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Effect of the inclusion of *Ganoderma* spp. on gut morphometry and growth performance of broiler chickens

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ABSTRACT - We conducted an experiment to evaluate the effect of different inclusion rates and routes of administration of *Ganoderma* spp. on growth performance and gut morphology in broilers. We randomly assigned 320 one-day-old male broilers (Ross 308) to eight treatments with the same basal diet. Performance parameters were food intake (FI), body weight gain (BWG), and feed conversion ratio (FCR). Treatments were a basal diet (NC) with 55 ppm of bacitracin methylene disalicylate BMC (PC), basal diet with *Ganoderma* at 50 ppm, 100 ppm, and 150 ppm in drinking water (WG50, WG100, WG150), and feed (FG50, FG100, FG150). Body weight gain was higher for FG150 compared with NC. Treatment FG150 and PC had the best indicators of intestinal morphometry, showing significant differences on villi height to crypt depth ratio compared with other treatments. *Ganoderma* supplementation may be an alternative for the replacement of growth-promoting antibiotics because it offers comparable results to those generated by bacitracin methylene disalicylate (BDM).

Keywords: broiler, *Ganoderma* spp., intestinal morphology, performance

1. Introduction

The global poultry industry is one of the fastest growing markets, with the potential to become the main source of animal proteins (Carvalho et al., 2021), as a result of the increasing demand of a growing world population for food. While the use of antibiotic-based growth promoters has significantly improved performance in global poultry production over the past 50 years (Gadde et al., 2017), there have been growing concerns about the side effects of prophylactic use of these drugs, such as the pro-apoptotic, pro-inflammatory, genotoxic effect (Gallo et al., 2017; Di Cerbo et al., 2018; Pacelli et al., 2020), the emergence of antibiotic-resistant forms of microorganisms, and the possibility of generating residues in the products of treated birds (Fard et al., 2014; Gadde et al., 2017).

This situation has motivated the development of alternative dietary strategies in poultry to improve growth performance and metabolic and health status (Lee et al., 2014). Research suggests that mushrooms can help improving the health and performance of poultry.

The basidiocarps, mycelia, and spores belonging to the genus *Ganoderma* spp., P. Karst., contain a variety of bioactive compounds, which mainly include triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, proteins, and trace elements such as aluminum, arsenic, barium cadmium, calcium, cobalt, copper, iron, magnesium, mercury, plumbum, potassium, selenium, vanadium, and zinc (Liu et al., 2015).

Despite the several trials with broilers (Guo et al., 2004; Willis et al., 2007; Giannenas et al., 2010; Giannenas et al., 2011; Kavyani et al., 2012; Hines et al., 2013; Shang et al., 2014; Abro et al., 2016; Fanhani et al., 2016) and laying hens (Willis et al., 2008; Willis et al., 2009; Hwang et al., 2012; Lee et al., 2015; Wang et al., 2015), studies remain scarce and involve fungal species other than *Ganoderma*. In addition, until now the use of mushroom polysaccharides as growth promoters in poultry has been quite limited, and inclusion rates are not clearly defined, which limits the use and integration of fungi in nutritional plans on an industrial scale. Further studies are needed to investigate the effects of these fungi to improve production performance in chickens and to illuminate the possible modes of action and methods of administration (Khan et al., 2019). Therefore, this study aimed to evaluate the best administration route and the effect of the dietary supplementation of biomass of *Ganoderma* spp. on growth performance and gut morphometry of broiler chicks.

2. Material and Methods

2.1. Experimental design, diets, and management

The experiment was conducted in Bogotá D.C., Colombia (04°38'13" S, 74°05'16" W, 2554 m altitude). Research was approved by the Institutional Committee on Animal Use (protocol number 023-2021). A total of 320 one-day-old male broiler chickens (Ross 308) were randomly assigned to eight treatments of four replicates (10 chickens each). Birds were housed in batteries, kept in a strictly isolated room, with a controlled environment (relative humidity and light), where the ambient temperature was gradually reduced from 33 to 25 °C from day 1 to day 21. Feed and water were provided *ad libitum* throughout the experiment.

All animals were fed the same basal diet formulated with the tested corn, vitamins, and minerals to meet the requirements of broilers from 11 to 21 d of age, according to Rostagno et al. (2011) (Table 1). The treatments were basal diet without additives (negative control; NC); basal diet with 55 ppm of Bacitracin methylene disalicylate (positive control; PC); and six diets supplemented with *Ganoderma* fungal biomass at 50 ppm, 100 ppm, and 150 ppm in drinking water (WG50, WG100, WG150) and in feed (FG50, FG100, FG150). The supplements were administered over 21 days.

Table 1 - Ingredients and nutrient specifications of experimental diets applied in starter and finisher periods

Ingredient (%)	Pre-starter diet (1–7 d)	Starter diet (8–21 d)
Corn (7.88%)	58.9	54.9
Soybean meal (45%)	23	29
Whole soy	12	10
Soybean oil	2	2.2
Dicalcium phosphate	1.76	1.64
Calcium carbonate	1	0.97
DL-Methionine	0.34	0.35
Salt	0.35	0.3
L-Lysine HCl	0.39	0.27
L-Threonine	0.17	0.1
Choline HCl	0.09	0.09
Mineral premix	0.1	0.1
Vitamin premix	0.05	0.05

2.2. Sampling and measurements

Performance parameters feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were evaluated weekly. On day 21 of age, all birds were slaughtered, and intestinal samples of

2 cm were taken immediately from the jejunum to evaluate the villus height, crypt depth, and villus height:crypt depth ratio (VH:CD). The samples were flushed with saline and fixed in buffered formalin (pH 7.0). The fixed intestinal samples embedded in paraffin were sectioned in 5.0 μm, stained with hematoxylin-eosin, and examined by light microscope (Nikon Eclipse E400) in 4 X. Villus height was measured from the tip of the villus to the villus-crypt junction according to Uni et al. (2003), and crypt depth was measured from the base upward to the region of transition between the crypt and villus (Uni et al., 1998). All measures were taken in micrometers (μm).

2.3. Statistical analysis

Statistical analysis was performed using SAS software (Statistical Analysis System, version 9.4). Data regarding parameters of growth performance (FI, BWG, and FCR) at the third week and intestinal morphology were expressed as mean ± standard deviation. Statistical differences between treatments were assessed by One-Way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test. A P<0.05 was considered significant. Normality of the residuals and homogeneity of variances were assessed by the Shapiro–Wilk Test and Barlett's test, respectively. The proposed mathematical model was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}, \tag{1}$$

in which Y_{ij} = value observed for the response variable Y in treatment i and its repetition j, μ = overall mean of all observations, T_i = treatment effect (WG50, WG100, WG150, FG50, FG100, FG150), and e_{ij} = experimental error associated with the observed value Y_{ij}.

3. Results

The PC group showed the lowest cumulative FI (827.3 g per bird) and FCR (1.01) compared with the other experimental groups (P<0.05) (Table 2). At 21 days of age, the FG150 group showed the best BW compared with the NC (P<0.05), but there was no difference between the *Ganoderma* and PC groups. Villi height and depth and VH:CD were similar in FG150 and PC, and these groups showed an increase (P<0.05) in villi dimension compared with the other groups (Table 3).

These results show that the effect of the fungal extract at the inclusion rate of 150 ppm on the intestinal villus was similar compared with the antibiotic. However, the other treatments with and without different inclusion rates of biomass did not show the same behavior.

Table 2 - Effect of different levels of *Ganoderma* spp. inclusion on growth performance of broilers over the period of 0-21 days; data are shown as SEM

	Body weight				Feed intake			Accumulated	Feed conversion ratio		
Treatment/age (d)	1	7	14	21	7	14	21	21	7	14	21
NC	46.3	158.6a	411	826.03b	125.8	280.8a	571.3a	977.8a	0.793	0.989a	1.18a
PC	45.7	154.7ab	408	831.4ab	114.6	239.7b	473.0b	827.3b	0.741	0.869b	1.01b
WG50	46.2	154.0ab	412	850.84ab	119.4	270.4a	568.0a	957.7a	0.776	0.962a	1.13a
FG50	46.2	158.7a	422	868.25ab	118.6	278.6a	584.4a	981.6a	0.748	0.947a	1.13a
WG100	46.2	155.8ab	419	855.8ab	123.1	276.7a	573.6a	973.4a	0.791	0.955a	1.11a
FG100	46.2	158.3a	409	856.91ab	117.2	272.1a	585.4a	967.2a	0.74	0.952a	1.14a
WG150	46.2	153.0b	411	834.14ab	121.4	273.3a	576.1a	970.7a	0.794	0.960a	1.17a
FG150	46.2	151.6b	412	875.71a	120.5	277.7a	587.9a	986.1a	0.795	0.966a	1.12a
SEM	0.28	1.66	5.73	14.4	2.51	5.23	9.23	13.9	0.016	0.02	0.04

NC - negative control; PC - positive control; WG50 - 50 ppm mushroom in water; FG50 - 50 ppm mushroom in feed; WG100 - 100 ppm mushroom in water; FG100 - 100 ppm mushroom in feed; WG150 - 150 ppm mushroom in water; FG150 - 150 ppm mushroom in feed; SEM - standard error of the mean.
a-b - Mean values with different letters within a column differ significantly (P<0.05).

Table 3 - Effect of different inclusion levels of *Ganoderma* spp. on intestinal morphology of broilers at day 21; data are shown as SEM

Parameter (µm)	Villi height	Villi depth	Height + depth	VH:CD
NC	916.84b	125.58bc	996.24bc	11.77ab
PC	1082.03a	148.56a	1171.05a	12.44a
WG50	804.64d	110.60bc	1171.05a	8.39c
FG50	917.05bc	123.99bc	1006.62bc	10.42b
WG100	796.52cd	110.07bc	895.37c	8.25c
FG100	938.56b	123.08bc	1024.24b	11.28ab
WG150	938.64b	108.7c	1051.12b	8.59c
FG150	1082.21a	131.94ab	1169.14a	12.63a
SEM	3.25	4.74	26.33	0.42

NC - negative control; PC - positive control; WG50 - 50 ppm mushroom in water; FG50 - 50 ppm mushroom in feed; WG100 - 100 ppm mushroom in water; FG100 - 100 ppm mushroom in feed; WG150 - 150 ppm mushroom in water; FG150 - 150 ppm mushroom in feed; SEM - standard error of the mean.
a,b,c,d - Different letters within a column indicate significant difference ($P<0.05$).

4. Discussion

Our results confirm that the diet supplementation with *Ganoderma* spp. at an inclusion rate of 150 ppm had a positive effect on villi morphometry and had the highest BWG and BW at 21 days of age. In a healthy gut, a greater intestinal absorption surface is expected to allow a more efficient use of nutrients and, consequently, better productive performance. The best response due to supplementation with 150 ppm of *Ganoderma* fungal biomass could be explained by the existence of polysaccharides (β-glucans) and triterpenoids, compounds that modulate the effect on the intestinal microbiota especially on *Bifidobacterium* populations (Chou et al., 2013), a beneficial bacterium with functional effects to the intestine. In addition, the presence of sugars and indigestible crude fiber and low fat play a beneficial role in the digestive tract of chickens, increasing the growth of this type of beneficial bacteria (Sundu et al., 2006).

The gut mucosal architecture was influenced by mushroom intake, improving the villus height and depth, with a significant increase in villus. According to Giannenas et al. (2011), the structure of the intestinal mucosa can reveal information on gut health. The effects on intestinal villus height and depth in FG150 and PC are consistent with those reported by Fard et al. (2014), who found a significant increase in villus height and crypt depth in jejunum in birds fed 1% (1239 and 187 µm) and 2% (1223 and 209 µm) of *P. ostreatus* fungal extracts. Giannenas et al. (2011) reported an increase in villus height in duodenum, jejunum, and ileum by effect of feed supplementation with 1 and 2% of *A. bisporus* in turkeys. In contrast, the same authors also reported no effect of intestinal villus by feed supplementation with *A. bisporus* in broilers under the same experimental conditions as turkeys (Giannenas et al., 2010). In addition, no effects were reported on body weight, daily gain, daily feed intake, and feed conversion ratio when broilers were supplemented or not with *G. lucidum* for 35 days (Chen and Yu, 2020).

A strong correlation between increased villus height with high absorptive efficiency and gut health has been reported (Shamoto and Yamauchi, 2000; Giannenas et al., 2010; Abuajamieh et al., 2020). Cook and Bird (1973) and Schneeman (1982) reported shorter villus and deeper crypts when the counts of pathogenic bacteria increased in the gastrointestinal tract.

In contrast with results of the current study, in which treatment FG150 had the best indicators of growth performance, Willis et al. (2013) found that the *Pleurotus* mushroom (5%) produced the highest average BWG compared with *G. lucidum* (5 and 10%) and *C. inensis* (10%). Similar results were reported in laying hens with the supplementation of 0.1 or 2% of *Ganoderma* mushroom, (Ogbe et al., 2009). Giannenas et al. (2010) found that 2% of dried *A. bisporus* mushroom in broiler chicken feed increased body weight and improved FCR at 42 days of age. In our study, mushroom supplementation also resulted in a better growth performance. However, the feed conversion was better in PC group.

Bacitracin methylene disalicylate reduced intake, and there was a direct association between FI and FCR.

Limited evidence exists on the mechanisms through which *Ganoderma* exerts growth-promoting activities. However, it is well known that their polysaccharides enhance feed efficiency of broilers (Khan et al., 2019). Although the effect on the intestinal microbiota was not evaluated in this study, other studies reported more efficient use of dietary nutrients and increased proliferation of epithelial cells at the intestinal level as the results of reduction in the intestinal pathogens, coupled with increased intestinal absorptive area (Topping, 1996; Józefiak et al., 2007), which promotes both feed digestibility and animal performance (Rehman et al., 2006; Rehman et al., 2007a,b).

From a nutritional point of view, *Ganoderma* is a source of crude protein (16.79%) and carbohydrates (63.27%). Glucose accounted for 11% and metals 10.2% of dry mass (K, Mg, and Ca are the major trace components) that can positively affect the performance parameters (Bao et al., 2001; Ogbe et al., 2008; Ogbe and Obeka, 2013; Willis et al., 2013); however, currently there is little information about the effects of this mushrooms on performance parameters in broilers.

There is not enough information in the literature to determine which is the best route of administration of mushrooms as supplements in poultry feed. As demonstrated in the present study, the best route of administration was the supplementation of fungi in the feed because of the best growth performance and the best intestinal morphometry obtained. The differential effect observed between water and feed administration may be due to a possible dilution effect of *Ganoderma* when supplemented in drinking water, which could reduce the concentration of polysaccharides and triterpenoids. Additionally, it is not ruled out that these bioactive compounds will be degraded in drinking water, decreasing their action potential. However, it is necessary to perform more tests to determine this.

5. Conclusions

The results presented in this paper suggest that *Ganoderma* spp. may be able to improve both growth performance and gut morphometry of broiler chickens. The diet supplemented with inclusion rates of FG150 significantly improved body weight and weight gain at day 21 of broilers. Besides, we determined that feed is the best route of administration. Thus, *Ganoderma* spp. can be included in the diets of broilers at commercially relevant rates with beneficial effects on their performance. The greater area of intestinal absorption observed with inclusion doses of 150 ppm in feed was associated with better growth performance, showing that *Ganoderma* spp., can have a growth-promoting effect comparable to that produced by the antibiotic Bacitracin methylene disalicylate (BMD). This research showed promising results for the supplementation of *Ganoderma* in poultry feed. However, there are still unresolved aspects as the mechanism of action and the type of microbiota that proliferate in the intestine after *Ganoderma* administration, such as the immunostimulant effect on broilers.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: D.M. Álvarez-Mira and L. L. Betancourt-López. Data curation: D.M. Álvarez-Mira and L. L. Betancourt-López. Formal analysis: C.A. Pinzón-Osorio, D.M. Álvarez-Mira and L. L. Betancourt-López. Funding acquisition: D.M. Álvarez-Mira and L. L. Betancourt-López. Investigation: C.A. Pinzón-Osorio, D.M. Álvarez-Mira and L. L. Betancourt-López. Methodology: D.M. Álvarez-Mira and L. L. Betancourt-López. Project administration: D.M. Álvarez-Mira and L. L. Betancourt-López. Supervision: D.M. Álvarez-Mira and L. L. Betancourt-López. Validation: D.M. Álvarez-Mira and L. L. Betancourt-López. Writing – original draft: C.A. Pinzón-Osorio. Writing – review & editing: D.M. Álvarez-Mira and L. L. Betancourt-López.

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