

Anticoccidial and performance effects of adding papaya seed to broiler diet

Efeito anticoccidiano e de desempenho da adição de semente de mamão em ração para frangos

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Summary

The objective was to evaluate the anticoccidial effects of papaya (*Carica papaya* L.) seed and the performance of broilers fed diets containing papaya seed. 525 male day-old broilers were distributed in a completely randomized design, with five treatments and seven replications, with 15 broilers. The treatments 1: basal diet with added performance enhancer (the antimicrobial zinc bacitracin) and an added anticoccidial; treatment 2: basal diet without bacitracin, salinomycin; treatment 3: basal diet with 0.5% papaya seed; treatment 4: basal diet with 1.0% papaya seed; and treatment 5: basal diet with 2.0% papaya seed. Were evaluated broiler performance using the Tukey test, the number of oocysts using the Kruskal-Wallis test, and injury scores using the Friedman test. The starter phase, including up to 2.0% papaya seed in broiler diets did not compromise performance. During the total study period (42 days), the inclusion of 0.5% powdered papaya seed had the same effects as inclusion of zinc bacitracin. Papaya seed to broiler diets did not affect the injury scores nor the number of oocysts. Although we found that adding 0.5% papaya seed to broiler diets did not compromise the broilers' performance, we were unable to confirm anticoccidial effects of papaya seed.

Keywords: antimicrobial; bacitracin; salinomycin.

Resumo

Objetivou-se avaliar a ação anticoccidiana e desempenho de frangos alimentados com rações contendo semente de mamão (*Carica papaya* L.) em pó. 525 pintos de corte macho de um dia foram distribuídos em delineamento inteiramente casualizado, com cinco tratamentos e sete repetições com 15. Os tratamentos foram: tratamento 1 – ração basal com melhorador de desempenho (bacitracina de zinco) e anticoccidiano e sem semente do mamão; tratamento 2 – ração sem bacitracina e sem semente do mamão; tratamento 3 – Ração com 0,5% de semente de mamão; tratamento 4 – Ração com 1% de semente de mamão; tratamento 5 – Ração com 2% de semente do mamão. As médias de desempenho foram comparadas pelo teste Tukey, dados do número de oocistos ao teste de Kruskal-Wallis e escore de lesões pelo teste de Friedman. Na fase inicial até 2% de inclusão não comprometeu o desempenho. No período total de criação a inclusão de 0,5% de semente de mamão em pó proporcionou mesmo resultado que o antimicrobiano. A adição de 0,5% de semente de mamão não compromete o desempenho dos frangos, mas não foi possível confirmar atividade anticoccidiana.

Palavras-chave: antimicrobiano, bacitracina; salinomycin.

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1. Introduction

Antimicrobials are often used as additives in the poultry feed industry, as they are able to modulate the intestinal microbiota and improve nutrient absorption efficiency, which results in better weight results, including increased weight gain, and feed conversion. As such, antimicrobials are classified as performance improvers. However, more recently, poultry farmers have aimed to reduce the use of antimicrobials in poultry diets. Thus, it is necessary to study possible alternatives, especially phyto-genic additives that are derived from plants or spices.

The restrictions on antibiotic growth promoters has stimulated interest in medicinal plants, which was revived in recent times because of their efficacy in providing cost effective therapy to several diseases because of secondary metabolites abundant in plants (Muazu e Paiko, 2020).

Papaya (*Carica papaya* L.) is one of the most produced plants in the world, and has interesting nutritional properties in seeds, leaves, and fruits, such as vitamins (A, B, and C), minerals, crude protein, and fiber (Nwofia *et al.*, 2012; Chukwuka *et al.*, 2013; Tarun and Prashar, 2015; Ugo *et al.*, 2019), and immunomodulatory and antioxidative activities (Sugiharto, 2020). Papaya also has the ability to eliminate intestinal parasites (Nideou *et al.*, 2017), and has antibacterial effects (Peter *et al.*, 2014). For example, Eke *et al.* (2014) described the in vitro antibacterial activity of the ethanolic extract of papaya seed against *Escherichia coli*, *Bacillus cereus* and *Pseudomonas aeruginosa*. In a study by Mohammed *et al.* (2014), papaya seed was found to be effective in reducing the protozoan *Entamoeba histolytica* in mice. *Carica papaya* leaf has been as a natural source of papain, chymopapain A and B, and papaya peptidase A. Papain is proteolytic and thus capable of enhancing protein digestion (Oloruntola *et al.*, 2018).

Based on these promising results, studies should be conducted on poultry to evaluate the anti-protozoan effect of adding papaya leaves and seed to poultry diets. Dakpogan *et al.* (2019) evaluated the anticoccidial effect of papaya seed added to water, and reported a decrease in the number of *Eimeria* sp. oocysts in broiler excreta, highlighting that papaya seed may have the potential to be as effective as conventional anticoccidials.

Some previous studies have found that adding papaya seed to poultry diets can provide the same performance results as antimicrobials (e.g. Bolu *et al.*, 2009). Bolu *et al.* (2009) observed that broilers fed papaya seed showed greater weight gain and feed consumption, and recommended the inclusion of dry papaya seed in the feed at up to 5% of the diet.

Thus, the objective of this study was to evaluate the anticoccidial and performance effects of feeding broilers diets containing papaya seed.

2. Material and Methods

The experiment was approved by the Ethics Committee on the Use of Animals (CEUA) under protocol number 1861061117. A total of 525 one-day-old male broiler chicks of the Cobb variety were used,

distributed in a completely randomized design, with five treatments and seven replicates, with 15 broilers per replicate.

Basal diets (Table 1) were formulated following the recommendations of Rostagno *et al.* (2017), and divided according to nutritional requirements in the different phases of broiler development. Water and feed were provided *ad libitum*, twice a day.

The final feed was provided to all broilers, without performance enhancer nor anticoccidials. The treatments consisted of a diet made with corn and soybean meal, and the addition of different levels of papaya seed to replace the inert material of the basal diet. The five treatments were as follows:; treatment 1: basal diet with added performance enhancer (the antimicrobial zinc bacitracin) and anticoccidial (salinomycin), but without papaya seed (this treatment was considered the positive control); treatment 2: basal diet that did not contain performance enhancer, anticoccidial, nor papaya seed (this treatment was considered the negative control); treatment 3: basal diet with 0.5% powdered papaya seed; treatment 4: basal diet with 1.0% powdered papaya seed; and treatment 5: basal diet with 2.0% powdered papaya seed.

Table 1 - Percentage and calculated composition of the basal diet used in the different phases of broiler development.

Ingredients	Pre-starter	Starter	Grower	Finisher
	1-7 days	8-21 days	22-35 days	36-42 days
Corn	54.119	57.916	60.643	67.311
Soybean meal 45%	37.619	34.254	30.969	27.174
Soybean oil	2.165	2.239	3.223	2.716
Bicalcium phosphate	1.914	1.505	1.261	1.040
Limestone	0.816	0.840	0.792	0.731
Inert material (starch)	2.000	2.000	2.000	-----
DL-methionine 98%	0.364	0.300	0.261	0.239
L-lysine HCL 99%	0.339	0.233	0.209	0.237
Salt (NaCl)	0.300	0.400	0.350	0.350
L-threonine 99%	0.122	0.071	0.050	0.052
Vitamin premix ¹	0.100	0.100	0.100	0.100
Mineral premix ²	0.050	0.050	0.050	0.050
Anticoccidial ³	0.055	0.055	0.055	-----
Zinc bacitracin	0.037	0.037	0.037	-----
Total	100.0	100.0	100.0	100.0
Nutritional composition				
Metabolizable energy (kcal/kg)	2.950	3.000	3.100	3.150
Crude protein (%)	22.20	20.80	19.50	18.00
Calcium (%)	0.920	0.819	0.732	0.638
Available phosphorus (%)	0.470	0.391	0.342	0.298
Available lysine (%)	1.333	1.174	1.078	1.01
Available methionine + cystine (%)	0.944	0.853	0.787	0.737
Available methionine (%)	0.647	0.571	0.519	0.485
Available threonine (%)	0.852	0.763	0.701	0.656

¹ Vitamin premix contained folic acid (min) 700 mg, pantothenic acid (min) 8,000 mg, biotin (min) 60 mg, niacin (min) 30 g, selenium (min) 400 mg, vit. A (min) 8,000,000 UI, vit. B1 (min) 3,000 mg, vit. B12 (min) 10,000 cg, vit. B2 (min) 4,000 mg, vit. B6 (min) 2000 mg, vit. D3 (min) 2,000,000 UI, vit. E (min) 15,000 UI, vit. K3 (min) 2,000 mg. ² Mineral premix contained: zinc (min) 125 g, copper (min) 12.6 g, iodine (min) 2.520 mg, iron (min) 105 g, manganese (min) 126 g. ³ Anticoccidial: salinomycin sodium (min) 12 g. ^{1 and 2} Agroceresmultimix®

Papaya seeds were washed briefly with running water to remove the pulp residue. Then, they were placed in an oven for drying at 40°C for 48 hours. After the drying process, the seeds were ground in a Willey mill, and passed through a 3 mm sieve. Subsequently, ground seeds were pooled, and 200 g of the total powdered seed was used for bromatological analysis, according to Silva and Queiroz (2002).

To evaluate performance, all broilers were weighed weekly, and the leftover feed was recorded in the feeders. At the end of each development phase (starter, 21 days and finisher 42 days), feed consumption, weight gain, feed conversion and final weight were evaluated.

At 13 days, the broilers were challenged with a live attenuated coccidiosis vaccine in water, with a dosage four (four mL of vaccine in 1 liter of water) times greater than that recommended by the manufacturer (one mL of vaccine in 1 liter of water for 100 chickens). The vaccine contained in its composition the following species in the genus *Eimeria*: *E. acervulina*, *E. maxima*, *E. tenella*, *E. brunetti*, *E. necatrix*, *E. praecox*, and *E. mitis*, being each mL with 30-50 thousand oocysts. Before the challenge, the broilers were subjected to a three-hour water fast to stimulate the consumption of the vaccine as soon as possible.

Eight days after the vaccine challenge was conducted, broiler excreta were collected from five plots per treatment, totaling 25 samples, to perform oocyst counts per gram of excreta, following the methodology of Hodgson (1970).

At 21 days, five broilers in each treatment were euthanized by cervical dislocation to assess the

scores for the type of lesion, color, and mucus volume in duodenum, jejunum/ileum and cecum, being two grams of excreta diluted in 60 ml of saline solution and filtered through gauze were used. With the aid of a Pasteur pipette, an aliquot of the supernatant was transferred to a Mc'Master chamber with each cabinet with 0.15 mL of volume with 0.005 g of analyzed excreta. Thus, each oocyst found during the count represented 200 OPG, and the the score of intestinal injuries according to the methodology of Johnson and Reid (1970).

The performance data were submitted to analysis of variance (ANOVA), using the statistical program R, and the means were compared using the Tukey test, with significance set at $P < 0.05$. The data on the number of oocysts were subjected to the Kruskal-Wallis test, and the injury scores were evaluated using the Friedman test.

3. Results

Based on the results of the bromatological analysis (Table 2), we found that the papaya seed contained a high proportion of crude protein (26%).

Regarding the performance results in the starter phase (body weight at 21 days) (Table 3), no significant effect of increasing levels of powdered papaya seed was found. That up to 21 days, the inclusion of 2.0% of the papaya seed did not affect broiler performance results was found.

Table 2 - Bromatological analysis (%), based on natural matter, of powdered papaya seed.

Moisture	6.00
Crude protein	26.2
Ethereal extract	21.0
Crude fiber	31.5
Mineral matter	5.1
Calcium	0.84
Phosphorus	0.48
Acid detergent fiber (ADF)	34.2

Table 3 - Performance of broilers fed diets containing different levels of powdered papaya seed, 21 days of age.

Treatment	Body weight (kg)	Feed intake (kg)	Body weight gain (kg)	Feed conversion ratio
Positive control	0.891	1.011	0.849	1.135
Negative control	0.857	1.024	0.815	1.200
0.5% papaya seed	0.910	0.948	0.867	1.045
1.0% papaya seed	0.910	1.018	0.872	1.113
2.0% papaya seed	0.900	0.997	0.853	1.110
Coefficient of variation (%)	5.06	12.57	5.29	12.84
P value	0.175	0.796	0.165	0.398

At 42 days of age, (Table 4), inclusion of papaya seed to broiler feed was found to affect the final weight and weight gain results.

Broilers fed diets containing 0.5% powdered papaya seed had the same weight and weight gain results as those fed diets containing performance enhancer (zinc bacitracin). Based on this result, it can be inferred that adding 0.5% of powdered papaya seed to broiler feed does not compromise the performance of the broilers. However, adding higher

levels of papaya seed (1.0% or 2.0%) compromised the results of final weight and weight gain, with similar results as found for broilers in the negative control treatment.

Regarding the results of the oocyst counts (Table 5), there was no effect of the papaya seed after the vaccine challenge. Similarly, there was no effect of adding papaya seed to broiler diets on the intestinal injury (duodenum, jejunum/ileum, cecum) score results (Table 5).

Table 4 - Performance of broilers fed different levels of powdered papaya seed, 42 days.

Treatment	Body weight (kg)	Feed intake (kg)	Body weight gain (kg)	Feed conversion ratio
Positive control	3.23 ^a	5.036	3.189 ^a	1.560
Negative control	3.079 ^b	4.887	3.037 ^b	1.588
0.5% papaya seed	3.108 ^{ab}	4.862	3.066 ^{ab}	1.565
1.0% papaya seed	3.043 ^b	4.912	3.000 ^b	1.616
2.0% papaya seed	3.083 ^b	4.913	3.040 ^b	1.594
CV* (%)	3.01	6.19	3.05	6.71
P value	0.008	0.848	0.008	0.859

* Coefficient of variation; averages followed by different letters in the same column differ from each other using the Tukey test (significance set at $P < 0.05$).

Table 5 - Values of oocyst count per gram of excreta and score* of coccidiosis lesions in the intestines of 21-day-old broilers receiving diets with or without powdered papaya seed.

Treatment	Number of oocysts	Score		
		Duodenum	Jejunum/ileum	Cecum
Positive control	660	1.4	1.2	0.2
Negative control	1400	0.8	0.4	0.0
0.5% papaya seed	3180	1.4	0.2	0.2
1.0% papaya seed	1420	1.6	0.2	0.0
2.0% papaya seed	1420	1.0	0.2	0.0
P value	0.882	0.706	0.227	0.963

*considering a scale of 1 to 4, according to Johnson & Reid (1970).

4. Discusson

The high proportion of crude fiber which can limit the use of papaya seed as a food source, since a higher percentage of fiber increases the viscosity of the digestate, which reduces the performance of digestive enzymes. The results of the bromatological analysis are similar to those obtained by Malacrida *et al.* (2011), who found that papaya seed is a source of protein (25.6%) and fiber (30.0%). However, these results are different from those found by Chukwuka *et al.* (2013), who found that proportions of crude protein (14.00%) and crude fiber (5.32%) in papaya seed depended on the ripening period of the fruit. These

variations in nutritional composition are expected, as they

are related to soil conditions, climate, and geographic location (Unigwe *et al.*, 2014).

Ogbuokiri *et al.* (2014) found that chickens fed diets containing 2.5% papaya leaves had greater daily weight gain, better feed conversion, and greater feed consumption at 21 days. According to these authors, the higher feed consumption may have been explained by the fact that the diet containing 2.5% dry papaya leaves had a higher fiber percentage (7.2% crude fiber according to bromatological analysis), which may have made the feed more bulky. However,

the results reported by Ogbuokiri *et al.* (2014) are not consistent with those of the present study, since the papaya seed had a higher percentage of crude fiber (31.5%) than leaves, but this did not result in greater feed consumption.

In a study conducted by Bolu *et al.* (2009) broilers fed with dried papaya seed had better weight gain results than broilers that did not receive dried papaya seed. According to these authors, dried papaya seed can be included in chicken feed at up to 5% of the diet (Bolu *et al.*, 2009). In other studies using papaya leaves (Ogbuokiri *et al.*, 2014; Unigwe *et al.*, 2014), better performance results were reported. However the inclusion of pawpaw latex decreased growth performance but maintained carcass yield, improved immune response and survivability of broiler chickens (Haruna e Odunsi, 2018).

According to Muazu and Piko (2020) it may be concluded that feeding broiler chickens with diets mixed with papaya seed powder significantly and positively reduced serum lipid peroxidation profile without negatively affecting feed efficiency, growth performance and serum biochemical parameters. In a study conducted by Erwan and Irawati (2020) the inclusion of papaya leave meals up to 9% into pellet ration may not alter broiler performance.

The period for the evaluation of the oocyst count (21 days) was the same as used by Cardozo and Yamamura (2006), who found a higher peak of oocyst elimination at 21 days when chickens raised in a free-range system were vaccinated against coccidiosis in the hatchery. The use of live vaccine in the first days of the bird's life provides contact with a small amount of oocyst, which leads to mild infection. Conversely, Dakpogan *et al.* (2019) found that adding papaya seed to broiler feed was effective in reducing the number of *Eimeria* sp. oocysts from broiler excreta when infected orally, and the authors emphasized that addition of papaya seed had the same effects as conventional anticoccidials. In a study by Al-Fifi (2007), chickens raised in a free-range system were infected with *E. tenella*, and papaya leaves were found to reduce the count of oocysts per gram of excreta by 53% when compared to chickens in the control treatment.

On the other hand, Al-Fifi (2007) found that the addition of papaya leaves to chicken feed resulted in greater efficiency in reducing the intestinal injury scores, and resulted in less blood in the excreta of chickens raised in a free-range system when compared with addition of *Vernonia amygdalina* (a perennial herb belonging to the *Asteraceae* family) and neem, *Azadirachta indica*. In a study carried out using papaya leaves, Nghonjuyi *et al.* (2015) found that the ethanolic extract of the leaves, added to the chicken feed at between 0.32 g and 1.44 g/chicken/day, reduced the number of oocysts in the excreta, and was as effective as a synthetic anticoccidial (sulfaquinoxaline). According to Nghonjuyi *et al.* (2015), the anti-inflammatory properties of vitamin A present in papaya leaves may have contributed to greater protection of the epithelial cells of the cecum, which resulted in a decrease in the

reproductive activity of the coccidia, and improved the chickens' resistance to coccidiosis.

In the present study, all broilers were challenged orally with a live vaccine at a dosage four times greater than that recommended by the manufacturer. However, this challenge was not enough to cause injury or result in increased excretion of oocysts. The injury scores observed in the intestine were low, and there was also no blood in the cecal content. Future studies could be conducted with increased dosages of the live vaccine containing oocysts of the protozoan *Eimeria* spp., and working with reused untreated litter. This would increase the intensity of the vaccine challenge, and increase the chances of determining whether papaya seed can actually have an anticoccidial effect, as the studies mentioned above found for papaya leaves.

5. Conclusion

Papaya seed can be added to broiler feed at up to 0.5% without compromising the performance of broilers, but the addition of papaya seed was not found to result in significant anticoccidial activity.

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