ISSN 1682-8356
ansinet.com/ijps

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2019.641.647

Research Article
Nutraceutical Effect of *Ganoderma lucidum* Fungus on Neonatal Broilers Diet

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Abstract
Background and Objective: The daily use of sub-therapeutic antibiotics in birds causes microbial resistance and cross resistance to other microorganisms, which directly affects poultry production. The objective of this study was to evaluate the nutraceutical effect of the *Ganoderma lucidum* fungus on growth performance, relative weight of the visceral organs and the count of cecal lactic acid bacteria of neonatal broilers. **Materials and Methods:** Three experimental treatments were applied: a basal diet (T0); T0+0.25% of *Ganoderma lucidum* and T0+0.50% of *Ganoderma lucidum*. **Results:** Supplementation with *Ganoderma lucidum* did not change the initial body weight, viability, feed intake and feed conversion, however, the addition of 0.25% promoted a higher body weight compared to the control. Also, this natural product significantly reduced ($p<0.05$) the relative weight of the liver, thymus, small intestine and large intestine, without differences ($p>0.05$) for the other organs. In addition, the dietary use of this fungus up to 0.5% did not modify irregular white bacilli, green bacilli with white halo and white coccus. **Conclusion:** Dietary supplementation with 0.25% improved the body weight of the broilers, without modifying the feed intake and feed conversion, as well as modified some digestive and immune organs, although without changes for the count of the cecal lactic acid bacteria.

Key words: Biological response, broiler, cecal pH, fungus, secondary metabolite

Citation: D.S. Molina, J.B. Gutiérrez, O.D. Machado, D. Más and Y. Martínez, 2019. Nutraceutical effect of *Ganoderma lucidum* fungus on neonatal broilers diet. Int. J. Poult. Sci., 18: 641-647.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Due to its great development and flexibility, in comparison with other food production systems of animal origin, the poultry sector is the most important in world livestock[1]. This activity causes to be highly competitive and require the poultry farmer to implement sustainable production systems[2]. In addition, chicken meat is considered one of the most consumed worldwide, because it contains a high nutritional value and lower price compared to other meats[3]. Today, broiler meat production has developed and expanded on a large scale, which covers all region and different climates in Latin America, due to its high adaptability, profitability, market approval and easy access of finding chickens with good genetic expression and high productivity[4].

To reach this food efficiency, since the 50s of the past centuries, antibiotics have been used as growth promoters in bird diets, although many countries restrict their sub-therapeutic use, in Latin America these preventive antibiotics are of frequently used, which causes microbial resistance, cross resistance to other microorganisms and directly affects animal welfare and environmentally friendly productions[5]. Moreover, many studies associated the consumption of meat from animals that use daily dietary antibiotics to microbial resistance in humans. The future premise of poultry producers is to provide the modern consumer with food safety and nutritional quality. In addition, many countries have proposed policies for the substitution or reduction of the use of these antibiotics in the diets of farm animals[6].

In recent years, research have shown that the use of nutraceutical products such as probiotics, prebiotics, phytobiotics and acidifiers may be an effective alternative to the indiscriminate use of sub-therapeutic antibiotics[7]. In this sense, fungi have been one of the most viable alternatives, according to Bederska-Łojewska *et al.*[8] fungi such as *Lentinula edodes*, *Agaricus bisporus*, *Agaricus blazei*, *Hericium caput-medusae*, *Pleurotus ostreatus*, *Pleurotus eryngii*, *Fomitella fraxinea*, *Flammulina velutipes*, *Cordycepsinensis*, *Cordyceps militaris* and *Ganoderma lucidum* are the most researched and consumed worldwide.

Specifically, *Ganoderma lucidum* commonly called Reishi or Linghzi, is a type of mushroom (fungus) used, researched and analyzed in humans and animals. Wadt *et al.*[9] identified that this fungus contains triterpenes, saponins, steroids, alkaloids, ganodic acid and β-glucans, which consumes the immune system and contributes to the detoxification of the organism[10]. Studies in birds showed that the alcoholic extract of this fungus used at a dose of 18 mg kg^{-1} BW^{-1} improved feed conversion and viability[11]. Also, Liu *et al.*[7] reported that *G. lucidum* decreases the damage caused by mycotoxins in broilers. To our knowledge, few studies have been developed to demonstrate adequate levels of this fungus in apparently healthy neonatal broilers, being the most critical stage of these animals. This research work was carried out with the objective of evaluating the effect of the dietary supplementation of the *Ganoderma lucidum* fungus on the growth performance, relative weight of the organs and quantification of the lactic acid bacteria of neonatal chickens.

MATERIALS AND METHODS

The study was carried out in June 2019 at the Poultry Research and Teaching Center of the Pan-American Agricultural School, Zamorano, located in Valle del Yeguare at km 32 of the Tegucigalpa road to Danlí. The place is 800 m above sea level with an average temperature of 26°C.

A total of 720 chickens of the Ross 308® genetic line were placed for 10 days according to a completely randomized design with three dietary treatments, five repetitions for each treatment and 48 birds per repetition. Dietary treatments consisted of:

- T0: Basal diet for broilers without additive
- T1: Basal diet for broilers+0.25% of *Ganoderma lucidum*
- T2: Basal diet for broilers+0.50% of *Ganoderma lucidum*

The well-developed fungus was taken in eco-trails of the Pan-American Agricultural School, Zamorano and was identified in the mycology laboratory of that university. Then, it was crushed to 1 mm in a Thomas Wiley® model 02 parallel blade electric mill. The samples were stored at room temperature, in tightly closed bags for 30 days[12]. In the feed mill, the fungus was mixed together with the premix of minerals and vitamins. The results of Aroche *et al.*[13] were considered to select the levels of addition as nutraceutical. The diets were formulated according to the requirements reported by the Ross 308® parenting manual (Table 1).

Each repetition consisted of a room with a deep wood chip bed and 11 birds m^{-2}. Feed and water were offered *ad libitum* in hopper feeders and nipple drinkers, respectively. The temperature and ventilation inside the house were controlled by gas heaters, curtain handling and fans. No medications or therapeutic veterinary care were used during the entire experiment. The chickens were vaccinated against Marek and Smallpox (first day).

The viability was determined by live animals among those existing at the beginning of the experiment. The feed conversion was calculated as the amount of feed ingested, for a gain of 1 g of body weight (BW). The initial and final weight was carried out individually on the first day and at 10 days of age, on a Mettler Toledo® IND226 industrial balance with accuracy ±1.00 g, respectively. Cumulative feed intake (F1) was calculated daily using the offer and rejection method.
At 10 days of age, 10 birds/fasting treatment for 6 hours were sacrificed by the bleeding method in the jugular vein, followed by the viscera (liver and heart), immune organs (thymus, spleen and Bursa of Fabricius) and the intestines (small and large) and weighed using a digital scale Truweigh blaze digital SCALE BL-100-01-BK with accuracy ±0.1 g.

After the sacrifice, the pH of the small intestine and left cecum of 10 birds/fasting treatment was determined using an Oakton® digital pH potentiometer model 700, calibrated with pH buffer solutions at 1.68, 4.01, 7.00, 10.01 and 12.45.

During the sacrifice, the left cecum of 10 birds/treatment was taken and a mucosal scraping was done with a scalpel for microbiological culture. The cecal content of each sample (1 g), was deposited in a tube containing 9 mL of sterile peptone water (Cultimed Parnreac-Química-SAU), homogenized in distilled water at a rate of 1/10 (w/v) and from it serial dilutions (1/10) were made until dilution 10^6. From each dilution 0.1 mL was taken and seeded in Petri dishes with MRS agar (Difco Laboratories, Detroit, Mich.) and pH 5.6 at 37°C for 48 h in anaerobiosis (Gas Pak system, BBL, Cockeysville, USA). For the determination of lactic acid bacteria; three repetitions were performed for each dilution; subsequently, the visual count of the colonies was performed. The tests were conducted in the microbiology laboratory of the Pan-American Agricultural School, Honduras.

The data were processed by one-way analysis of variance (ANOVA) of simple classification in a completely randomized design, before carrying out the analysis of variance we proceeded to verify the normality of the data by the Kolmogorov Smirnov test and for the uniformity of the variance, Bartlett's test, where necessary, the Duncan's test[14] was used to determine the differences between means, according to the statistical software SPSS version 17.1[15]. Differences of $p<0.05$ were considered statistically significant.

Table 1: Ingredient and contributions of the diet (1-10 days)

Ingredients (%)	Basal diet
Cornmeal (CP, 7.79%)	46.91
Soymeal (CP, 48.00%)	39.67
Mineral and vitamin premixes[1]	0.50
Sodium chloride	0.50
Soy oil	8.20
Choline	0.08
DL-Methionine	0.37
L-Threonine	0.10
L-Lysine	0.27
Calcium carbonate	1.10
Monocalcium phosphate	1.63
Mycofix plus® 5.0	0.12
Lumis Lbzyme® X50 Enzymes	0.50
Coccidiostat	0.05
Nutritional contributions (%)	
Metabolizable energy (kcal kg^{-1} DM)	3000.00
Crude protein	23.43
Crude fiber	3.28
Ashes	6.35
Ca	0.96
P available	0.48
Lysine	1.28
Methionine+cystine	0.95
Threonine	0.67
Valine	0.89
Isoleucine	0.79
Leucine	1.71
Histidine	0.65
Arginine	1.30
Tryptophan	0.23
Phenylalanine	0.93

[1]Each kg contains; Vitamin A: 13,500 IU, Vitamin D3: 3,375 IU, Vitamin E: 34 mg, B2: 6 mg, Pantothenic acid: 16 mg, Nicotinic acid: 56 mg, Cu: 2,000 mg, Folic acid: 1.13 mg, Vitamin B12: 34 mg, Mn: 72 mg, Zn: 48 mg

RESULTS AND DISCUSSION

Table 2 shows the growth performance of newborn broilers fed diet supplemented with *Ganoderma lucidum* powder. Initial body weight, viability, feed intake and feed conversion ratio did not indicate statistical differences ($p>0.05$) between experimental treatments. However, dietary supplementation with 0.25% of *Ganoderma lucidum* significantly increased ($p<0.05$) the body weight of neonatal broilers (up to 10 days) in relation to the control treatment.

One of the main objectives of our study was to verify if this natural product rich in secondary metabolites could improve the body weight of broilers, (especially in the first days of life), being the main justification to replace

Table 2: Effect of dietary supplementation with *Ganoderma lucidum* powder on growth performance of broilers (1-10 days)

	Experimental treatments				
Items	T0	T1	T2	SEM±	p-value
Initial BW(g)	45.58	46.69	47.41	0.673	0.197
Final BW (g)	244.54^{b}	258.17^{a}	250.63^{ab}	3.155	0.005
Weight gain (g)	198.93^{b}	211.49^{a}	203.22^{ab}	3.580	0.048
Viability (%)	97.92	97.50	97.50	1.605	0.978
Feed intake (g)	248.75	253.04	247.10	10.679	0.921
FCR	1.25	1.20	1.22	0.046	0.712

^{a,b}Means with different letters in the same row differ to $p<0.05$, T0: Control, T1: 0.25% of *Ganoderma lucidum*, T2: 0.50% of *Ganoderma lucidum*, FI: Feed intake, FCR: Feed conversion ratio sub- therapeutic antibiotics in the diets of the chickens. In this sense, the use of 0.25% of *G. lucidum* improved in 14 g the body weight compared to the basal diet until 10 days of age. According to Jayachandran *et al.*[16] this fungus has a high content of polysaccharides, especially β-glucans and other beneficial secondary metabolites with anti-inflammatory, immuno-stimulant, antimicrobial and antioxidant effects, which favors the animal's feed efficiency[17,18].

Note that, to our knowledge, few studies have been developed to know the nutraceutical effects of the *G. lucidum* fungus on the diets of non-ruminant animals, especially chickens. Studies in humans have shown that this fungus due to its chemical characteristics induces the cell cycle and apoptosis to reduce some types of cancer[19], because they are rich sources of polysaccharides. In an *in vitro* study it was found that the purified polysaccharides of *Ganoderma* spp. have antioxidant activity by the elimination of 1,1-diphenyl-2-picryyl-hydrazyl free radicals (DPPH), being similar to standard such as vitamin C and BHT[20].

In this sense, Liu *et al.*[7] reported that *G. lucium* reduced the negative effect of aflatoxin B1 in chickens, due to the increase in total antioxidant capacity, catalase, glutathione peroxidase and hydroxyl radical scavenging activity in the liver and spleen of broilers. Also, Willis *et al.*[21] stated that this fungus increased the excretion of parasitic oocysts and improved body weight in broilers. In addition, Ogbe and Affiku[11] had recommended the mixed use of *Moringa oleifera* and *G. lucidum* to promote a better productive response, without affecting the hematological indicators and the edible portions in broilers. In addition, this natural product did not cause a higher mortality (Table 2), confirming that the use of this non-toxic fungus is harmless, especially in young birds, due to the high susceptibility of these animals.

Table 3 shows that dietary supplementation of *G. lucidum* powder did not cause significant changes (p>0.05) for the relative weight of the pancreas, heart, bursa of Fabricius, spleen and cecal pH. However, this natural product (*G. lucidum*) increased (p<0.05) the body weight at slaughter, although it significantly reduced (p<0.05) the relative weight of the liver. In addition, dietary supplementation with 0.25% of *G. lucidum* significantly reduced (p<0.05) the relative weight of the small and large intestine in relation to T0 and T1, respectively. Also, T2 caused a reduction in the relative weight of the thymus (p<0.05) compared to T0 and T1.

As shown in Table 2, the *G. lucidum* fungus increased the body weight, however, this may not be related to a greater enzymatic activity of the viscera, since the relative weight of the liver decreased and the relative weight of the heart and pancreas was not different by effect of the natural product. Results of Aguilar *et al.*[22] found that the dietary use of a natural product rich in secondary metabolites modified the relative weight of the digestive, visceral and immune organs. Our results showed that higher supplementation of this fungus (0.5%) rich in chemical bioactives decreased the relative weight of the thymus. Note that despite the variability of the relative weight of the organs, the animals had no apparent conditions.

According to Fang *et al.*[23], an increase in the relative weights of the thymus and the bursa of Fabricius could be associated with a more active immune system in birds. However, studies with phytochemicals in birds have found that lymphoid organ growth is sometimes not related to a better experimental treatment[22]. On the other hand, it is known that an excess of secondary metabolites causes symptoms related to anti-nutritional factors[12], which can affect the immune system of the birds, it seems that the supplementation with 0.5% of *G. lucidum* did not promote a higher body weight, perhaps related to a greater incorporation of these secondary metabolites. In turn, an excessive increase in the activity of viscera such as the spleen, liver and pancreas could depress growth performance, which decreases the organic and enzymatic function of birds. In the case of the small intestine, a decrease in relative weight

Table 3: Effect of dietary supplementation with *Ganoderma lucidum* powder relative weight of digestive, visceral and immune organs in broilers (10 days)

	Experimental treatments				
Items	T0	T1	T2	SME±	p-value
BW at sacrifice (g)	233.20[b]	247.00[a]	246.00[a]	1.515	<0.001
Liver (g kg^{-1} de BW)	3.83[a]	3.16[b]	3.32[b]	0.272	0.028
Pancreas (g kg^{-1} de BW)	0.43	0.48	0.46	0.034	0.500
Heart (g kg^{-1} de BW)	0.80	0.79	0.72	0.046	0.378
Bursa of Fabricius (g kg^{-1} de BW)	0.14	0.18	0.17	0.018	0.322
Thymus (g kg^{-1} de BW)	0.87[a]	0.80[a]	0.50[b]	0.011	0.035
Spleen (g kg^{-1} de BW)	0.13	0.10	0.14	0.021	0.107
Small intestine (g kg^{-1} de BW)	10.76[a]	8.90[b]	10.01[ab]	0.440	0.021
Large intestine (g kg^{-1} de BW)	1.09[a]	0.88[b]	1.10[a]	0.073	0.043
Cecal pH	5.73	6.14	6.45	0.259	0.199

[a,b]Means with different letters in the same row differ to p<0.05, T0: Control, T1: 0.25% of *Ganoderma lucidum*, T2: 0.50% of *Ganoderma lucidum*

Table 4: Effect of dietary supplementation with *Ganoderma lucidum* powder on the count of cecal lactic acid bacteria in broilers (10 days)

	Experimental treatments				
Lactic acid bacteria (CFU g^{-1})[1]	T0	T1	T2	SEM±	p-value
Bacilli[2]	5.75	4.00	4.19	0.644	0.165
Bacilli[3]	6.84	5.30	5.89	0.468	0.115
Cocci[4]	7.23	6.42	6.36	0.462	0.374

[1]The count was expressed as Log10, [2]Irregular white, [3]Green with white halo, [4]White. T0: Control, T1: 0.25% of *Ganoderma lucidum*, T2: 0.50% of *Ganoderma lucidum*

in T1 could be related to greater digestibility and reduced retention of nutrients at an early age in broilers, which represented a greater body weight[24] (Table 2).

The decrease in intestinal pH is determined by the increase in the colonization of lactic acid bacteria (LAB) such as *Lactobacillus* and *Bifidobacterium* in GIT, which increases the production of volatile fatty acids (VFA), which emit protons and acidify the intestine[25]. The use of up to 0.50% of fungi in fattening diets did not increase the proliferation of LAB in such a way that it modified the intestinal pH ($p<0.05$), as observed in Table 3. In young birds, there are some contradictions on the effect of phytochemicals on intestinal pH, mainly due to the late proliferation of LAB and because an excess of these chemical compounds causes metabolic alterations; therefore, their effects will depend on the concentration of these secondary metabolites in the biological material and their supplementation in the diet.

Table 4 shows the count of cecal lactic acid bacteria in newborn broilers. The use of the fungus *Ganoderma lucidum* did not modify statistically ($p<0.05$) irregular white bacilli, green bacilli with white halo and white coccus. However, a non-significant reduction in the cecal beneficial bacterial population is observed.

Although, the results obtained in the trial do not reflect notable changes ($p>0.05$) on the count of cecal lactic bacteria that grew in MRS broth, a possible mitigation of both Bacilli and Coccus is observed in the treatments with the supplementation of *G. lucidum* (T1 and T2), apparently this product modifies bacterial growth. These results differ from those obtained by Khan *et al.*[26], these authors indicate that the prebiotic properties of mushrooms increase the count of bacteria such as *Lactobacillus* and *Bifidobacterium* (lactic acid bacteria).

On the other hand, in the first days of life, chickens are exposed to various stressful conditions such as weather, pathogenic bacteria and management that cause intestinal inflammation, mainly postprandial that depresses the animal response[23]. Currently, the scientific community is discussing the main mode of action of natural products. Some research has reported that positive results are due to its anti-inflammatory properties that decrease inflammation of the small intestine and increase the absorption of nutrients[27], other studies have shown that inclusions of products rich in secondary metabolites reduce the proliferation of pathogenic bacteria in gastrointestinal tract, which increases gut health and promotes greater digestibility of nutrients and animal response[28]. Apparently, the beneficial effect of this fungus could be related to the intestinal anti-inflammatory effect due to the in vitro results corroborated by O'Brien and Sali[29], however, more studies are needed to corroborate this hypothesis.

However, the uses of secondary metabolites currently gain more territory when addressing challenges during the production process in the poultry industry[30]. One of its main causes would be the negative impact on consumers of meat or poultry products due to residual effects, which has led to the prohibition of the use of antibiotics as growth promoters since 2006 by the European Union[11]. Thus, the *Ganoderma lucidum* fungus could be in an excellent nutraceutical and sustainable alternative in the world's poultry production.

CONCLUSION

Dietary supplementation with 0.25% of the *Ganoderma lucidum* fungus improved body weight and weight gain, without modifying other productive indicators. Also, dietary use up to 0.5% decreased the relative weights of some organs, such as the liver, thymus and intestines and this natural product did not modify the count ofcecal lactic acid bacteria in broilers.

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