



ANIMAL SCIENCE

Dry residue of cassava on slow-growing broiler diets, with or without the addition of carbohydrases

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Abstract: Dry residue of cassava was studied on the digestibility, performance, intestinal measurements, with or without inclusion of carbohydrases, of slow-growing broilers. 160 Label Rouge broiler chickens, 21-d-old, were distributed in a randomized, 2x5 factorial arrangement (male and female x 0, 10, 20, 30 and 40% residue) (metabolism trial). 1,100 male chicks were distributed in a 2x5 factorial arrangement (with/without carbohydrases x 0; 2.5; 5.0; 7.5; and 10.0% residue), with five replicates (performance trial). Increasing residue levels led to increases in energetic values. Feed intake from 1–21-d-old and 1–63-d-old decreased linearly. At 42 d-old, feed intake and weight gain levels exhibited a quadratic response, which predicted a highest value at 3.32% and 4.77%, respectively, for diets without carbohydrases. For 21- and 42-d-old chickens, the inclusion of carbohydrases reduced the weight and length of the small intestine. The energetic values of the diets were positively influenced by the residue and had similar digestibility values for both sexes. Inclusion of up to 10% of residue in slow-growing broiler diets does not impaired performance and intestinal morphology. The addition of carbohydrases reduced the viscosity of the digesta but it was not enough to improve the performance of the birds.

Key words: Alternative feed, fiber, label rouge, performance.

INTRODUCTION

There is continuing interest in the identification of alternative feeds, such as agro-industrial residues, that can replace the ingredients commonly used for feeding poultry in order to reduce the cost of production, without compromising animal quality. In addition, exogenous enzymes have been incorporated into the diets to improve their performance and facilitate the use of alternative ingredients (Ravindran 2013). In this context, processed byproducts of cassava (*Manihot esculenta* Crantz), such as dry residue of cassava (DRC) is an alternative ingredient, which can be used

as an alternative energetic source with the potential to partially replace corn.

Starch mass is a product resulting from the process of producing manioc starch, which is characterized as a fibrous material that contains starch unable to be extracted throughout processing (Leonel & Cereda 2000). This residue is considered problematic for the processing industries as a result of its high level of humidity, which is difficult to utilize. Some processing units dehydrate this material, producing the DRC, which can serve as an alternative for preservation and nutritional use, but increases acquisition costs (Fernandes et al. 2015, Broch et al. 2018).

The addition of alternative fibrous feeds in the diets of broilers may compromise their performance due to antinutritional factors including non-starch polysaccharides (NSPs). The effects of soluble and insoluble NSPs in the gastrointestinal tract of the animals and consequently in the nutrient digestibility and absorption are different. The soluble NSPs increases the digesta viscosity and the intestinal transit time (Fuente et al. 1998); whereas the insoluble NSPs may alter characteristics of the digestive tract, decreasing the intestinal transit time and intestinal villi, and having an abrasive action, scraping mucin from the mucosa (Montagne et al. 2003, Boonsinchai et al. 2016, Singh et al. 2019). Khempaka et al. (2009) reported that DRC has a high level of insoluble fiber mainly cellulose and xylan, approximately 20 and 4.2%, respectively (Kosugi et al. 2009). The main sugars in the insoluble fraction of cassava pulp is xylose, galactose and mannose (Chauynarong et al. 2015).

However, feeds with moderate levels of insoluble fiber that are associated with carbohydrases may be beneficial to the animals, promoting gizzard development, improving intestinal motility, and enhancing the digestibility of non-fibrous compounds (Jiménez-Moreno et al. 2013, Jiménez-Moreno et al. 2019). In addition, it has been reported that insoluble NSPs influences gut microbiota and volatile fatty acid production (De Maesschalck et al. 2019).

The objective of this study was to evaluate the effects of different levels of DRC in diets for slow-growing broilers on digestibility of both males and females. In addition, the effects of the inclusion of different levels of DRC, with or without inclusion of carbohydrases, on performance, weight of gastrointestinal organs, length of both the small and large intestines,

intestinal morphometry and viscosity of digesta, were assessed.

MATERIALS AND METHODS

One metabolism and one performance trial were conducted to evaluate inclusion of DRC into the diets of slow-growing broilers. The protocols of the experiments were previously approved by the Committee on Ethics in the Use of Animals of Western Parana State University (number 29/2014). The procedures for slaughter and the collection of biological materials were carried out in accordance with the CFMV resolution n°. 1000/2012. The animals were slaughtered according to Normative Instruction n°.3 (January 17, 2000) of the DSA/MAPA, which establishes Methods of Desensitization for Humanitarian Abatement.

For the determination of DRC energy values, 160, 21-day-old birds of the Label Rouge strain were distributed in a completely randomized factorial design 2 x 5 (containing males and females; and a reference basal diet and four levels of substitution of the basal diet by DRC: 10, 20, 30, 40%), with four replications and four birds per experimental unit (EU). The reference basal diet was formulated based on corn and soybean meal, according to recommendations of Rostagno et al. (2011). The percentage and calculated composition of the basal diet was the same of those used in the performance trial during the grower phase (22 to 63 days) with no DRC and no enzyme inclusion (Table III). The birds were raised in a poultry house, receiving a basal diet of corn and soybean meal until they were 21 days old, and were later transferred to metabolic cages (50 x 50 cm) equipped with movable trays to collect excreta, individual gutter feeders, and nipple drinker systems. The metabolic cages were allocated in a room with temperature control by using air conditioning following

the strain's recommendations (24 - 27°C). The lighting program consisted of continuous 23 h light and 1 h of darkness (30 lux light intensity) throughout the whole experimental period.

After a five-day adaptation period to diets and cages, five days of total excreta collection were performed. The excreta were stored in plastic bags and stored in a freezer at -20°C. At the end of the experimental period, feed intake, and the total amount of excreta produced within each EU were determined. Subsequently, the excreta were thawed, homogenized, weighed, and pre-dried in a forced ventilation oven at 55°C for 72 h. After pre-drying, the samples were ground, and an analysis of dry matter (DM), gross energy (GE), and nitrogen (N) was performed, according to AOAC methods (2019). Based on the results of these analyses, apparent metabolizable energy (AME) and AME corrected by nitrogen balance (AME_n) were calculated, by means of equations proposed by Matterson et al. (1965):

$$AME_{\text{test diet}} \text{ (kcal kg}^{-1}\text{)} = (\text{gross energy intake} - \text{gross energy excreted}) / \text{feed intake}$$

$$AME_{\text{reference basal diet}} \text{ (kcal kg}^{-1}\text{)} = (\text{gross energy intake} - \text{gross energy excreted}) / \text{feed intake}$$

$$AME_{\text{ingredient}} \text{ (kcal kg}^{-1}\text{)} = AME_{\text{reference basal diet}} + [(\text{AME}_{\text{test diet}} - \text{AME}_{\text{reference basal diet}}) / \% \text{ of inclusion of ingredient test}]$$

$$AME_{n \text{ test diet}} \text{ (kcal kg}^{-1}\text{)} = [(\text{gross energy intake} - \text{gross energy excreted}) - (8.22 \times \text{nitrogen balance})] / \text{feed intake}$$

$$AME_{n \text{ reference basal diet}} \text{ (kcal kg}^{-1}\text{)} = [(\text{gross energy intake} - \text{gross energy excreted}) - (8.22 \times \text{nitrogen balance})] / \text{feed intake}$$

$$AME_{n \text{ ingredient}} \text{ (kcal kg}^{-1}\text{)} = AME_{n \text{ reference basal diet}} + [(\text{AME}_{n \text{ test diet}} - \text{AME}_{n \text{ reference basal diet}}) / \% \text{ of inclusion of ingredient test}]$$

$$\text{Nitrogen balance} = \text{nitrogen consumed} - (\text{nitrogen excreted} - \text{endogenous nitrogen excreted})$$

The apparent metabolizable coefficients (AMC and AMC_n) were calculated based on gross

energy, AME, and AME_n values (AMC = AME/GE x 100; AMC_n = AME_n/GE x 100).

The performance trial was conducted in an experimental poultry house, subdivided into 1.76 m² boxes, which contained tubular feeders, nipple drinkers, and concrete floors covered with pine wood (first use). A total of 1,100 male, day-old broilers of the Label Rouge strain were randomly distributed in a 2x5 factorial design (with and without the addition of exogenous carbohydrases and five levels of DRC: 0.0; 2.5; 5.0; 7.5; and 10.0%) in which there were five replicates and 22 birds per EU. Feed was provided in mash form, and birds had free access to both food and water. The lighting program consisted of continuous 23 h light and 1 h of darkness (30 lux light intensity) throughout the whole experimental period, with a maximum and minimum temperature of 28°C and 23°C, respectively, and a maximum and minimum humidity of 65% and 47%, respectively. The temperature inside the shed was controlled using exhaust fans and nebulizers.

Experimental diets were corn and soybean based and were formulated using the chemical composition and the nutritional requirement values recommended for broilers of standard performance by Rostagno et al. (2011) (Tables I, II, and III). The chemical composition of DRC was 89.86% dry matter, 60.73% starch, 0.31% ether extract, 1.12% crude protein, 13.57% crude fiber, 20.82% acid detergent fiber, 38.22% neutral detergent fiber, 1.53% mineral matter, and 1,703 kcal kg⁻¹ AME (average value of the DRC substitution levels of 30 and 40%, obtained in the metabolism trial).

The inclusion of carbohydrases was in accordance with the manufacturer's recommendation (DSM Nutritional Products) as follows: 0.04% Ronozyme A® (CT) α-amylase and endo-1,3:1,4-β-glucanase(400gton⁻¹offeed);0.02% Ronozyme VP® (CT) endo-1,3(4)-β- glucanase,

Table I. Percentage and calculated composition of experimental diets containing different levels of dry residue of cassava, with or without the addition of carbohydrases, used during the pre-starter phase (1 to 7 days) for slow-growing broilers.

Ingredient (%)	Without carbohydrases					With carbohydrases				
	0.0	2.5	5.0	7.5	10.0	0.0	2.5	5.0	7.5	10.0
Corn, 7.88%	53.93	49.90	45.87	41.85	37.82	54.95	50.92	46.89	42.87	38.84
Soybean meal, 46%	37.01	37.69	38.37	39.04	39.72	36.84	37.52	38.20	38.87	39.55
Poultry by product meal	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Dry residue of cassava	0.00	2.50	5.00	7.50	10.00	0.00	2.50	5.00	7.50	10.00
Soybean oil	1.37	2.25	3.13	4.00	4.89	0.46	1.34	2.21	3.09	3.97
Monobicalcium phosphate	1.35	1.35	1.36	1.36	1.36	1.35	1.35	1.36	1.36	1.36
Limestone	0.89	0.88	0.86	0.84	0.82	0.89	0.88	0.86	0.84	0.83
NaCl	0.47	0.47	0.47	0.47	0.48	0.47	0.47	0.47	0.47	0.48
DL-methionine, 98%	0.31	0.31	0.32	0.32	0.33	0.31	0.31	0.32	0.32	0.33
L-lysine sulphate, 50.7%	0.30	0.28	0.26	0.24	0.22	0.30	0.28	0.27	0.25	0.23
L-threonine, 98%	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047	0.047
¹ Vitamin and Mineral Premix	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170
Carbohydrase enzymes combination ²	0.000	0.000	0.000	0.000	0.000	0.065	0.065	0.065	0.065	0.065
Choline chloride	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Salinomycin	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Antioxidant	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
Avilamycin	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Calculated composition										
Metabolizable energy (Kcal kg ⁻¹)	2.925	2.925	2.925	2.925	2.925	2.925	2.925	2.925	2.925	2.925
Crude protein (%)	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Calcium (%)	0.92	0.92	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	0.47	0.47
Sodium (%)	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Digestible lysine (%)	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Digestible met+cis (%)	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94	0.94
Digestible threonine (%)	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85

¹ Vitamin and Mineral premix for birds, levels per kilogram product: Vit. A (min) 9000000.00 UI, Vit. D3 (min) 2500000.00 UI, Vit. E (min) 20000.00 UI, Vit. K3 (min) 2500.00 mg, Vit. B1 (min) 1500.00 mg, Vit. B2 (min) 6000.00 mg, Vit. B6 (min) 30000.00 mg, Vit. B12 (min) 12000.00 mg, Pantothenic acid (min) 12 g, Niacin (min) 25g, Folic acid (min) 800.00 mg, Biotin (min) 60.0 mg, Selenium (min) 250.0 mg; Copper (min) 20g, Iron (min) 100g, Manganese (min) 160g, Cobalt (min) 2000 mg, Iodine (min) 2000 mg, Zinc (min) 100g. ² Carbohydrase enzymes combination: Ronozyme® VP (CT) (200 g ton⁻¹ of diet), Ronozyme® A (CT) (400 g ton⁻¹ of diet), and Ronozyme® WX (CT) 2000 (50 g ton⁻¹ of diet). For the use of carbohydrases, metabolizable energy was valued at 50 kcal kg⁻¹

pentosanase, hemicellulase, and pectinase (200 g ton⁻¹ of feed); and 0.005% Ronozyme WX 2000® (CT) endo-1,4- xylanase (50 g ton⁻¹ of feed). The nutritional matrix of the enzymes was evaluated at 50 kcal kg⁻¹ (Tables I, II, and III). Weight gain (WG), feed intake (FI), and feed conversion ratio (FCR) values were determined when broilers were 21, 42, and 63 days old. Mortality was determined daily to correct FI and FCR values, according to Sakomura & Rostagno (2016). The mortality rate was considered normal for this facility, being less than 2% for all groups.

At 21 and 42 days, two birds per EU, with a mean group weight (\pm 5%), were individually weighed and euthanized by electronarcosis followed by exsanguination in order to assess jejunum morphometry via light microscopy, gastrointestinal organ weight, and small and large intestine weight. To assess jejunum morphometry, a 2 cm fragment of the segment was opened longitudinally, washed in saline solution, fixed in buffered formalin solution (10%), dehydrated in a series of increasing concentrations of alcohol, diaphanized in xylol

Table II. Percentage and calculated composition of the experimental diets used during the starter phase (8 to 21 days) for slow-growing broilers fed diets containing different levels of dry residue of cassava, with or without the addition of carbohydrases.

Ingredients (%)	Without carbohydrases					With carbohydrases				
	0.0	2.5	5.0	7.5	10.0	0.0	2.5	5.0	7.5	10.0
Corn, 7.88%	60.83	56.78	52.72	48.66	44.61	61.86	57.80	53.75	49.69	45.63
Soybean meal, 46%	30.35	31.05	31.75	32.46	33.16	30.17	30.88	31.58	32.28	32.98
Poultry by product meal	4.50	4.50	4.50	4.50	4.50	4.50	4.50	5.00	4.50	4.50
Dry residue of cassava	0.00	2.50	5.00	7.50	10.00	0.00	2.50	5.00	7.50	10.00
Soybean oil	1.12	2.00	2.89	3.77	4.66	0.21	1.09	1.97	2.86	3.74
Monocalcium phosphate	0.88	0.89	0.89	0.89	0.89	0.88	0.88	0.89	0.89	0.89
Limestone	0.94	0.92	0.91	0.89	0.88	0.94	0.92	0.91	0.89	0.88
NaCl	0.44	0.44	0.44	0.44	0.45	0.44	0.44	0.44	0.44	0.45
DL-methionine, 98%	0.26	0.27	0.27	0.27	0.28	0.26	0.27	0.27	0.27	0.28
L-lysine sulphate, 50.7%	0.31	0.29	0.27	0.25	0.23	0.32	0.30	0.28	0.26	0.24
L-threonine, 98%	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043	0.043
¹ Vitamin and Mineral Premix	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170
Carbohydrase enzyme combination ²	0.000	0.000	0.000	0.000	0.000	0.065	0.065	0.065	0.065	0.065
Choline chloride	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Salinomycin	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Antioxidant	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
Avilamycin	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Calculated composition										
Metabolizable energy (Kcal kg ⁻¹)	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000
Crude protein (%)	21.30	21.30	21.30	21.30	21.30	21.30	21.30	21.30	21.30	21.30
Calcium (%)	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Available phosphorus (%)	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Sodium (%)	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Digestible lysine (%)	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
Digestible met+cis (%)	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Digestible threonine (%)	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76

¹ Vitamin and Mineral Premix, levels per kilogram product: Vit. A (min) 9000000.00 UI, Vit. D3 (min) 2500000.00 UI, Vit. E (min) 20000.00 UI, Vit. K3 (min) 2500.00 mg, Vit. B1 (min) 1500.00 mg, Vit. B2 (min) 6000.00 mg, Vit. B6 (min) 30000.00 mg, Vit. B12 (min) 12000.00 mg, Pantothenic acid (min) 12 g, Niacin (min) 25g, Folic acid (min) 800.00 mg, Biotin (min) 60.0 mg, Selenium (min) 250.0 mg, Copper (min) 20g, Iron (min) 100g, Manganese (min) 160g, Cobalt (min) 2000 mg, Iodine (min) 2000 mg, Zinc (min) 100g. ² Carbohydrase enzymes combination: Ronozyme® VP (CT) (200 g ton⁻¹ of diet), Ronozyme® A (CT) (400 g ton⁻¹ of diet), and Ronozyme® WX (CT) 2000 (50 g ton⁻¹ of diet). For the use of carbohydrases, metabolizable energy was valued at 50 kcal kg⁻¹

and embedded in paraffin (Luna 1968). After semi-seriate microtomy (cuts of seven µm), the sections were stained with hematoxylin and eosin. Morphometric analyses (30 readings/sample) were performed using a Motic Image Plus 2.0 imaging system. Villus height was measured from its basal region, which coincides with the upper portion of crypts, its the apex; and the crypt depth was measured from its base to cryptic villi within the transition region. The gastrointestinal organs (proventriculus, gizzard,

small intestine, large intestine, pancreas, and liver) were cleaned with physiological saline solution, dried with filter paper and weighed. The weight of each organ was expressed relative to the total body weight (g/100 g BW). The length of the intestine was also measured (cm).

Two 63 d-old birds per EU were used to determine the viscosity of digesta, weight of gastrointestinal organs, and length of the small and large intestine. Contents of the small intestine of birds were removed for viscosity analysis. The

Table III. Percentage and calculated composition of the experimental diets used during the grower phase (22 to 63 days) for slow-growing broilers fed diets containing different levels of dry residue of cassava, with or without the addition of carbohydrases.

Ingredients (%)	Without carbohydrases					With carbohydrases				
	0	2.5	5.0	7.5	10	0	2.5	5.0	7.5	10
Corn, 7.88%	68.72	64.66	60.61	56.55	52.49	69.75	65.69	61.63	57.58	53.52
Soybean meal, 46%	21.82	22.52	23.22	23.93	24.63	21.64	22.35	23.05	23.75	24.45
Poultry by product meal	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50	5.50
Dry residue of cassava	0.00	2.50	5.00	7.50	10.00	0.00	2.50	5.00	7.50	10.00
Soybean oil	1.65	2.53	3.42	4.30	5.18	0.73	1.62	2.50	3.38	4.27
Monobasic calcium phosphate	0.35	0.35	0.35	0.35	0.36	0.35	0.35	0.35	0.35	0.35
Limestone	0.67	0.66	0.65	0.63	0.62	0.68	0.66	0.65	0.63	0.62
NaCl	0.32	0.33	0.33	0.33	0.33	0.32	0.32	0.33	0.33	0.33
DL-methionine, 98%	0.21	0.22	0.22	0.22	0.23	0.21	0.21	0.22	0.22	0.23
L-lysine sulphate, 50.7%	0.34	0.32	0.30	0.28	0.26	0.34	0.32	0.30	0.28	0.26
L-threonine, 98%	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029	0.029
¹ Vitamin and Mineral Premix	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
Carbohydrase enzymes combination ²	0.000	0.000	0.000	0.000	0.000	0.065	0.065	0.065	0.065	0.065
Choline chloride	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Salinomycin	0.055	0.055	0.055	0.055	0.055	0.055	0.055	0.055	0.055	0.055
Antioxidant	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Avilamycin	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Calculated composition										
Metabolizable energy (Kcal kg ⁻¹)	3.150	3.150	3.150	3.150	3.150	3.150	3.150	3.150	3.150	3.150
Crude protein (%)	18.60	18.60	18.60	18.60	18.60	18.60	18.60	18.60	18.60	18.60
Calcium (%)	0.638	0.638	0.638	0.638	0.638	0.638	0.638	0.638	0.638	0.638
Available phosphorus (%)	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298	0.298
Sodium (%)	0.195	0.195	0.195	0.195	0.195	0.195	0.195	0.195	0.195	0.195
Digestible lysine (%)	1.010	1.010	1.010	1.010	1.010	1.010	1.010	1.010	1.010	1.010
Digestible met+cis (%)	0.737	0.737	0.737	0.737	0.737	0.737	0.737	0.737	0.737	0.737
Digestible threonine (%)	0.656	0.656	0.656	0.656	0.656	0.656	0.656	0.656	0.656	0.656

¹Vitamin and Mineral Premix, levels per kilogram product: Vit. A (min) 9000000.00 UI, Vit. D3 (min) 2500000.00 UI, Vit. E (min) 20000.00 UI, Vit. K3 (min) 2500.00 mg, Vit. B1 (min) 1500.00 mg, Vit. B2 (min) 6000.00 mg, Vit. B6 (min) 30000.00 mg, Vit. B12 (min) 12000.00 mg, Pantothenic acid (min) 12 g, Niacin (min) 25g, Folic acid (min) 800.00 mg, Biotin (min) 60.0 mg, Selenium (min) 250.0 mg, Copper (min) 20g, Iron (min) 100g, Manganese (min) 160g, Cobalt (min) 2000 mg, Iodine (min) 2000 mg, Zinc (min) 100g. ² Carbohydrase enzymes combination: Ronozyme® VP (CT) (200 g ton⁻¹ of diet), Ronozyme® A (CT) (400 g ton⁻¹ of diet), and Ronozyme® WX (CT) 2000 (50 g ton⁻¹ of diet). For the use of carbohydrases, metabolizable energy was valued at 50 kcal kg⁻¹

contents were homogenized and centrifuged at 4,000 rpm for 10 min. Then, supernatants were transferred to tubes and stored in a freezer for further analysis. The viscosity was determined using a Brookfield DV-E viscometer using a low viscosity (UL/Y) adapter (Brookfield Engineering Laboratories, Inc, Stoughton, MA, USA). Samples were kept at a temperature of 40°C and a shear rate that ranged from 30 to 100 s⁻¹. The apparent viscosity was presented as a mean shear rate of 100 s⁻¹.

The energy values, as well as their respective metabolizable coefficients, were subjected to an

analysis of variance, which assessed individual effects of sex (male or female) and DRC content (excluding reference basal diet, 0% of DRC), as well as the interaction between both. The effect of the inclusion level was determined by polynomial regression analysis. The effects of DRC levels, carbohydrases supplementation, and the interaction between these factors were assessed using an analysis of variance. The F test was used to compare means of samples produced from experimental groups in which carbohydrases were either added or not added to feed. The effect of the levels of DRC on the

characteristics evaluated was determined using a polynomial regression. A level of significance of $P < 0.05$ was adopted in all analyses, which were performed using the SAS statistical program, version 9.1 (2014).

RESULTS

There was no interaction ($P > 0.05$) between the DRC levels included and the sex of the birds regarding AME, AME_n, and their metabolizable coefficients (AMC and AMC_n). As DRC levels increased, an increase in AME, AME_n, AMC and AMC_n values were observed (Table IV).

In 1–21-d-old broilers, no interaction ($P > 0.05$) between carbohydrase addition and DRC levels was observed on FI, WG and FCR values (Table V). Broilers fed diets with carbohydrases possessed the lowest levels of WG ($P = 0.007$) and FCR ($P = 0.054$). A linear reduction in FI ($P = 0.016$) and FCR ($P = 0.001$) values were observed as DRC content increased. Carbohydrase addition and DRC levels in 1–42-d-old broilers affected FI ($P = 0.031$) and WG ($P = 0.054$) values. FI and WG showed a quadratic adjustment and the levels that provided maximum responses were estimated at 3.32% and 4.77% DRC, respectively,

without the supplementation of carbohydrase. For FCR, a linear reduction ($P = 0.001$) was observed in all samples, regardless of whether carbohydrase was included. The addition of carbohydrases to the diets increased FCR in birds ($P = 0.037$), this occurred independently of the co-product levels added. When comparing means using the F test, greater FI was observed when birds were fed 2.5% DRC without the addition of carbohydrases compared to those receiving the same level of DRC supplemented with enzymes. Also, broilers fed diets with 2.5% and 5.0% DRC without carbohydrases were heavier than those receiving the same levels of DRC supplemented with enzymes (Table V). From 1–63 days, carbohydrase addition and DRC levels did not affect any of the variables assessed that were related to the performance of broilers ($P > 0.05$). However, DRC levels correlated with linear reductions ($P = 0.041$) in FI, regardless of the use of carbohydrases (Table V).

In 21- and 42-d-old birds, no interaction ($P > 0.05$) was observed between the addition of carbohydrases and DRC levels for the relative weight of the organs of the gastrointestinal tract and length of the small and large intestine ($P > 0.05$) (Table VI). However, a linear increase

Table IV. Apparent metabolizable energy (AME), nitrogen balance-corrected AME (AMEn) and apparent metabolizable coefficient (AMC) and nitrogen balance-corrected AMC (AMCn) of slow-growing broilers, male and female, fed diets containing different levels of dry residue of cassava.

DRC Levels (%)	AME (kcal kg ⁻¹)	AME _n (kcal kg ⁻¹)	AMC	AMC _n
10	676	698	19.22	19.82
20	1232	1256	35.09	35.76
30	1569	1593	44.59	45.27
40	2009	2046	57.08	58.14
Male	1387	1410	39.42	40.06
Female	1357	1387	38.57	39.43
Mean	1371.8	1398.2	39.00	39.75
SEM	92.77	94.54	2.64	2.69
L x S	0.156	0.252	0.154	0.251
Level (L)	<0.001	<0.001	<0.001	<0.001
Sex (S)	0.634	0.746	0.644	0.757
Linear	<0.001 ¹	<0.001 ²	<0.001 ³	<0.001 ⁴
Quadratic	0.365	0.457	0.358	0.450

¹ $Y=288.583 + 43.3289x$, $R^2= 0.88$; ² $Y=302.882 + 43.8126x$, $R^2= 0.87$; ³ $Y=8.2256 + 1.23079x$, $R^2= 0.88$; ⁴ $Y=8.6328 + 1.24451x$, $R^2= 0.87$.

($P = 0.001$) in the relative weight of the small intestine was observed in both periods, regardless of the use of carbohydrases, as the levels of DRC in feed increased. In 21-d-old birds given feed supplemented with carbohydrases, increased relative weights of the small intestine were observed ($P = 0.037$), and a contrary effect was observed in 42-d-old birds. Regression equations evaluating the relative weight of the large intestine of 42-d-old birds best fit using a quadratic adjustment ($P = 0.053$) and the levels that provided the maximum responses were estimated to correspond to 3.44% DRC regardless of carbohydrase supplementation.

Addition of carbohydrases and increased DRC levels in 63-d-old birds, interacted with small intestine length ($P = 0.014$), with a linear reduction of intestine length occurring as DRC levels in the diets of birds supplemented with carbohydrases increased ($P=0.008$). A greater length was observed of the small intestine of the birds fed diets containing 5.0% and 10.0% of DRC that were not supplemented with carbohydrases in comparison with those fed the same levels of DRC supplemented with carbohydrases (Table VI).

No interaction ($P > 0.05$) was observed between the addition of carbohydrases and DRC levels for all the studied variables of intestinal morphometry and viscosity of the digesta, independently of the period (Table VII). There was a quadratic response observed for crypt depth ($P = 0.001$) in 21-d-old birds, with its lowest depth estimated to correspond to 5.74% DRC, a value that did not depend on carbohydrase supplementation. A decrease in the viscosity of the digesta was observed with the inclusion of carbohydrase, regardless of the levels DRC provided (Table VII).

DISCUSSION

The cost of poultry production using slow-growing strains is high due to the extended production cycle, which underscores the importance of research investigating the use of alternative ingredients capable of decreasing cost without affecting animal performance. Dehydrated residue derived from the processing of cassava contain 50% residual starch and is considered a potential alternative to corn- and soybean-based feed. Dehydrated residues contain about 43% insoluble NSPs (Raupp et al. 1999).

NSPs are commonly considered anti-nutritional factors because they increase the viscosity of the digesta and reduce nutrient digestibility and absorption (Singh et al. 2019). Nevertheless, it has been shown that the insoluble form of this component can bring benefits to the gastrointestinal tract of birds, resulting in better performance. Many of these benefits are related to improved intestinal health, increased endogenous enzymatic action, gizzard development, and functionality (Shakouri et al. 2006, Bao & Choct 2010). In addition, modulation of the intestinal microbiota may be a result of the physicochemical action of fiber on bacteria, hindering and removing pathogenic bacteria from the intestinal mucosal wall surface, favoring the development of beneficial bacteria (Abazari et al. 2016).

In this context, the results of AME and AME_n and their respectively coefficients revealed that fiber content was not a limiting factor for the birds. Dietary fiber presents a potential to modulate gut microbiota, and the cecal populations appear to be able to ferment different fiber sources included in the diet. In addition, the genetic may also affected the bacterial community influencing the responses in the digestibility of nutrients by the dietary

Table V. Performance of slow-growing broilers fed diets containing different levels of dry residue of cassava, with or without the addition of carbohydrases

	Feed intake (g)		Weight gain (g)		Feed conversion ratio
1 – 21 days					
Without enzyme	800.42		519.81 ^a		1.540 ^b
With enzyme	795.43		506.50 ^b		1.571 ^a
DRC Levels (%)					
0.0	820.81		518.34		1.584
2.5	821.29		516.16		1.592
5.0	802.15		519.11		1.547
7.5	780.71		507.14		1.540
10.0	764.66		505.01		1.515
(E x L)	0.173		0.076		0.606
Enzyme (E)	0.513		0.007		0.054
Level (L)	0.001		0.204		0.016
Linear	0.001 ¹		0.039		0.016 ²
Quadratic	0.270		0.458		0.666
SEM	4.89		2.69		0.009
1 - 42 days					
Without enzyme					1.847 ^b
With enzyme					1.871 ^a
DRC Levels (%)	Without	With	Without	With	
0.0	3066.81 ^a	3163.20 ^a	1640.05 ^a	1670.36 ^a	1.882
2.5	3280.38 ^a	3113.88 ^b	1727.32 ^a	1663.20 ^b	1.885
5.0	3128.77 ^a	3048.38 ^a	1708.99 ^a	1610.86 ^b	1.863
7.5	3011.90 ^a	3073.27 ^a	1655.83 ^a	1657.89 ^a	1.836
10.0	3021.52 ^a	3078.86 ^a	1662.95 ^a	1675.11 ^a	1.828
(E x L)	0.031		0.054		0.152
Enzyme (E)					0.037
Level (L)	0.001	0.340			0.004
Linear					0.001 ⁵
Quadratic	0.036 ³		0.038 ⁴		
SEM	16.97		8.24		0.007
1 - 63 days					
Without enzyme	5738.94		2672.96		2.134
With enzyme	5793.14		2672.38		2.169
DRC Levels (%)					
0.0	5804.32		2680.00		2.167
2.5	5875.32		2747.01		2.142
5.0	5794.09		2677.53		2.148
7.5	5620.20		2612.12		2.154
10.0	5736.27		2676.68		2.145
(E x L)	0.522		0.249		0.382
Enzyme (E)	0.308		0.496		0.133
Level (L)	0.048		0.101		0.703
Linear	0.041 ⁶		0.189		0.958
Quadratic	1.000		0.751		0.758
SEM	27.38		15.80		0.011

In the same line, means followed by different lowercase letters indicate statistical difference by the F test (P<0.05). ¹Y= 828.504-6.11548x, R²= 0.94; ²Y= 1.59407-0.00767564x, R²= 0.88; ³Y= 3120.38+28.2832x-4.26463x², R²= 0.47, maximum point 3.32%; ⁴Y= 1656.28+21.2746x-2.23012x², R²= 0.50, maximum point 4.77%; ⁵Y= 1.89064-0.0634392x, R²=0.91; ⁶Y= 5844.28-15.6487x, R²= 0.42.

fiber (Ricke & Rothrock Jr 2020). Mtei et al. (2019) observed that the coefficients of apparent ileal digestibility of nutrients were influenced by the dietary fiber content. However, the responses to the increased dietary fiber content were improved in layers compared with broilers and pullets. Lumpkins et al. (2010) also found differences in intestinal development between a modern multipurpose broiler strain, a high-yield strain, and a historic strain of bird. Therefore, differences in the gastrointestinal microbial activities and function can be correlated to differentiated digestion and absorption of nutrients as well as with the genetic of the bird used in the study.

Although gender is considered a factor that can affect energy utilization, which correlates with differences in the physiology of the gastrointestinal tract as well as the intestinal microbiota of birds (Wu et al. 2019), gender-based effects were not observed in this study. This result demonstrates that similar utilization of residue nutrients occurred between the two sexes.

The inclusion of moderate amounts of fiber in broiler diets can prevent excessive FI, improve gizzard function, and provide increased feed efficiency without impairing performance (Mateos et al. 2012). In fact, in addition to decreasing FI at all stages tested, ingredients with high crude fiber content can alter dietary density, which and the space occupied in the digestive tract of the bird can become a limiting factor for FI (Sundu et al. 2006). DRC intake promoted increased WG and improved FCR. These results differed from Picoli et al. (2014) that reported that the inclusion of 2% of DRC compromised performance.

On the other hand, it was observed a impairment in FCR of broilers fed the carbohydrase at 21 and 42 days of age. The enzyme benefits depend on the magnitude of

any challenge under which birds are grown, such as digestibility of the diet, the concentration of viscous NSP in the diet, degree of intestinal insult and even the rearing conditions (environment and stress factors) (Aftab & Bedford 2018). Silva et al. (2019) observed that the inclusion of up to 10% DCR, when associated with carbohydrases may be used in broiler diets while maintaining performance. Considering results reported by these authors, the slow-growing broilers may respond differently than fast-growing birds, when diets are supplemented with enzymatic complexes, and the nutritional matrix of the enzymes evaluated at 50 kcal kg⁻¹ for the Label Rouge strain may have been overestimated. In addition, the effects of the addition of enzymes are related to the concentration of NSP in the diet, as observed by Smeets et al. (2018).

The inclusion of DRC in the diets of 21- and 42-d-old broilers resulted in a linear increase in the relative weight of the small intestine. Dietary fiber can increase the intestinal wall tissue mass and intestinal layer muscle thickness, resulting in morphological and physiological changes of the gastrointestinal tract, increasing intestinal weight (Svihus et al. 2010, Silva et al. 2019). This mechanism can potentially lead to reductions in the activities of endogenous enzymes and nutrient absorption (Teirlynck et al. 2009). Throughout the same period, regardless of DRC levels, the enzyme effectively minimized changes in the small intestine caused by the inclusion of fiber in the diet, via reductions in intestinal weight and length. The same results were not observed in the initial (starter) period (1–21-d-old birds). This can be explained by the increased development of the organ in 42-d-old birds. However, after 63 days, carbohydrases addition was associated with linearly decreasing effects on the length of the small intestine, demonstrating an increased efficiency of the

Table VI. Relative weight of gastrointestinal organs, and length of the small and large intestine (cm) of slow-growing broilers fed diets containing levels of dry residue of cassava, with or without the addition of carbohydrases

21 days										
	Gizzard	Proventriculus	Pancreas	Liver	Small intestine	Large intestine	Heart	Length small intestine	Length large intestine	
Without enzyme	2.86	0.72	0.38	2.69	6.84 ^b	1.26	0.64	117.36	29.20	
With enzyme	2.93	0.70	0.39	2.71	7.33 ^a	1.19	0.62	119.66	28.29	
DRC Levels (%)										
0.0	3.02	0.62	0.39	2.67	6.44	1.17	0.61	117.50	28.53	
2.5	2.73	0.71	0.40	2.56	6.88	1.12	0.64	117.70	28.42	
5.0	2.88	0.65	0.41	2.70	6.82	1.21	0.61	118.45	29.57	
7.5	2.96	0.71	0.40	2.79	7.52	1.39	0.65	119.70	28.95	
10.0	2.89	0.86	0.35	2.77	7.75	1.23	0.64	119.20	28.25	
(E x L)	0.369	0.185	0.293	0.367	0.369	0.407	0.661	0.451	0.171	
Enzyme (E)	0.605	0.662	0.412	0.822	0.037	0.457	0.280	0.353	0.329	
Level (L)	0.430	0.073	0.169	0.442	0.004	0.341	0.394	0.732	0.611	
Linear	0.934	0.017	0.243	0.263	0.001 ¹	0.244	0.256	0.536	1.000	
Quadratic	0.466	0.282	0.037	0.987	0.727	0.716	0.965	0.929	0.451	
SEM	0.059	0.029	0.008	0.052	0.132	0.045	0.008	1.155	0.461	
42 days										
Without enzyme	1.99	0.51	0.27	2.15	4.07 ^a	0.83	0.51	160.76 ^a	38.56	
With enzyme	1.99	0.49	0.27	2.07	3.63 ^b	0.89	0.55	151.04 ^b	40.08	
DRC Levels (%)										
0.0	2.11	0.44	0.25	2.15	3.39	0.88	0.52	154.00	38.40	
2.5	1.93	0.49	0.27	2.16	3.56	0.72	0.53	151.20	36.20	
5.0	2.15	0.48	0.27	2.15	3.86	0.79	0.55	155.22	38.90	
7.5	1.95	0.47	0.27	2.10	4.27	0.95	0.54	158.40	41.40	
10.0	1.81	0.60	0.28	2.02	4.18	0.97	0.52	160.70	41.70	
(E x L)	0.369	0.249	0.369	0.452	0.115	0.118	0.338	0.421	0.407	
Enzyme (E)	1.000	0.581	0.447	0.273	0.006	0.274	0.218	0.030	0.314	
Level (L)	0.326	0.164	0.502	0.458	0.002	0.029	0.515	0.445	0.134	
Linear	0.151	0.053	0.269	0.201	0.001 ²	0.041	0.861	0.185	0.031	
Quadratic	0.493	0.359	0.522	0.460	0.015	0.053 ³	0.314	0.606	0.445	
SEM	0.057	0.022	0.007	0.035	0.094	0.031	0.013	2.182	0.762	
63 days										
Without enzyme	1.37	0.31	0.18	1.95	3.91	0.75	0.41		54.23	
With enzyme	1.38	0.29	0.18	1.91	3.59	0.71	0.42		52.72	
DRC Levels (%)								Without	With	
0.0	1.29	0.31	0.17	1.88	3.59	0.72	0.40	188.87 ^a	199.80 ^a	52.23
2.5	1.37	0.30	0.19	1.96	3.88	0.74	0.43	187.87 ^a	195.73 ^a	55.27
5.0	1.41	0.29	0.17	1.85	3.87	0.72	0.40	195.07 ^a	182.53 ^b	52.50
7.5	1.35	0.31	0.18	1.97	3.78	0.73	0.43	184.93 ^a	194.20 ^a	54.17
10.0	1.44	0.30	0.18	1.99	3.65	0.74	0.42	194.20 ^a	179.87 ^b	53.20
E x L	0.335	0.532	0.569	0.592	0.518	0.794	0.299	0.014		0.405
Enzyme (E)	0.846	0.169	0.399	0.407	0.074	0.112	0.609	0.939		0.106
Level (L)	0.362	0.565	0.401	0.188	0.502	0.662	0.163	0.364	0.017	0.217
Linear	0.163	0.857	0.695	0.128	0.975	0.730	0.431	0.606	0.008 ⁴	0.801
Quadratic	0.719	0.624	0.796	0.538	0.208	0.929	0.345	0.857	0.806	0.369
SEM	0.027	0.004	0.003	0.022	0.084	0.012	0.005	1.607		0.468

In the same line, means followed by different lowercase letters indicate statistical difference by the F test ($P < 0.05$); ¹Y= 6.427558-0.130933x, R²= 0.92; ²Y= 3.39492-0.0911480x, R²= 0.90; ³Y= 0.847638+0.0362105x-0.00526393x², R²= 0.70, minimum point 3.44%; ⁴Y= 198.707-1.65600x, R²= 0.56.

enzyme for facilitating the digestion of nutrients as fiber levels increased in the final stage.

Olkowski et al. (2005) assessed the effects of high-fiber diets, determining that they were associated with increased weights and relative lengths of the small intestine and ceca of broilers. These authors associated results with decreased availability of nutrients resulting from characteristics such as adaptive hyperplasia of the intestinal mucosa. Although similar results were observed in the present study, it is worth mentioning that the adaptation affecting the relative weight and size of organs did not negatively affect the performance of birds.

According to these results, the inclusion of carbohydrases in the diets did not interfere with the intestinal morphometry of birds. Though it efficiently decreased the viscosity of the digesta, this reduction did not improve broilers performance. The high fiber content of feed may

be responsible for increases in the viscosity of the digesta in the small intestine of birds, which caused a decrease in the digestibility and absorption of other nutrients (Hetland et al. 2004). The inclusion of carbohydrases in the diets of birds can reduce digesta viscosity, as well as reduce the encapsulation effect of nutrients on cell walls and, in turn, result in increased availability and absorption of protein and starch and increase energy utilization (Slominski 2011).

The quadratic effect for crypt depth observed in 21-d-old birds may be a result of the size of the villi, i.e., smaller villi require less cell turnover (proliferation and differentiation). However, it has been speculated that insoluble fiber can positively affect the integrity and function of the mucosa (Kalmendal et al. 2011). The inclusion of DRC in feed did not cause significant changes in the height of the villi nor did it affect the relationship between the height

Table VII. Villus height (um), crypt depth (um) and villus-to-crypt ratio of jejunum at 21 and 42 days of age and viscosity of the digesta (mPa.s) at 63 days of slow-growing broilers fed diets containing levels of dry residue of cassava, with or without the addition of carbohydrases.

	21 days			42 days			63 days
	Villus height	Crypt depth	V:C	Villus height	Crypt depth	V:C	Viscosity
Without enzyme	285.37	36.90	8.17	461.49	54.32	9.64	4.36 ^a
With enzyme	277.90	38.36	7.74	478.89	54.07	9.99	4.26 ^b
DRC Levels (%)							
0.0	317.22	45.14	7.44	508.21	55.09	10.65	4.23
2.5	272.09	33.94	8.36	460.80	48.678	10.37	4.32
5.0	254.83	35.15	7.52	454.79	57.60	8.72	4.32
7.5	267.79	35.41	8.01	454.57	55.98	9.37	4.30
10.0	296.24	38.53	4.44	472.60	53.63	9.98	4.31
(E x L)	0.405	0.256	0.398	0.575	0.353	0.382	0.806
Enzyme (E)	0.685	0.434	0.486	0.530	0.957	0.952	0.023
Level (L)	0.224	0.003	0.501	0.481	0.551	0.429	0.546
Linear	0.478	0.079	0.450	0.467	0.794	0.473	0.507
Quadratic	0.025	0.001 ¹	0.891	0.246	0.894	0.252	0.435
SEM	9.126	1.067	0.292	12.811	2.420	0.409	0.028

In the same column, means followed by different lowercase letters indicate statistical difference by the F test ($P < 0.05$); ¹Y= $43.9422 + 3.63574x - 0.316542x^2$, $R^2 = 0.84$, minimum point 5.74%

of villi and the depth of crypts determined in both periods. The villi:crypt relationship is indicative of the capacity of intestinal digestion, and an increase in this relationship corresponds to possible improvements in digestion and absorption and decreased energy expenditure with cell renewal (Bivolarski & Vachkova 2014). In this context, Husvéth et al. (2015) suggested that the beneficial effects of fiber included in whole-grain feeding are mediated by increased digestive enzyme activities more substantially than by changes in the tissue structure of the gut.

CONCLUSION

DRC most positively affected energetic values when it was provided at mean values of 1.371,8 kcal kg⁻¹ for AME and 1.398,2 kcal kg⁻¹ for AME_n, and similar digestibility levels were observed between the sexes. Inclusion of up to 10% DRC in slow-growing broiler diets does not impaired performance and intestinal morphology. The addition of carbohydrases was effective in reducing the viscosity of the digesta, regardless of levels of DRC provided in the diets of birds. However, these effects were not enough to improve the performance of broilers.

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