Original Articles

Evaluation of a Feed Plant Additive for Coocidiosis Control in Broilers Herbals for Coccidiosis Control

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ABSTRACT

Coccidiosis negatively impacts health and productive performance in broilers. Anticoccidial resistance has prompted alternatives to prevent and control this disease. Therefore, the objective of this study was to evaluate a plant feed additive consisting of *Acacia concinna* containing saponins (Peptasan®) and compare its effectiveness with salinomycin in terms of performance of broilers challenged with *Eimeria*. In the first experiment, 300 Ross chicks (1 day old) were randomly distributed into a negative (uninfected and untreated), and positive control group (infected and untreated), and three levels of dietary Peptasan® (500, 750 and 1000 ppm). In the second experiment, the same number of chicks were distributed in two control groups (negative and positive), two levels of Peptasan® (500 and 750 ppm) and one level of salinomycin (550 ppm). In both experiments chicks were challenged at day 21 of age with 100,000 oocysts of *Eimeria acervulina*, 50,000 oocysts of *Eimeria maxima* and 50,000 oocysts of *Eimeria tenella*. Dietary Peptasan® improved productive performance, reduced mortality and the number of oocysts in feces, as well as increased yellow pigmentation of the skin of challenged chickens. The plant feed additive Peptasan® containing *Acacia concinna* can be effectively used for preventing coccidiosis in broilers.

INTRODUCTION

Avian coccidiosis is caused by protozoa of the genus *Eimeria* that infect the intestinal mucosa (Chapman, 2017) and cause nutrient malabsorption, inefficient feed utilization, impaired body weight gain and mortality (Lee *et al.*, 2012). The control of avian coccidiosis has depended on the application of attenuated vaccines and medication with ionophores or synthetic chemicals (Djemai *et al.*, 2016). Ionophores increase transmembran ion fluxes and dissipation of gradients to exert its anticoccidial effects (Novilla, 2018), but do not eradicate completely the parasites (Chapman, 2017). Ionophores have been banned in several countries and their frequent use has increased the presence of resistant *Eimeria* strains (Djemai *et al.*, 2016) which has generated the need to develop safe and inexpensive methods to control poultry coccidiosis (Mohiti-Asli & Ghanaatparast-Rashti, 2015).

Herbal products are emerging as a strategy to combat coccidiosis (Muthamilselvan *et al.*, 2016; Abbas *et al.*, 2017a). They are characterized by having bioactive components such as phenolic acids, alkaloids, terpenes, tannins and flavonoids (Abbas *et al.*, 2012a), with antioxidant properties and anticoccidial activity (Abbas *et al.*, 2017b) comparable to that of synthetic drugs (Mohiti-Asli & Ghanaatparast-Rashti, 2015).

Peptasan® is a commercial poly-herbal product containing several phytochemical compounds (Kumar *et al.*, 2007), and is composed of

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several plants including the legume *Acacia concina*, which has high concentration of saponins (Jelassi *et al*., 2016), active compounds against coccidia that may be lethal to the parasites by inducing oxidative stress (Abbas *et al*., 2012b). Due to the presence of these active phytochemical compounds, Peptasan® could be an alternative in the control of avian coccidiosis. Therefore, the objective of the study was to evaluate the herbal additive Peptasan® and compare it with salinomycin in performance of broilers challenged with *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella*.

MATERIALS AND METHODS

The study consisted of two experiments of 35 days each one, in which 300 day-old Ross ST308 chicks were used and challenged with *E. acervulina*, *E. maxima* and *E. tenella*. Both experiments were conducted in the experimental facilities of the Consultancy and Advisory Services in Animal Parasitology located in Jiutepec, Morelos, Mexico. In each experiment, the chicks were distributed in a completely randomized design in five groups, with six replicate cages with 10 birds per cage. The cages were provided with gutter-type feeders and tray type automatic drinkers.

Broilers were managed according to Mexican official standars (NOM-062-ZOO-1999; NOM-051-ZOO-1995), and wildlife law of Morelos state (2017, Capitule 3, Articles 20 – 25; Capitule 5, Articles 32 – 35, and Capitule 8, Articles 44 – 52).

Experiment 1

At day 1, the chicks were distributed into five treatments: negative control group (uninfected and untreated birds), positive control group (birds infected with *Eimeria* and untreated) and three challenged groups, treated with three feed plant additive levels (500, 750 and 1000 ppm) of Peptasan® (Technofeed Mexico, Nuproxa Suiza, Indian Herbs Co.) in the feed (Crude protein 19.1%; Metabolizable energy 3.027 Mcal kg^{-1}; Ether extract 29.06%). On day 21 of the experiment, each bird was challenged with a known number of sporulated oocysts (*E. acervulina*: 100,000 oocysts, *E. maxima*: 50,000 oocysts and *E. tenella*: 50,000 oocysts). The inoculum was orally inoculated mixed in 150 g of feed. The excreta of each treatment were collected daily from days 1 to 14 post- challenged, to determine the oocysts per gram of feces according to the methodology proposed by Johnson and Reid (1970). The amounts of feed offered and refusals were recorded daily to estimate the average daily feed intake. The individual body weight was measured every 7 days. Weight gain was calculated from the difference between initial and final weight in each period. The feed conversion ratio was calculated as the proportion of average feed intake: average daily gain. The mortality was recorded daily and expressed as the proportion of the number of dead per total number of chicks.

Experiment 2

In this experiment Peptasan® was compared with the commercial ionophore salinomycin. The chicks were distributed in a negative control group (uninfected and untreated birds), a positive control group (birds infected with *Eimeria* and untreated) and three challenged groups, two of which were treated with 500 and 750 ppm of Peptasan®, and the third with salinomycin (12%) at 550 ppm. On day 21, the treatments to be infected received the challenge as described in the previous experiment. The feed intake, weight gain and feed conversion were measured according to the methodology described in experiment 1. At day 27 of the study, five birds per repetition (cage) selected at random were slaughtered by cervical dislocation. The anticoccidial efficacy was evaluated based on the injury index (Long *et al*., 1982). To calculate this index, the regions of the upper duodenum, middle jejunum and caecum were examined, because these are natural predilection sites for *E. acervulina*, *E. maxima* and *E. tenella*, respectively (Mohiti-Asli & Ghanaatparast-Rashti, 2015). The number of oocysts per gram of feces was evaluated as described in experiment 1. The skin *b** values were measured on the right apterial latero-pectoral area by using a reflectance colorimeter.

Statistical analyses

Data from both experiments were analyzed as a completely randomized design with JMP (Sall *et al*., 2012) and the normality of the variables was evaluated. The initial live weight was tested as a covariate and orthogonal contrasts were performed to test the linear and quadratic effect of the levels of the herbal product in the first experiment and those of the ionophore salinomycin in the second trial (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Productive performance

In the experiment 1, Peptasan® at 750 ppm showed consumption levels similar to the negative control

group (Table 1). This same trend was observed in the final weight and daily weight gain (*p*=0.23), as well as the feed conversion (*p*=0.18) (Table 1). However, in this experiment the level of inclusion of Peptasan® in the diet seems to be important in terms of response of the bird because 750 ppm showed better response compared to 1000 ppm (Table 1). Kukhetpitakwong *et al.* (2006) referred to the toxicity of the extract of

A. concinna, the major component of Peptasan®, that should be taken into consideration in animal use. *A. concinna* is rich in saponins, and their inclusion level can be beneficial or harmful depending on whether it is at low or high concentrations (Shi *et al.*, 2013). Nevertheless, Peptasan® has been used in doses 10 times higher than those recommended without presenting toxicity in rats (Kumar *et al.*, 2007).

Table 1 – Body weight, feed intake, body weight gains and feed conversion ratio of chicks unchallenged and challenged with *Eimeria spp* and fed with and without Peptasan®

Variable*	Treatments					SEM	*p-value*	L	Q
	N[a]	I[b]	P500[c]	P750	P1000				
Final BW, g	1546	1423	1493	1548	1432	23.05	0.24	0.77	0.12
Feed intake, g d^{-1}	69.12	69.88	68.4	69.06	68.77	0.12	0.001	0.24	0.07
ADG, g	42.9	39.4	41.5	42.8	39.5	0.66	0.24	0.77	0.12
Feed conversion	1.62	1.77	1.65	1.62	1.75	0.03	0.18	0.62	0.19

*Data are presented at day 35. [a] Negative control group (uninfected and without additive in diet).

[b] Positive control group (infected and without additive in diet). [c] Challenged chicks fed Peptasan® (P: dietary Peptasan®, ppm). BW: body weigth, ADG: average daily gain, SEM: standard error of mean, P: probability value, L: linear effect Peptasan®, Q: quadratic effect Peptasan®.

In experiment 2, it was observed that at seven days of the experiment the positive and negative control groups recorded higher body weight (*p*=0.0001) compared with chickens whose diets included Peptasan® and salinomycin (Table 2). On days 14 and 21 the body weight was similar among treatments and on days 28 and 35 challenged chickens that were fed with Peptasan® improved body weight (*p*<0.01), feed intake (*p*<0.001) and daily weight gain (*p*<0.001) (Table 2).

Coccidiosis depresses growth by reducing feed intake and increases mortality due to a metabolic imbalance caused by the proliferation of the *Eimeria* and the destruction of the absorption surface in the intestine resulting in malabsorption of nutrients (Wajiha *et al.*, 2018). In both experiments the inclusion of Peptasan® in the diet of chickens inoculated with coccidia allowed them to respond better to the challenge. This may be explained by the saponins *in Acacia conccina*, which include monoterpenoids

Table 2 – Body weight, feed intake, body weight gains and feed conversion ratio of chicks unchallenged and challenged with *Eimeria spp* and fed with and without Peptasan® and salinomycin.

	Treatments					SEM	*p-value*	L	Q	L*
	N[a]	I[b]	P500[c]	P750	S550[d]					
Body weight, g										
Day 1	45.83	45.33	45.83	44.83	45.33	0.189	0.44	1	0.34	0.41
Day 7	200.17	194.83	182.67	186.17	188.67	1.618	0.0009	0.0001	0.004	0.006
Day 14	496.17	495.67	494.83	486	479.33	4.213	0.67	0.92	0.75	0.23
Day 21	856.17	867.5	840.67	848	822.17	6.482	0.24	0.44	0.51	0.09
Day 28	1439	1195.33	1355.17	1325.83	1312.21	18.487	0.0001	0.04	0.42	0.003
Day 35	2043	1776.75	1978.52	1905.67	1907.5	20.298	0.0003	0.16	0.90	0.007
Feed intake, g d^{-1}	56.43	52.39	57.18	56.28	56.20	0.345	0.0001	0.13	0.06	0.64
ADG, g d^{-1}	52.49	45.23	51.31	49.13	49.11	0.575	0.0004	0.37	0.63	0.01
Feed conversion	1.092	1.167	1.117	1.145	1.143	0.01	0.29	0.48	0.93	0.14

[a] Negative control group (uninfected and without additive in diet). [b] Infected control group (infected and without additive in diet). [c] Challenged chicks fed Peptasan® (P: Dietary Peptasan®, ppm). [d] Challenged chicks fed salinomycinin diet (ppm). ADG: Average daliy gain, SEM: standard error of mean, P: probability value, L: linear effect Peptasan®, Q: quadratic effect Peptasan®. *Linear effect of treatment with salinomycin.

like citronellal and sabinene (Lee-Rangel *et al.*, 2017) and other compounds such as flavonoids, tannins, gums and mucilage with capacity to affect several microorganisms (Todkar *et al.*, 2011) as well as immunomodulating properties (Kukhetpitakwong *et al.*, 2006; Applegate, 2009) that improve overall

health and productivity (Giannenas & Kyriazakis, 2009; Pirali Kheirabadi *et al.*, 2014; Wang *et al.*, 2018). These properties may explain the better response of chickens treated with Peptasan® in body weight at seven days post challenge in comparison with chickens treated with salinomycin.

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Anticoccidial activity

Table 3 shows that the inclusion of Peptasan® reduced the mortality in linear form ($p<0.05$) and a correlation of 40% ($p=0.008$) was detected between the number of oocysts in feces and the percentage of mortality. The excretion of oocysts in the negative control group was ruled out and there was a downward trend in reducing the oocysts count in feces with Peptasan® ($p=0.06$) with a high effectiveness against *E. acervulina* and *E. tenella* (99%) and to a lesser extent against *E. maxima* (85%) (Table 3).

Table 3 – Oocyst count and mortality of chicks unchallenged and challenged with *Eimeria* spp and fed with and without Peptasan®

Variable	Treatments					SEM	p-value	L	Q
	N[a]	I[b]	P500[c]	P750	P1000				
Mortality[d], %	0	10	1.11	7.5	1.25	1.04	0.002	0.03	0.06
Oocysts[e]	0	701667	61040	19875	9533	95793	0.07	0.90	0.90
E. acervulina	0	368356	22500	6244	3344	50956	0.083	0.94	0.93
E. maxima	0	23100	10567	6500	3325	4035	0.44	0.51	0.69
E. tenella	0	196417	6967	4342	1108	38998	0.44	0.96	0.98

[a] Negative control group (uninfected and without additive in diet). [b] Positive control group (infected and without additive in diet). [c] Challenged chicks fed Peptasan® (P: Dietary Peptasan®, ppm). [d] Ratio of the number of dead per total number of chicks (%). [e] Oocysts per gram of feces. SEM: standard error of mean, P: probability value, L: linear effect Peptasan®, Q: quadratic effect Peptasan®.

Mortality, in the second experiment, was reduced ($p=0.0001$) to zero with Peptasan® and salinomycin compared to the infected control group where it was 25% (Table 4). Mortality was positively correlated 45% ($p=0.012$), 43% ($p=0.019$) and 56% ($p=0.001$) with the lesion index in the duodenum, jejunum and caecum, respectively.

Mortality depends on the level of infection (Christaki *et al.*, 2004). The reduction in the excretion of oocysts in faeces is indicative of a lower degree of infection (Kucukyilmaz *et al.*, 2012). The chickens with Peptasan® presented reduction of mortality, lower excretion of oocysts and a better productive performance, at seven days after inoculation when the maximum peak of excretion of oocysts occurs (Du & Hu, 2004).

In contrast, the infected control group showed lesions in the duodenum and jejunum, with damage mainly in the caecum, with an index of lesions of 4 (Table 4), where *E. tenella* is associated with hemorrhages in the lumen of the caecum and bloody stools (Crespo & Senties-Cue, 2015). Peptasan® reduced lesion score in duodenum, jejunum and cecum with the same efficacy as salinomycin. The lesion index in cecum was 0.83 for 500 ppm and 1.5 for 750 ppm, while in the treatment with salinomycin (550 ppm) the index was 1.83 ($p=0.0001$).

The polyphenolic components of Peptasan® interrupt the lipid structure of the cell membrane of parasites (Hassan *et al.*, 2007), affecting the enzymatic and metabolic activity and resulting in cell death. This causes a toxic effect in the mature enterocytes in the intestinal mucosa causing cells infected with sporozoites to be released before the merozoite phase of the protozoa, responsible for the main clinical signs of coccidiosis (Mohiti-Asli & Ghanaatparast-Rashti, 2015; Muthamilselvan *et al.*, 2016). The end result

Table 4 – Anticoccidial activity in broiler chickens unchallenged and challenged with *Eimeria spp* and fed with and without Peptasan® and salinomycin.

	Treatments					SEM	p-value	L	Q	L[f]
	N[a]	I[b]	P500[c]	P750	S550[d]					
Mortality[e], %	0.0	25.0	0.0	0.0	0.0	0.228	0.0001	1	1	1
Lesion index, day 27										
Duodenum	0.0	1.50	0.17	0.0	0.83	0.149	0.0006	0.63	0.58	0.02
Jejunum	0.0	2.17	0.0	0.0	1.00	0.194	0.0001	0.99	0.99	0.01
Cecum	0.0	4.00	0.83	1.50	1.83	0.319	0.0001	0.23	0.88	0.01
Pigmentation										
L*	64.59	64.77	64.39	63.83	64.83	0.177	0.39	0.73	0.69	0.67
a*	3.92	3.96	4.29	4.06	4.30	0.142	0.87	0.42	0.44	0.41
b*	14.44	11.05	12.14	9.35	11.61	0.342	0.0001	0.01	0.77	0.004

[a] Negative control group (uninfected and without additive in diet). [b] Infected control group (infected and without additive in diet). [c] Challenged chicks fed Peptasan® in diet (P: dietary Peptasan®, ppm). [d] Challenged chicks fed salinomycin in diet (ppm). [e] Ration of the number of dead per total number of chicks (%). L*: Lightness, a*: Redness, b*: Yellowness, SEM: standard error of mean, P: probability value, L: linear effect, C: quadratic effect. [f] Linear effect of treatment with salinomycin.

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was a reduction in damage to the intestinal tissue and increased absorption of nutrients (Morris *et al.*, 2007), which highlights the coccidicidal potential of the polyphenolic components in herbal products (Abbas *et al.*, 2017a).

Pigmentation of the skin

The challenge with *Eimeria* strains reduced linearly (p=0.019) the parameter $b*$, related to the yellow pigmentation of the skin (Table 4). However, 500 ppm of Peptasan® improved the value of the parameter $b*$ in the skin of the chickens challenged with *Eimeria* (p=0.0001) (Table 4).

The increase in pigmentation of the skin of chickens treated with Peptasan® is due to the reduction in the rate of lesions in the intestinal tract. Frade *et al.* (2013) observed that in animals infected with coccidia the absorption of carotenoids was reduced by desquamation and shortening of the villi of the intestinal mucosa, and a continuous decrease in plasma carotenoids due to blood loss derived from hemorrhages in the cecal mucosa.

CONCLUSIONS

Peptasan® as a herbal additive improved animal performance, and represents a natural alternative in coccidiosis control in poultry. Peptasan® when supplemented at 500 ppm is effective against coccidiosis by reducing, oocysts excretion, intestinal lesions and mortality with the same efficacy as salinomycin (12 %) supplemented at 550 ppm.

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