicks with an average initial weight of 48 ± 2 g were distributed in a 2 x 2 factorial arrangement by a completely randomized design, providing four treatments with eight replicates and 10 birds per replicate. Treatments comprised feeds containing animal- or plant-based meals with or without supplementary protease enzyme: i) basal vegetable feed (BVF), ii) BVF + protease (BFP), iii) basal vegetable feed + animal by-product meal (BFA), and iv) BFA + protease. The experimental period consisted of three days for adaptation and four days for data collection. Experimental diets were formulated according to the nutritional requirements proposed by Rostand *et al*. (2011), modified by a reduction of 7% in all amino acids, 50 kcal of energy, and use of the protease enzyme as a substitute for starch. Diets contained no growth promoters or anticoccidians. Nutritional and percentage composition are shown in Tables 1 to 3.

Birds were housed in batteries of 32 experimental cages, each 0.40 × 0.50 m in size and constructed with galvanized wire. Each was equipped with manual drinkers and feeders, and an excreta-collecting tray. Ventilation was controlled with curtains. Feed and water were provided ad libitum for the entire experimental period. The sheds were monitored for temperature and relative air humidity. Lighting was constant with the use of incandescent lamps.

Metabolism was assayed from the fourth to the seventh day, using total excreta collection (Sakomura & Rostagno, 2007). Excreta were collected twice a day, and samples were stored in identified plastic bags and frozen. For bromatological analyses, samples were pre-dried in a forced ventilation oven at 55 ± 5 °C and then ground in a Wiley mill. Analyses were performed according to the methods proposed by Silva and Queiroz (2002). The authors calculated nutritional balances as proposed by Matterson *et al*. (1965), and metabolizability coefficients as proposed by Batal and Parsons (2002). The nutrient metabolizability (M) was determined by the equation:

$$M\ (\%) = \frac{NI - NE}{NI} x100$$

Where: NI = ingested nutrient, and
NE = excreted nutrient.

For the second experiment, 720 one-day-old male Cobb 500® chicks, with an average initial weight of 43 ± 1 g, were distributed in a 2 x 2 factorial arrangement by a completely randomized design, providing four treatments with six replicates and 30 birds per replicate. Treatments were the same as those used in the first experiment, but the experimental period was 42 days, divided into four phases, namely pre-initial, initial, growth, and termination. Experimental diets were formulated as before, and diets contained no antibiotics or anticoccidians. Percentage and nutritional compositions are shown in Table 2 and Table 3.

Birds were reared on the floor, stored in 24 experimental concrete boxes (2.10 × 2.50 m), each equipped with pendular drinkers and feeders, and rice hull litter. Birds were reared under the same management conditions as the first experiment. At 7, 14, 21, 34, and 42 days, the authors evaluated feed intake (g), average weight (g), weight gain (g), food conversion (kg/kg), and liveability (%). Feed intake (g) was calculated as the weight difference between the feed provided and the leftovers. Average weight was calculated by the total weight of chickens in the batch, divided by the number of chickens. Weight gain was obtained through the difference between birds' initial average weight and final average weight. Feed conversion was calculated as the relationship between weight gain and feed consumption. To calculate feed consumption and feed conversion variables, the authors considered mortality rates, which were recorded daily. Liveability was determined through the equation:

$$Livability\ (\%) = \frac{final\ number\ birds\ x\ 100}{initial\ number\ of\ birds}$$

Table 1 Composition of experimental feeds containing protease and animal by-product meals for broilers in the pre-starter phase, days 1 to 7

Ingredients, g/kg	Treatments			
	BF	BFP	BFA	BFAP
Corn	60.13	60.13	65.31	65.31
Soybean meal	35.53	35.53	24.41	24.41
Viscera meal			3.73	3.73
Feather meal			1.67	1.67
Meat and bone meal			2.00	2.00
Dicalcium phosphate	1.89	1.89	0.76	0.76
Starch	0.10	0.05	0.10	0.05
Protease		0.05		0.05
Limestone	1.04	1.04	0.64	0.64
Vitamin supplement[1]	0.10	0.10	0.10	0.10
Mineral supplement[2]	0.05	0.05	0.05	0.05
Salt	0.45	0.45	0.36	0.36
DL-Methionine	0.37	0.37	0.35	0.35
L-Lysine	0.24	0.24	0.38	0.38
L-Threonine	0.08	0.08	0.09	0.09
Calculated composition, %				
Metabolizable energy, Kcal/kg	2900	2900	2900	2900
Crude protein	21.85	21.85	21.85	21.85
Digestible threonine	0.79	0.79	0.79	0.79
Digestible methionine+cysteine	0.95	0.95	0.94	0.94
Digestible methionine	0.66	0.66	0.64	0.64
Digestible lysine	1.21	1.21	1.21	1.21
Calcium	1.00	1.00	0.93	0.93
Available phosphorus	0.46	0.46	0.47	0.47
Sodium	0.22	0.22	0.22	0.22

BVF: basal vegetable feed, BFP: BVF + protease, BFA: basal vegetable feed + animal by-product meal, BFAP: BFA + protease (BFAP). [1]selenium 0.30 mg, vitamin A 10.000 UI, vitamin D_3 2.500 UI, vitamin E 25 mg, vitamin K_3 2 mg, vitamin B_1 2.50 mg, vitamin B_2 6.50 mg, vitamin B_6 3.50 mg, vitamin B_{12} 18 mcg, folic acid 1.20 mg, pantothenic acid 15 mg, niacin 42 mg, biotin 80 mcg, ethoxyquin 166 mg
[2]manganese 90 mg, zinc 75 mg, iron 60 mg, copper 9.75 mg, iodine 1.20 mg

Table 2 Composition of experimental feeds containing protease and animal by-product meals in pre-starter (1 to 7 days) and starter (8 to 21 days) phases for broilers

Ingredients, g/kg	Treatments							
	Pre-starter phase				Starter phase			
	BF	BFP	BFA	BFAP	BF	BFP	BFA	BFAP
Corn	60.13	60.13	65.31	65.31	63.26	63.26	67.62	67.62
Soybean meal	35.53	35.53	24.41	24.41	32.92	32.92	23.71	23.71
Viscera meal			3.73	3.73			2.58	2.58
Feather meal			1.67	1.67			1.00	1.00
Meat and bone meal			2.00	2.00			3.03	3.03
Dicalcium phosphate	1.89	1.89	0.76	0.76	1.76	1.76	0.47	0.47
Soy oil					0.20	0.20		
Starch	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05
Protease		0.05		0.05		0.05		0.05
Limestone	1.04	1.04	0.64	0.64	0.82	0.82	0.54	0.54
Vitamin supplement[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral supplement[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.45	0.45	0.36	0.36	0.43	0.43	0.35	0.35
DL-Methionine	0.37	0.37	0.35	0.35	0.24	0.24	0.24	0.24
L-Lysine	0.24	0.24	0.38	0.38	0.09	0.09	0.20	0.20
L-Threonine	0.08	0.08	0.09	0.09				
Calculated composition, %								
Metabolizable energy, Kcal/kg	2900	2900	2900	2900	2950	2950	2950	2950
Crude protein	21.85	21.85	21.85	21.85	20.65	20.65	20.65	20.65
Digestible threonine	0.79	0.79	0.79	0.79	0.69	0.69	0.67	0.67
Digestible methionine+cysteine	0.95	0.95	0.94	0.94	0.80	0.80	0.80	0.80
Digestible methionine	0.66	0.66	0.64	0.64	0.53	0.53	0.53	0.53
Digestible lysine	1.21	1.21	1.21	1.21	1.03	1.03	1.03	1.03
Calcium	1.00	1.00	0.93	0.93	0.87	0.87	0.87	0.87
Available phosphorus	0.46	0.46	0.47	0.47	0.44	0.44	0.44	0.44
Sodium	0.22	0.22	0.22	0.22	0.21	0.21	0.21	0.21

BVF: basal vegetable feed, BFP: BVF + protease, BFA: basal vegetable feed + animal by-product meal, BFAP: BFA + protease

[1]Selenium 0.30 mg, vitamin A 10.000 UI, vitamin D3 2.500 UI, vitamin E 25 mg. vitamin K3 2 mg, vitamin B1 2.50 mg, vitamin B2 6.50 mg, vitamin B6 3.50 mg, vitamin B12 18 mcg, folic acid 1.20 mg, pantothenic acid 15 mg, niacin 42 mg, biotin 80 mcg, ethoxyquin 166 mg

[2]Manganese 90 mg, zinc 75 mg, iron 60 mg, copper 9.75 mg, iodine 1.20 mg

Table 3 Composition of experimental feeds containing protease and animal by-product meals in growing (22 to 35 days) and final (36 to 42 days) for broilers

Ingredients, g/kg	Treatments							
	22 to 35 days				36 to 42 days			
	BF	BFP	BFA	BFAP	BF	BFP	BFA	BFAP
Corn	66.91	66.91	72.49	72.49	69.40	69.40	73.64	73.64
Soybean meal	28.59	28.59	17.95	17.95	26.04	26.04	17.97	17.97
Viscera meal	-	-	2.00	2.00	-	-	2.59	2.59
Feather meal	-	-	2.67	2.67	-	-	1.00	1.00
Meat and bone meal	-	-	2.00	2.00	-	-	1.00	1.00
Dicalcium phosphate	1.60	1.60	0.67	0.67	1.45	1.45	0.79	0.79
Soy oil	0.99	0.99	0.54	0.54	1.25	1.25	1.20	1.20
Starch	0.10	0.05	0.10	0.05	0.10	0.05	0.10	0.05
Protease	-	0.05	-	0.05	-	0.05	-	0.05
Limestone	0.78	0.78	0.63	0.63	0.75	0.75	0.63	0.63
Vitamin supplement[1]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral supplement[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.41	0.41	0.33	0.33	0.38	0.38	0.33	0.33
DL-Methionine	0.24	0.24	0.13	0.13	0.26	0.26	0.26	0.26
L-Lysine	0.20	0.20	0.32	0.32	0.22	0.22	0.28	0.28
L-Threonine	-	-	0.01	0.01	-	-	0.02	0.02
Calculated composition, %								
Metabolizable energy Kcal/kg	3050	3050	3050	3050	3100	3100	3100	3100
Crude protein	19.10	19.10	19.10	19.10	18.16	18.16	17.74	17.74
Digestible threonine	0.63	0.63	0.63	0.63	0.60	0.60	0.60	0.60
Digestible methionine+cysteine	0.77	0.77	0.67	0.67	0.77	0.77	0.77	0.77
Digestible methionine	0.51	0.51	0.39	0.39	0.52	0.52	0.51	0.51
Digestible lysine	1.02	1.02	0.97	0.97	0.97	0.97	0.92	0.92
Calcium	0.81	0.81	0.81	0.81	0.75	0.75	0.75	0.75
Available phosphorus	0.40	0.40	0.40	0.40	0.37	0.37	0.37	0.37
Sodium	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19

BVF: basal vegetable feed, BFP: BVF + protease, BFA: basal vegetable feed + animal by-product meal, BFAP: BFA + protease

[1] Selenium 0.30 mg, vitamin A 10.000 UI, vitamin D3 2.500 UI, vitamin E 25 mg, vitamin K3 2 mg. vitamin B1 2.50 mg, vitamin B2 6.50 mg, vitamin B6 3.50 mg, vitamin B12 18 mcg, folic acid 1.20 mg, pantothenic acid 15 mg, niacin 42 mg, biotin 80 mcg, ethoxyquin 166 mg

[2] Manganese 90 mg, zinc 75 mg, iron 60 mg, copper 9.75 mg, iodine 1.20 mg

To determine the biometry of the digestive tract, on the 21st day, one bird per replicate was identified and subjected to a six-hour fasting period, after which it was weighed and subsequently euthanized by cervical dislocation. Birds were eviscerated and organs were collected and weighed according to these steps: weight of the pro-ventricle, gizzard, pancreas weight, liver weight, small intestine weight, and large intestine weight. The values obtained were used to calculate the relative weight for each organ (RWO), through the equation:

$$RWO\ (\%) = \left(\frac{organ\ weight}{live\ weight}\right) x\ 100$$

Data on metabolizability, performance, and biometry of the digestive tract were evaluated through an analysis of variance. When effects were deemed significant (α = 0.05) Tukey's test was used to compare the means. All statistical analyses were performed using R software. The statistical model used was:

$$y_{ijk} = m + a_i + b_j + (ab)_{ij} + e_{ijk}$$

Where: y_{ijk}= an observation from the k^{th} replicate (k = 1,2,…,8),
m = the overall mean,
a_i = the fixed effect accounting for the addition of animal by-product meal,
b_i = the fixed effect accounting for the addition of protease,
$(ab)_{ij}$ = the interaction effect of factors a and b, and
e_{ijk} = the random error with mean 0 and variance σ^2.

Results and Discussion

Significant differences were observed (Table 4) for ether extract metabolizability (EEMC) and balance (EEB), and for the metabolizability of nitrogen (NMC) and dry matter (DMMC). Treatments with animal-based meals showed the best values for the metabolizability coefficients. Nitrogen balance (NB) was did not differ across the treatments (P >0.05). Interaction effects were observed (P <0.05) between protein source and protease enzyme for EEMC and EEB. The plant-based protein source without protease supplementation decreased EEMC (P <0.05) relative to the other treatments which were similar. Protease supplementation improved EEB when the protein source was plant based. However, protease supplementation decreased EEB when the protein source was animal based.

Means for performance attributes of broiler chicks fed diets supplemented with protease and animal by-product meal from 1 to seven days old are shown in Table 5. Birds that were fed animal-based meals grew more rapidly. Otherwise, no significant differences were found (P >0.05).

Means for performance attributes of broiler chicks fed diets supplemented with protease and animal by-product meal from 1 to 21 days old are shown in Table 6. Protease supplementation increased FI when the protein source was plant based. For birds fed animal-origin ingredients, the FI did not differ significantly whether or not protease was included in their diet, although the addition of protease did result in a numerical decrease in FI. For the remaining variables, no significant differences were found.

Means for performance attributes of broiler chicks fed diets supplemented with protease and animal by-product meal from 1 to 42 days old are shown in Table 7. Broilers fed diets with plant-based meals had better feed conversion (FC). Those birds that did not receive supplemental protease had improved liveability.

Means for relative weights of gastrointestinal organs of broiler chickens at 21 days old are shown in Table 8. Relative weight of the pancreas was higher in birds that received animal-based meals. Otherwise, no significant differences in relative weights of the gastrointestinal organs were observed.

Table 4 Metabolizability (%) of dry matter, nitrogen and ether extract by broiler chicks; and nitrogen and ether extract balances for feeds containing protease and animal by-product meal

	NB (g)	EEB (g)	NMC (%)	EEMC (%)	DMMC (%)
Treatments					
BVF	29.35	43.34[b]	75.59	86.70[b]	75.59
BFP	27.26	48.66[a]	75.23	89.18[a]	75.23
BFA	28.98	46.13[a]	79.34	90.88[a]	79.34
BFAP	27.22	40.49[b]	79.29	90.10[a]	79.29
Meals					
Animal	28.10	43.31[b]	79.32[a]	90.49[a]	79.32[a]
Vegetable	28.10	46.00[a]	75.41[b]	87.94[b]	75.41[b]
Protease					
Without	29.17	44.73	77.47	88.79	77.57
With	27.24	44.58	77.26	89.64	77.26
P-value					
Meals	0.841	0.032	0.003	<0.001	0.003
Protease	0.059	0.896	0.866	0.166	0.866
Interaction	0.866	<0.001	0.903	0.011	0.903
CV, %	9.83	7.57	4.39	1.90	2.99

BVF: basal vegetable feed, BFP: BVF + protease, BFA: basal vegetable feed + animal by-product meal, BFAP: BFA + protease, DMMC: dry matter metabolizability, NMC: nitrogen metabolizability, EEMC: ether extract metabolizability, NB: nitrogen balance, EEB: ether extract balance
[a,b] Within a classification means with a common superscript do not differ at P =0.05

Table 5 Feed intake, average weight gain, final average weight, feed conversion and liveability of broiler chicks fed diets containing protease and animal by-product meal from 1 to 7 days old

	FI, g	AWG, g	FAW, g	FC, kg/kg	Liveability, %
Treatments					
BVF	164.5	120.2	163.3	1.31	96.1
BFP	159.6	119.8	162.8	1.29	96.1
BFA	159.2	122.2	164.8	1.24	96.1
BFAP	160.8	124.2	167.2	1.26	96.6
Meals					
Animal	160.0	123.2[a]	166.0	1.252	96.3
Vegetable	162.1	120.0[b]	163.0	1.302	96.1
Protease					
With	160.2	122.0	165.0	1.28	96.3
Without	161.9	121.2	164.1	1.28	96.1
P-value					
Meals	0.551	0.048	0.061	0.170	0.570
Protease	0.631	0.600	0.556	0.989	0.570
Interaction	0.349	0.430	0.335	0.647	0.570
CV, %	4.54	2.81	2.04	5.74	1.23

BVF: basal vegetable feed, BFP: BVF + protease, BFA: basal vegetable feed + animal by-product meal, BFAP: BFA + protease, FI: feed intake, AWG: average weight gain, FAW: final average weight, FC: feed conversion ratio
[a,b] Within a classification means with a common superscript do not differ at P =0.05

Table 6 Feed intake, average weight gain, final average weight, feed conversion and liveability of broilers fed diets containing protease and animal by-product meal from 1 to 21 days old

	FI, g	AWG, g	FAW, g	FC, kg/kg	Liveability, %
Treatments					
BVF	1259.7[a]	776.4	822.3	1.46	92.9
BFP	1295.8[b]	770.8	822.0	1.49	93.6
BFA	1252.8[ac]	776.2	839.5	1.45	92.8
BFAP	1230.5[c]	765.0	813.0	1.44	94.2
Meals					
Animal	1241.7[b]	770.6	826.2	1.444	93.5
Vegetable	1277.7[a]	773.6	822.1	1.477	93.3
Protease					
With	1263.2	767.9	817.5	1.465	93.9
Without	1256.3	776.3	830.9	1.457	92.9
P-value					
Meals	0.019	0.702	0.458	0.149	0.537
Protease	0.500	0.294	0.269	0.700	0.315
Interaction	0.009	0721	0.294	0.445	0.396
CV, %	1.96	2.35	1.37	3.33	1.16

BVF: basal vegetable feed, BFP: BVF + protease, BFA: basal vegetable feed + animal by-product meal, BFAP: BFA + protease, FI: feed intake, AWG: average weight gain, FAW: final average weight, FC: feed conversion ratio
[a,b,c] Within a classification means with a common superscript do not differ at P =0.05

Table 7 Feed intake, average weight gain, final average weight, feed conversion and liveability of broilers fed diets containing protease and animal by-product meal from 1 to 42 days old

	FI, g	AWG, g	FAW, g	FC, kg/kg	Liveability, %
Treatments					
BVF	5184.2	2620.2	2748.3	1.71	92.9
BFP	5165.3	2610.8	2757.8	1.70	93.6
BFA	5157.3	2608.7	2725.4	1.76	928
BFAP	5045.8	2595.2	2737.0	1.75	94.2
Meals					
Animal	5101.6	2602.0	2731.2	1.76[a]	93.3
Vegetable	5174.8	2615.5	2753.1	1.70[b]	93.4
Protease					
With	5105.6	2603.0	2747.4	1.730	93.8[a]
Without	5179.8	2614.5	2736.9	1.736	92.9[b]
P-value					
Meals	0.208	0.646	0.520	0.032	0.909
Protease	0.260	0.697	0.755	0.756	0.010
Interaction	0.418	0.945	0.975	0.947	0.324
CV, %	2.29	2.26	2.48	3.33	1.16

BVF: basal vegetable feed, BFP: BVF + protease, BFA: basal vegetable feed + animal by-product meal, BFAP: BFA + protease, FI: feed intake, AWG: average weight gain, FAW: final average weight, FC: feed conversion ratio
[a,b] Within a classification means with a common superscript do not differ at P =0.05

Table 8 Relative weights of digestive system organs of broiler chickens at 21 days old when fed with diets containing protease and animal by-product meal

	Pancreas	Gizzard	Proventricle	Liver	Intestine
Treatments					
BVF	0.28	2.50	0.52	2.64	7.48
BFP	0.34	2.44	0.52	2.67	7.37
BFA	0.36	2.60	0.56	2.76	7.60
BFAP	0.35	2.53	0.57	2.88	8.00
Meals					
Animal	0.36^{a}	2.57	0.56	2.82	7.80
Vegetable	0.31^{b}	2.47	0.52	2.66	7.43
Protease					
With	0.35	2.49	0.55	2.78	7.68
Without	0.32	2.55	0.54	2.70	7.54
P-value					
Meals	0.018	0.520	0.205	0.132	0.312
Protease	0.173	0.658	0.856	0.490	0.691
Interaction	0.053	0.996	0.847	0.645	0.477

BVF: basal vegetable feed, BFP: BVF + protease, BFA: basal vegetable feed + animal by-product meal, BFAP: BFA + protease

^{a,b} Within a classification means with a common superscript do not differ at P =0.05

The present results for metabolizability may be compared with those of Laboissière (2008), who evaluated viscera and bone meals with different levels of humidity in the processing of broiler feed, and found that treatments with those meals showed improved results for DMMC and NMC. Contrary to the results that are presented here, Brito *et al*. (2006) observed 6% improvement in EEMC when they added a multi-enzymatic complex containing protease to broiler diets based on extruded soybean.

Laboissière (2008) also found better performance in seven-day-old pre-initial broilers fed viscera and bone meals. The results of the present study were similar to those observed by Bellaver *et al*. (2005), who compared the inclusion of 3% of viscera and bone meal in broiler diets with vegetable diets and observed no effects on performance at 21 days old.

These results differ from those found by Guichard and Djakalia (2008), who evaluated the substitution of animal-origin meals with plant-origin ones in diets for broiler chickens and found that animal by-product meals resulted in better feed conversions and higher weight gains in the same period. A possible explanation can be related to the origins and processing methods of the animal meal. Troni *et al*. (2016) stated that during the production of animal meal for the poultry industry, the inclusion of sources of by-products such as feathers, blood, and viscera may occur and can contribute to the nutrient level variability between these alternative feedstuffs.

In terms of protease use, better performance was expected for animals that received a supplemented diet, since protease supplementation enhances the digestibility of proteins and amino acids. Therefore, endogenous enzyme production is optimized, thus helping to improve ingredient quality, reducing their variability and ameliorating negative effects of trypsin inhibitors (Cowieson *et al*., 2016; Walk *et al*., 2018). Park and Kim (2018) observed that broilers fed diets supplemented with protease had better weight gain and feed conversion compared with birds fed diets without it. Furthermore, Freitas *et al*. (2011) evaluated a serine monocomponent protease and found better weight gain and feed conversion for birds fed diets containing protease compared with those with no enzymatic supplementation. On the other hand, Park and Kim (2018) did not observe any influence on seven-day-old broiler performance with feed containing protease combined with essential oils. Nor did Zotesso (2015) did not observe any effect of including protease on performance variables for broiler chickens between 1 and 42 days old.

Protease was expected to influence pancreas biometry because of the increased production of endogenous enzymes. Fidelis *et al*. (2011) reported that protease increased the effects of endogenous pancreatic enzymes, augmenting the hydrolysis and solubilization of the protein. However, in the present study, even though the authors observed the influence of diets on pancreas biometry, this related to the type of meal used and not to protease use. Although no protease influence on the organ biometry was observed, the results are consistent with those of Park and Kim (2018), who did not observe significant differences in relative organ weight in chickens fed diets with protease supplementation.

Conclusions

Use of animal by-product meal as a source of protein in the starter feed of broiler chickens improved digestibility and performance of broiler chicks. Use of protease during the starter rearing period is recommended mainly for vegetable-based diets.

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Authors' Contributions

DPC, NSML and JHS conceived and designed the experiments. DPC, HFO, MFP, KAT and PSA performed the experiments. MFP analysed the data. MAA, NSML and JHS contributed reagents, materials, and analytical tools. DPC and HFO wrote the paper. HFO edited the manuscript.

Conflicts of Interest Declaration

The authors declare they have no conflicts of interest regarding the work presented in this report.

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