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Original Article

Diets with Ganoderma lucidum Mushroom Powder and Zinc-Bacitracin on Growth Performance, Carcass Traits, Lymphoid Organ Weights and Intestinal Characteristics in Broilers

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ABSTRACT

In order to evaluate the effect of diets with *Ganoderma lucidum* mushroom powder and zinc-bacitracin on growth performance, carcass traits, lymphoid organ weights, and intestinal characteristics in broilers, a total of 600 one-day-old unsexed broilers from Cobb 500 MV × Cobb 500 FF genotype was analyzed for 28 days, following a completely randomized design with three dietary treatments, five replicates and 40 birds per replicate. The dietary treatments consisted of a basal diet (BD) without additives (T0) and the dietary inclusion of 2.5 g/kg of *Ganoderma lucidum* (T1) and 350 mg/kg of zinc bacitracin antibiotic (T2). The experimental groups did not change (p>0.05) the performance of the broilers. However, *G. lucidum* powder increased (p≤0.05) the carcass and breast yields (p≤0.05) and decreased the abdominal fat and liver yields (p≤0.05), although with no notable differences with the antibiotic group for the latter organ (liver) (p>0.05). Both additives (*G. lucidum* and antibiotic) increased breast meat moisture, protein, and redness; however, these treatments reduced L^* (lightness), and the zinc-bacitracin reduced breast yellowness (p≤0.05). Likewise, this medicinal mushroom (*G. lucidum*) increased the relative weight of bursa of Fabricius and the morphometry of the small intestine (p≤0.05), although with no changes for other immune and digestive organs or for the content of cecal lactic acid bacteria (p>0.05). The dietary inclusion with 2.5 g/kg of *Ganoderma lucidum* powder is recommended to improve breast yield, protein, and colorimetry without affecting performance and cecal traits of fast-growing broilers.

INTRODUCTION

Many countries have banned the use of growth-promoting antibiotics following their total elimination in the European Union on January 1, 2006. However, according to the World Organization for Animal Health (2019), 45 countries still commonly use these synthetic products in broiler diets. It is known that the daily use of growth promoting antibiotics causes microbial resistance, cross resistance to other microorganisms, and their bioaccumulation in muscles (Xiong *et al.*, 2018). Although many producers are against reducing or eliminating subtherapeutic antibiotics in chicken production, especially to control mortality and improve feed conversion ratio (Martínez *et al.*, 2021), many companies market natural products capable of partially or totally replacing these antibiotics from an early age.

Apparently, the best natural alternatives to eliminate the indiscriminate use of preventive antibiotics in birds have been probiotics, prebiotics, phytobiotics, and acidifiers (Grashorn *et al.*, 2010; Al-Khalaifa *et al.*, 2019; Zhang *et al.*, 2020). These products improve gut health by modulating the host's anti-inflammatory, antimicrobial, immune, and antioxidant response, which improves nutrient absorption and birds genetic expression (Grashorn *et al.*, 2010). Many mushrooms have been used within the context of traditional Chinese medicine for their antimicrobial, antitumor, antiatherosclerosis, antiinflammatory, hypolipidemic, antidiabetic, antioxidant, and antifungal effects. The most representative are *Lentinula* spp., *Agaricus* spp., *Hericium* spp., *Pleurotus* spp., *Fomitella* spp., *Flammulina* spp., *Cordyceps* spp. and *Ganoderma* spp. (Bederska-Łojewska *et al.*, 2017).

Ganoderma lucidum, a mushroom that is highly adaptable to different latitudes, is considered a rich source of secondary metabolites such as polysaccharides, triterpenes, saponins, steroids, alkaloids, ganodic acid, and β-glucans and both its extracts and spores have shown positive effects on bird viability and feed conversion ratio (Ogbe & Affiku, 2012). Furthermore, Ogbe *et al.* (2009) found that the aqueous extract of wild mushrooms (*Ganoderma lucidum*) reduced the number of fecal oocytes of *Eimeria tenella* in broilers and Liu *et al.* (2016) indicated a better performance when they used *Ganoderma lucidum* spores in broilers exposed to low levels of aflatoxin B1. In a previous study, Molina *et al.* (2019) demonstrated that dietary supplementation with 0.25% of *Ganoderma lucidum* powder improved growth performance, although with no changes for cecum traits.

To our knowledge, few studies have been conducted comparing the oral use of *Ganoderma lucidum* powder and an antimicrobial growth promoter that is frequently used in broiler diets, which will allow to verify whether this natural product could totally replace these synthetic products. This research work was carried out with the aim of evaluating the effect of diets with *Ganoderma lucidum* mushroom powder and zinc-bacitracin on growth performance, carcass traits, lymphoid organ weights, and intestinal characteristics in broilers.

MATERIALS AND METHODS

Study location

The study was carried out during February and March, 2020, at the Poultry Research and Teaching Center of Zamorano University, located 30 km southeast of Tegucigalpa, San Antonio de Oriente, Francisco Morazán department, Honduras. The annual average temperature is 24 °C and the average precipitation is 1100 mm per year.

Collection and chemical analysis of *G. lucidum*

2 kg of *G. lucidum* mushroom were collected in the Uyuca ecological reserve, San Antonio de Oriente, Honduras. The mushroom was deposited and stored in a fiber bag to avoid the humidity increases due to transpiration during transportation to the preparation area (Aroche *et al.*, 2018). The fungal samples were identified in the plant pathology lab of Zamorano University, Honduras. The mushroom was cleaned with distilled water to remove impurities. Afterwards, it was placed on a wide and dry paper-protected surface and allowed to naturally dry indoors for 5 days. Once dry, the mushrooms were transported to the Soil Laboratory of Zamorano University, Honduras and ground using a Thomas Wiley® model 02 parallel blade mill. The powder was stored in airtight bags with low humidity and temperature. In the feed mill, the mushroom was mixed together with the premix of minerals and vitamins (Molina *et al.*, 2019).

The following variables were analyzed to establish the chemical composition of the mushroom powder: dry matter, crude protein, ash, N, Ca, Mg, K, Cu, Fe, Mn, and Zn, according to AOAC 2001.11. (2006), and P was determined using the molybdenum blue colorimetry method. We had also found presence of alkaloids, coumarins, tannins, non-protein amino acids, flavonoids, sugars, triterpenes, and catechins; as well as the non-absence of saponins, resins, and mucilages (Martínez *et al.*, 2021a).

Experimental design, animals, and treatments

A total of 600 one-day-old unsexed chickens from Cobb 500 MV × Cobb 500 FF genotype were studied for 28 days according to a completely randomized design with three dietary treatments, five replicates, and 40 birds per replicate. The dietary treatments consisted of a basal diet (BD) without additives (T0), the dietary inclusion of 2.5 g/kg of *Ganoderma lucidum* (T1), and 350 mg/kg of Zinc bacitracin antibiotic (T2).

Table 1 – Chemical composition of *Ganoderma lucidum* powder (Dry matter).

Chemical composition	Means	Standard deviation	Coefficient of variation
Dry matter (%)	10.6	0.435	4.112
Crude protein (%)	9.81	0.216	2.206
Ash (%)	2.83	0.152	5.391
N (%)	1.57	0.034	2.206
Ca (%)	0.32	0.026	8.267
Mg (%)	0.186	0.015	8.183
K (%)	0.49	0.015	3.075
P (%)	0.10	0.005	5.587
Cu (mg/kg)	5.01	0.032	0.641
Fe (mg/kg)	168.66	13.051	7.737
Mn (mg/kg)	39.00	4.58	11.75
Zn (mg/kg)	32.67	3.511	10.750

The results of Molina *et al.* (2018) were considered to select the inclusion level of the fungus in this study. The ingredients and nutritional contributions of the diets are shown in table 2, 3, and 4.

Table 2 – Ingredients and nutritional contributions of broiler diets (0-8 days).

	Experimental treatments		
Ingredients (%)	Control	*G. lucidum*	Antibiotic
Cornmeal	50.13	49.88	50.095
Soybean meal	39.47	39.47	39.47
Mineral and vitamin premix	0.50	0.50	0.50
Sodium chloride	0.50	0.50	0.50
African palm oil	5.70	5.70	5.70
Choline	0.05	0.05	0.05
DL-methionine	0.38	0.38	0.38
L-threonine	0.10	0.10	0.10
L-lysine	0.25	0.25	0.25
Calcium carbonate	1.13	1.13	1.13
Biofos	1.57	1.57	1.57
Mycotoxin sequestrants	0.12	0.12	0.12
Multienzymes	0.05	0.05	0.05
Coccidiostat	0.05	0.05	0.05
Ganoderma lucidum	0.00	0.25	0.00
Zn bacitracin	0.00	0.00	0.035
Composición proximal (%)			
Metabolizable energy (kcal/kg)	3000	3000	3000
Crude protein	23.43	23.43	23.43
Crude fiber	2.59	2.59	2.59
Neutral fiber detergent	9.56	9.56	9.56
Crude fat	8.64	8.64	8.64
Ca	0.96	0.96	0.96
p available	0.48	0.48	0.48
Lysine	1.28	1.28	1.28
Methionine + cystine	0.95	0.95	0.95
Threonine	0.86	0.86	0.86
Valine	0.91	0.91	0.91
Isoleucine	0.80	0.80	0.80
Arginine	1.30	1.30	1.30
Tryptophan	0.24	0.24	0.24

[1]Each kg contains: vitamin A 11,550 IU, vitamin D3 4,300 IU, vitamin E 27.5 IU, vitamin K3 3.85 mg, vitamin B1 2.75 mg, vitamin B2 9.9 mg, vitamin B6 3.85 mg, vitamin B12 22.0 Mcg, niacin 49.5 mg, pantothenic acid 15.4 mg, folic acid 1.38 mg, biotin 166 Mcg; selenium 0.09 mg, iodine 0.18 mg, copper 3.00 mg, iron 36.0 mg, manganese 54.0 mg, zinc 48.0 mg, cobalt 0.12 mg.

Experimental conditions

Each replicate consisted of pens that were randomly distributed within the house, using a deep wood chip bed and 11 birds/m^2. Feed and water were offered *ad libitum* in hopper feeders and nipple waterers, respectively. The photoperiod distribution proposed by Cobb-Vantress (2018) was used, as follows: 0–7 days of age, 23L:1D; 8–28 days, 20L:4D. The temperature and ventilation inside the house were controlled by gas brooders, curtain management, and fans. The shed was disinfected according to environmental quality standards of Poultry Research and Training Center Protocol: the experimental area was disinfected with quaternary ammonium (5%) 24 h before chicks entered it. No medications or therapeutic veterinary care were administrated throughout the experimental stage. Birds were vaccinated against infectious bronchitis, Newcastle disease, and Gumboro diseases at birth.

Table 3 – Ingredients and nutritional contributions of broiler diets (9-18 days).

	Experimental treataments		
Ingredients (%)	Control	*G. lucidum*	Antibiotic
Cornmeal	62.47	62.22	62.435
Soybean meal	30.48	30.48	30.48
Mineral and vitamin premix	0.50	0.50	0.50
Sodium chloride	0.50	0.50	0.50
African palm oil	2.79	2.79	2.79
Choline	0.05	0.05	0.05
DL-methionine	0.30	0.30	0.30
L-threonine	0.08	0.08	0.08
L-lysine	0.27	0.27	0.27
Calcium carbonate	1.09	1.09	1.09
Biofos	1.25	1.25	1.25
Mycotoxin sequestrants	0.12	0.12	0.12
Enzymes	0.05	0.05	0.05
Coccidiostat	0.05	0.05	0.05
Ganoderma lucidum	0.00	0.25	0.00
Zn bacitracin	0.00	0.00	0.035
Metabolizable energy (kcal/kg)	3100	3100	3100
Crude protein	20.00	20.00	20.00
Crude fiber	2.50	2.50	2.50
Ca	0.84	0.84	0.84
P available	0.42	0.42	0.42
Lysine	1.12	1.12	1.12
Methionine+cystine	0.85	0.85	0.85
Threonine	0.73	0.73	0.73
Isoleucine	0.68	0.68	0.68
Arginine	1.18	1.18	1.18
Tryptophan	0.21	0.21	0.21

[1]Each kg contains: vitamin A 11,550 IU, vitamin D3 4,300 IU, vitamin E 27.5 IU, vitamin K3 3.85 mg, vitamin B1 2.75 mg, vitamin B2 9.9 mg, vitamin B6 3.85 mg, vitamin B12 22.0 Mcg, niacin 49.5 mg, pantothenic acid 15.4 mg, folic acid 1.38 mg, biotin 166 Mcg; selenium 0.09 mg, iodine 0.18 mg, copper 3.00 mg, iron 36.0 mg, manganese 54.0 mg, zinc 48.0 mg, cobalt 0.12 mg.

Growth Performance

The indicators of the broiler's growth performance were determined weekly. Viability was determined by counting living animals as compared to those existing at the beginning of the experiment. Feed intake was calculated using the offer-and-reject method. Feed conversion ratio was calculated as the amount of feed ingested for a 1kg body weight (BW) gain. The initial and final individual weight of each stage was measured using a Mettler Toledo IBOA224-15NP (Jiangsu, China) industrial scale with an accuracy of ±2 g.

Table 4 – Ingredients and nutritional contributions of broiler diets (19-28 days).

	Experimental diets		
Ingredients (%)	Control	*G. lucidum*	Antibiotic
Cornmeal	64.03	63.78	63.995
Soybean meal	28.33	28.33	28.33
Mineral and vitamin premixes	0.50	0.50	0.50
Sodium chloride	0.50	0.50	0.50
African palm oil	3.75	3.75	3.75
Choline	0.05	0.05	0.05
DL-methionine	0.26	0.26	0.26
L-threonine	0.05	0.05	0.05
L-lysine	0.22	0.22	0.22
Calcium carbonate	1.02	1.02	1.02
Biofos	1.07	1.07	1.07
Mycotoxin sequestrants	0.12	0.12	0.12
Enzymes	0.05	0.05	0.05
Coccidiostat	0.05	0.05	0.05
Ganoderma lucidum	0.00	0.25	0.00
Zn bacitracin	0.00	0.00	0.035
Metabolizable energy (kcal/kg)	3100	3100	3100
Crude protein	19.00	19.00	19.00
Crude fiber	2.45	2.45	2.45
Ca	0.76	0.76	0.76
P available	0.38	0.38	0.38
Lysine	1.02	1.02	1.02
Methionine+cystine	0.57	0.57	0.57
Threonine	0.66	0.66	0.66
Arginine	1.04	1.04	1.04
Tryptophan	0.19	0.19	0.19

[1]Each kg contains: vitamin A 11,550 IU, vitamin D3 4,300 IU, vitamin E 27.5 IU, vitamin K3 3.85 mg, vitamin B1 2.75 mg, vitamin B2 9.9 mg, vitamin B6 3.85 mg, vitamin B12 22.0 Mcg, niacin 49.5 mg, pantothenic acid 15.4 mg, folic acid 1.38 mg, biotin 166 Mcg; selenium 0.09 mg, iodine 0.18 mg, copper 3.00 mg, iron 36.0 mg, manganese 54.0 mg, zinc 48.0 mg, cobalt 0.12 mg.

Carcass traits

At 28 days of age, 10 broilers (5 males and 5 females) were selected for each experimental replicate, totaling 50 broilers per treatment. The broilers fasted for 6 h. The average BW of each treatment was considered to randomly select the broilers for slaughter. Broilers were euthanized by a certified veterinarian using the mechanical cervical dislocation (stunning) method, and the exsanguination technique was used once the birds were unconscious, the. The carcasses were suspended by the legs for 2 min during bleeding. Carcasses were then immersed in a scalder at 60°C with a water flow of 1 L/bird/min for 3 minutes. Afterwards, a circular plucker was used for 10 s. Evisceration was carried out manually. To determine the relative weight of edible portions, the broilers were weighed before slaughter on a Mettler Toledo IBOA224-15NP scale (Jiangsu, China) with an accuracy of ±2 g. Immediately after evisceration, carcass, liver (without gallbladder), heart, leg, gizzard (without content), bone-in breast, and abdominal fat were weighed. Subsequently, 6 breast meat samples (250 g) were randomly taken for each treatment from 3 male and 3 female broilers. The samples were then stored in plastic bags at a temperature of −15°C until laboratory analysis.

The chemical composition and colorimetry of breasts were determined for the previously selected samples (6 replicates per treatment) at the Zamorano Food Laboratory (LAAZ). The skinless breast meat was thawed and ground to a paste with a homogenizing blender. The content of moisture, ashes, fat, and protein in the samples was then prescribed, as per the methodology described by AOAC (2006). Furthermore, colorimetry in raw and skinless thawed breast meat was determined (6 samples for each treatment). Five grams of muscle pieces were placed inside the equipment per duplicate. Coordinates L^* (luminosity), a^* (red index), and b^* (yellow index) were evaluated using a sphere spectrophotometer Minolta CR-400/410 Chroma Meter (Konica Minolta Sensing Inc., Osaka Japan).

Absolute and relative weight of the digestive and immune organs and pH cecal

At the moment of broiler slaughter, the thymus, spleen, bursa of Fabricius, small intestine, and cecum were extracted and weighed using a BLAZE® BL scale with a precision ±0.01 g (100-01-BK scale, USA). Afterwards, the relative weight of the organs was calculated considering body weight at slaughter. In addition, the length of the small intestine and cecum was determined using a tape measure (Stanley Hand Tools 33-42, USA).

Cecal lactic acid bacteria

The left ceca of five birds/treatment were randomly picked and had their mucosa scraped with a scalpel for microbiological culture. Each sample's cecal content was placed in a sterile tube; weight was recorded and diluted with Butterfield's phosphate-buffered dilution water to a 1:9 ratio (w:v). Diluted cecal contents were homogenized, and serial dilutions (1/10) were made from it until the dilution of 10^5. Aliquots of 0.1 ml of each dilution were spread plated on the surface of MRS agar (Neogen Acumedia, Mich.) supplemented with methylene blue (0.016 g/1000 ml) at 37 °C with a pH of 5.6 for 48 hours in anaerobiosis (Gas Pak system, BBL, Cockeysville, USA). Lactic acid bacteria counts were reported as Log 10 CFU/g by colonies morphology on MRS + MB agar. Gram stain and catalase activity were tested for each type of colony, as reported by Molina *et al.* (2019). A Labomed model LX400 light microscope (California, USA) was used for the morphological characterization of bacterial colonies. Microbiological tests were performed in the Food Microbiology Laboratory of Zamorano University.

Statistical analysis

The data were processed by one-way analysis of variance (ANOVA) using a completely randomized design. Before this doing so, the normality of the data was verified using the Kolmogorov Smirnov test, the uniformity of the variance using Bartlett's test and, where necessary, Duncan's test was used to determine the differences between means ($p<0.05$). All analyzes were conducted using the statistical software SPSS, version 23.0.

RESULTS AND DISCUSSION

The effect on broilers (0-28 days) growth performance of the dietary inclusion of *G. lucidum* as compared to a diet without additive and growth-promoting antibiotics ($p<0.05$) is shown in table 5. No statistical differences were observed for body weight, feed intake, feed conversion ratio, or mortality in the productive stages ($p>0.05$).

The experimental treatments (*G. lucidum* and antimicrobial growth promoter) had no direct effect on broilers main productive indicators (Table 5). In this sense, Chen *et al.* (2020) did not find improvements in body weight and feed intake when they used an extract of *Ganoderma lucidum* on broiler diets, despite the fact that this natural product increased antibody titers and improved gut heath. According to Martínez *et al.* (2021b), natural or synthetic growth promoters have a better productive response in broilers when zootechnical conditions are not adequate. Apparently, the experimental conditions allowed the expression of broiler's genetic potential, considering that mortality (2 to 3%) and body weight were similar to those stipulated by Cobb-Vantress (2018) for this genetic line (Cobb 500).

Also, the results show that despite the fact that this mushroom has high concentrations of polysaccharides (especially β-glucans), alkaloids, and other secondary metabolites (Bidegain *et al.*, 2020; Gong *et al.*, 2020), its dietary use in small concentrations (0.25%) does not cause morbidity and mortality in broiler throughout their productive stage. Similar results in mortality were reported by Ogbe & Affiku (2012) and Molina *et al.* (2019) when they used *Ganoderma lucidum* extracts and whole powder in broiler diets, respectively. Ni *et al.* (2016) mentioned that high concentrations of secondary metabolites (polysaccharides, alkaloids, and polyphenols) in broiler diets can provoke adverse effects on performance, digestibility, and immune response.

Authors such as Molina *et al.* (2019) have found that dietary supplementation with 0.25% of *Ganoderma lucidum* powder in Ross 308 broilers increased body weight in 5.57%, without reducing feed intake and viability as compared to a diet without additive. These

Table 5 – Effect of diets with *Ganoderma lucidum* mushroom powder and Zinc-bacitracin on broilers' growth performance (0-28 days).

Items	Experimental treatments			SEM±	*P value*
	Control	*G. lucidum*	Antibiotic		
0-8 days					
Body weight (g)	195.67	202.65	196.68	2.653	0.493
Feed intake (g)	176.15	178.55	165.27	6.998	0.481
Feed conversion ratio (kg/kg)	1.18	1.14	1.10	0.042	0.450
Mortality (%)	0.00	0.50	0.50	0.408	0.756
9-18 days					
Body weight (g)	565.06	575.51	570.84	4.587	0.871
Feed intake (g)	540.04	528.47	530.60	10.535	0.083
Feed conversion ratio (kg/kg)	1.46	1.42	1.42	0.025	0.660
Mortality (%)	1.00	1.50	0.50	0.585	0.357
19-28 days					
Body weight (g)	1557.24	1556.80	1560.20	22.215	0.560
Feed intake (g)	1467.20	1494.69	1488.49	17.745	0.732
Feed conversion ratio (kg/kg)	1.48	1.53	1.51	0.050	0.596
Mortality (%)	1.52	1.02	1.01	0.598	0.777
0-28 days					
Feed intake (g)	2183.39	2201.71	2184.37	21.696	0.319
Feed conversion ratio (kg/kg)	1.45	1.46	1.44	0.019	0.850
Mortality (%)	2.00	3.00	2.00	1.080	0.422

results can be associated with the anti-inflammatory and antimicrobial effects of this natural product (Hahn *et al.*, 2006; Jayachandran *et al.*, 2017; Torki *et al.*, 2018; Zolj *et al.*, 2018). The positive results of *Ganoderma lucidum* powder in Ross 308 broiler diets (Molina *et al.*, 2019) may be due to the fact that this genotype suffers more stress and mortality in the initial stage than Cobb 500 broilers (evaluated in the present study). In this sense, El-Tahawy *et al.* (2017) compared the performance of Ross 308 and Cobb 500 broilers, reporting 3.55% more mortality for the first genotype. However, more other studies are necessary to confirm the hypothesis. Moreover, Liu *et al.* (2020) have demonstrated that the oral use of *Ganoderma lucidum* spores improved weight gain, free radical scavenging activity, antioxidant capacity, and broiler's immune function. It is known that due to its saprophytic character, *Ganoderma lucidum* mainly acquires secondary metabolites from the dead wood of flat-leaf trees (Shen *et al.*, 2017). Therefore, its beneficial effect on biological indicators in broilers could be associated with the concentration of secondary metabolites acquired from the plant substrate and the inclusion level of whole powder or extracts in the diets.

Table 6 shows that *G. lucidum* powder increased ($p\leq0.05$) carcass and breast yield and decreased ($p\leq0.05$) abdominal fat yield as compared to the control diet (without additive) and Zinc-bacitracin group. Likewise, both additives decreased ($p\leq0.05$) liver yield as compared to the basal diet. The leg, heart, and gizzard yields did not change ($p>0.05$) due to the effect of the experimental diets. Moreover, *G. lucidum* and antibiotic groups increased ($p\leq0.05$) the contents of moisture, protein, and a^* in breast meat; however, these treatments reduced L^* and fat, and the last group (antibiotic) significantly decreased b^* ($p\leq0.05$). No notable changes ($p>0.05$) were found for ash among experimental groups.

Although *G. lucidum* powder did not change ($p>0.05$) broiler's growth performance (Table 5), this natural product did promote a higher carcass yield, perhaps due to better intestinal health, which allowed for the increase of muscle synthesis. Other authors Farag & El-Rayes (2016) and Martínez *et al.* (2021b) reported similar results in carcass yields when they used small concentrations of natural alternatives rich in beneficial secondary metabolites (mainly tannic, coumarins, polysaccharides, flavonoids, and alkaloids). Azad *et al.* (2013) and Mashayekhi *et al.* (2018) have informed that the positive effects of these secondary metabolites on broiler carcass yields have been related to nutrient absorption, mainly that of essentials amino acids such as lysine, methionine, and threonine.

The scientific community has emphasized the role of *Ganoderma lucidum* polysaccharides in the immune and anti-inflammatory response *in vitro* and *in vivo* (Zhu *et al.*, 2007; Lu *et al.*, 2020). In this context, Xu *et al.* (2011) have reported that this mushroom affects immune cells and cells related to the immune system, including B lymphocytes, T lymphocytes, dendritic cells, macrophages, and natural killer cells. According to Niewold (2007), intestinal inflammation is directly related to a decrease in nutrient absorption and, consequently, poor development. Also, Khadem *et al.*

Table 6 – Effect of dietary supplementation with *G. lucidum* on broilers' carcass traits (28 days).

	Experimental treataments				
Ítems (%)	Control	*G. lucidum*	Antibiotic	SEM±	P value
Yield					
Carcass	68.51[b]	70.76[a]	68.91[b]	0.765	0.005
Breast	21.81[b]	23.01[a]	21.79[b]	0.904	0.030
Leg	10.30	10.54	10.88	0.221	0.199
Abdominal fat	1.24[a]	1.07[b]	1.28[a]	0.107	0.031
Liver	2.23[a]	1.98[b]	2.02[b]	0.072	0.049
Heart	0.65	0.58	0.60	0.031	0.241
Gizzard	1.68	1.80	1.80	0.070	0.400
Chemical composition of breast					
Moisture	70.68[b]	73.12[a]	73.71[a]	2.069	0.006
Protein	22.26[b]	24.85[a]	24.43[a]	0.739	0.049
Fat	7.06[a]	2.03[b]	1.86[b]	1.692	0.027
Ash	0.97	0.95	0.98	0.025	0.739
L^*	53.41[a]	48.62[b]	47.16[b]	0.843	<0.001
a^*	10.29[b]	12.79[a]	13.24[a]	0.512	0.001
b^*	20.85[a]	21.54[a]	17.92[b]	0.592	0.001

[a,b]Means with different letters in the same row differ at ($p\leq0.05$). L^*: lightness; a^*: redness; b^*: yellowness.

(2014) and Soler *et al.* (2016) have reported that natural products rich in secondary metabolites (mainly alkaloids) modulate the postprandial inflammatory response due to a reduction of the jejunal mucosal expression of inducible NO synthase and the concentration of IL8, IL -1β, and TNF-α, respectively. Therefore, the dietary inclusion of *Ganoderma lucidum* powder could reduce intestinal inflammation and consequently increase nutrient absorption in the intestinal lumen, which translates into better carcass yield.

On the other hand, breast yield, the main edible part of the chicken, increased with the mushroom group as compared to the other treatments (Table 6). Breast yield has been associated with the bioavailability of lysine, which favors breast synthesis in comparison to other muscles (Zampiga *et al.*, 2019). Thus, the oral use of *Ganoderma lucidum* powder and its possible anti-inflammatory effect on the intestinal cells could increase the absorption of this essential and limiting amino acid (lysine) in poultry. However, Toghyani *et al.* (2012) found no improvement in any portion yield when using 2 g/kg of a powdered mushroom in broiler diets. Also, although subtherapeutic antibiotics have anti-inflammatory and antimicrobial effects (Niewold, 2007), some studies have demonstrated that its prolonged use (as was the case in this experiment) provokes postprandial inflammation and an adverse effect on nutrient absorption (Al-Khalaifa *et al.*, 2019). However, more studies are needed to understand the intestinal inflammatory effect of long-term promoting antibiotics in fast-growing broilers.

Moreover, *G. lucidum* powder decreased abdominal fat in comparison to the antibiotic group and basal diet (Table 6). A decrease in this indicator is associated with the concentration of very low-density lipoproteins (VLDL) and its influence on intestinal inflammation (Van Den Borne *et al.*, 2015). Although many authors have reported the biological response of *Ganoderma lucidum* polysaccharides (Sang *et al.*, 2021), in a previous work we found that this product is also rich in alkaloids (Martínez *et al.*, 2021a). Khadem *et al.* (2014) found that isoquinolinic alkaloid compounds reduced pro-inflammatory cytokines and C-reactive proteins, and consequently abdominal fat yields. Apparently, oral use in small concentrations of this alkaloid-rich medicinal mushroom (*G. lucidum*) had a positive impact on gut inflammation, lipid absorption, and abdominal fat yield. However, more studies are needed to characterize the types of alkaloids in this mushroom. From a practical point of view, the reduction of abdominal fat in processing plants is very beneficial, since abdominal fat is an undesirable component (Fouad & El-Senousey, 2014).

On the other hand, studies with animals at laboratory level reported that this medicinal mushroom (*G. lucidum*) reduces the non-alcoholic liver, mainly due to its hypocholesterolemic properties (Jung *et al.*, 2018). Apparently this natural product has the same effect in broilers, since we found a decrease in relative liver weight (Table 6); which is important in this species since all lipid metabolism is clearly hepatic (Emami *et al.*, 2021). Furthermore, Hajjaj *et al.* (2005) identified 26-oxygenosterols ganoderol A, ganoderol B, ganoderal A, and ganoderic acid Y, which are sterols related to hypocholesterolemic effect in animals and humans. According to Ogbe *et al.* (2015) an excess of phytosterols causes hepatic hypertrophy, due to the increased function of this organ in synthesizing endogenous cholesterol. This did not happen in laboratory animals (Jung *et al.*, 2018) and in this study when we used *Ganoderma lucidum* powder.

Likewise, the oral use of *Ganoderma lucidum* powder apparently led to better broiler intestinal health and nutrient absorption, and Zn bacitracin increased the translocation of the proteins and amino acids provided in the diet (Mir *et al.*, 2017) to breast muscle, since both additives increased the concentration of breast protein in 2.59 and 3.17%, respectively (Table 6). Similarly, other authors have found an interaction between the bioavailability of lysine and the percentage of proteins in the breast and carcass (Nasr & Kheiri, 2012). In this sense, Tashla *et al.* (2019) have reported similar results in breast protein when they used Black pepper powder rich in the piperine alkaloid in broiler diets. However, Mourão *et al.* (2008) found no changes in the chemical composition of the breast when they used citrus pulp rich in polyphenolic compounds and dietary fiber (Rafiq *et al.*, 2018).

Also, table 6 shows that these additives (mushroom and antibiotics) reduce breast fat. Furthermore, Hajjaj *et al.* (2005) and Guo *et al.* (2018) have mentioned that due to the content of phytosterols and polysaccharides and the modulation of gut microflora, the mushroom *Ganoderma lucidum* has a proven hypolipidemic effect, which reduces the blood circulation of harmful lipids (mainly cholesterol). This apparently provokes a reduction in muscle lipids such as the breast (leaner meat) (Table 6). Also, fat is related to moisture content and both oral additives increased this variable (moisture). It is known that lipids are insoluble in water, thus both indicators are inversely proportional (Martínez *et al.*, 2021b). Despite the higher moisture, these breasts are in the acceptable range for chicken meat (Saelin *et al.*, 2017). It is important to note that an adequate protein/water ratio directly influences the organoleptic acceptance and finished product yields (Ang *et al.*, 1984).

Interestingly, the oral use of Zinc bacitracin as an antimicrobial growth promoter reduced breast fat (Table 6). Studies in humans have determined that some antibiotics such as metronidazole and ciprofloxacin can reduce serum lipids acutely due to the growth of lactic acid bacteria such as *Bifidobacterium* spp. (Jenkins *et al.*, 2005). However, these studies are not conclusive for poultry. Therefore, it is necessary to continue research to determine whether the dietary antibiotics commonly used in these animals have the same effect. Also, the ash content is directly related to the amount of minerals of a feed product (Martínez *et al.*, 2021). The experimental treatments did not change the translocation of the minerals, despite the fact that *Ganoderma lucidum* powder has essential minerals (Table 1) and they were supplied in the broiler diets (Tables 2, 3 and 4).

On the other hand, both additives (*Ganoderma lucidum* powder and Zinc bacitracin) increased *a**, perhaps due to a higher incorporation of myoglobin and red pigments on breast, which is indirectly proportional to *L**. Apparently, the intestinal anti-inflammatory effect of *Ganoderma lucidum* powder provoked a higher absorption of this pigment, which increases the antioxidant capacity of muscle (breast) and decreases oxidative rancidity in meat (Mir *et al.*, 2017). Also, *b** decreased with the antibiotic group (Table 6) and this treatment apparently increased breast pH. Fletcher *et al.* (2000) reported a negative statistical correlation between pH and yellowness in breast meat. According to Qiao *et al.* (2001), management prior to slaughter and genetic predisposition are the main factors influencing meat color. Despite the fact that all evaluated broilers had the same genotype and slaughter process, they presented different values for these colorimetry parameters (*L**, *a** and *b**).

Table 7 shows that dietary inclusion of 2.5 g/kg of *G. lucidum* powder increased the relative weight of bursa of Fabricius and decreased the morphometry of the small intestine compared to the control diet ($p \leq 0.05$), although without notable differences from the antibiotic group of ($p > 0.05$). Furthermore, the cecal lactic acid bacteria count and the relative weight of the thymus, spleen, small intestine, and cecum, as well as the morphometry of the latter organ, did not change as a result of the experimental treatments ($p > 0.05$).

One of the objectives of this experiment was to determine whether *Ganoderma lucidum* powder in fast-growing chicken diets could stimulate the immune system based on the relative weight of the lymphoid organs as occurs in laboratory animals (Huang *et al.*, 2010). Specifically, an increase in the relative weight of bursa of Fabricius, as found in this study (Table 7), has been related to a higher immunological activity in broilers, since this primary lymphoid organ produces immunological memory B lymphocytes (Sultana *et al.*, 2020).

However, Molina *et al.* (2019) did not report changes in the relative weight of this immune organ when they used 0.25% of *Ganoderma lucidum* powder on broiler diets (0-10 days), despite the fact that this natural product promoted body weight. Aguilar *et al.* (2013) have mentioned that an increase in relative weight of lymphoid organs in apparently healthy birds does not always correspond to a better immunological activity. These authors reported that the oral use of a phytobiotic increased the relative weight of the bursa of Fabricius and decreased the body weight gain in birds without disease, perhaps due to a higher energy expenditure for the production of lymphocytes. Our results indicate that the oral use of *Ganoderma lucidum* powder increased the relative weight of the

Table 7 – Effect of dietary supplementation with *G. lucidum* on organ weights and broiler's intestinal traits (28 days).

Ítems	Experimental treatments			SEM±	*p* value
	Control	*G. lucidum*	Antibiotic		
Bursa of Fabricious (%)	0.34[b]	0.50[a]	0.39[ab]	0.040	0.027
Thymus (%)	0.26	0.31	0.30	0.030	0.356
Spleen (%)	0.08	0.06	0.07	0.007	0.263
Small intestine (%)	3.67	3.52	3.74	0.129	0.489
Small intestine (cm)	175.10[a]	162.40[b]	170.30[ab]	3.691	0.050
Cecums (%)	0.75	0.89	0.75	0.048	0.073
Cecums (cm)	15.50	15.90	15.00	0.613	0.588
Cecal LAB (CFU/g)	7.73	7.25	7.89	0.346	0.433

[a,b]Means with different letters in the same row differ at ($p \leq 0.05$). LAB: lactic acid bacteria.

bursa of Fabricius in broilers (apparently healthy) by 32% as compared to the basal diet (Table 7), which apparently determined that no benefits were found in body weight at 28 days old (Table 5). However, more studies are needed to confirm this hypothesis.

Also, the thymus, which is a primary lymphoid organ participating in the production of immune defense T lymphocytes (Sultana *et al.*, 2020) had no changes to its relative weight as a result of dietary additives (*Ganoderma lucidum* and antibiotic; Table 7). Similar results were found by Molina *et al.* (2019) when they used this medicinal mushroom (0.25%) in the diet of neonatal broilers (0-10 days); however, a higher supplementation (0.30%) depressed relative thymus weight. Authors such as Wang *et al.* (2013) and Li *et al.* (2020) demonstrated that *Ganoderma lucidum* mushroom extracts rich in polysaccharides and alkaloids increased the thymus and spleen index in laboratory mice, which promoted hematopoiesis and improved serum IgA levels. This shows that the effect on the relative thymus weight will depend on the animal species and age, as well as on the formulation and level of inclusion of *Ganoderma lucidum.*

Likewise, the relative spleen weight did not change with the oral inclusion of the tested additives (*Ganoderma lucidum* and antibiotic; Table 7). The variations in relative weight and spleen immune activity have been related to blood-borne antigens and the presence of pathogenic bacteria and viruses that affect birds; since this organ participates in the cellular and humoral response through its role in the generation, maturation, and storage of lymphocytes (Perozo *et al.*, 2004). Therefore, the highest immunological activity in birds generally occurs with the involution of the primary lymphoid organs (Bursa de Fabricio and thymus). Moreover, Martínez *et al.* (2021b) have reported the same effect on relative spleen weight when they used natural products rich in secondary metabolites (mainly tannins, coumarins, flavonoids, and with reduced sugars) in broilers. Other studies have found that the *Ganoderma lucidum* mushroom increases the spleen index in apparently healthy and sick laboratory animals (rats and mice), indicating a higher immune response (Wang *et al.*, 2013; Zhu *et al.*, 2019; Li *et al.*, 2019; Li *et al.*, 2020). It is important to note that all the broilers remained apparently healthy during the experiment and did not present symptoms associated with any disease.

However, this study found a significant decrease ($p \leq 0.05$) in the morphometry of the small intestine of broilers fed with *Ganoderma lucidum* powder as compared to the diet without additive, although the relative weight of this organ changed between treatments (Table 7). Studies in laboratory animals have reported that distention of the small intestine is one of the most recurrent symptoms in postprandial inflammation (Chen *et al.*, 2017). Although our results are not conclusive, a decrease in this indicator due to the medicinal mushroom in apparently healthy young birds has been related to the higher functionality of the organ; with similar results found by Molina *et al.* (2019) when they used up to 0.50% of *Ganoderma lucidum* powder in neonatal broilers. These authors mentioned that a decrease in the length of this digestive organ was related to better digestibility and intestinal health, which improved some biological indicators in broilers. This study proved that this natural product (*G. lucidum*) modified carcass and breast yields, as well as the chemical composition of the latter (Table 6). It is also known that high levels of fiber in bird diets increase the morphometry and relative weight of the small and large intestine, given the higher permanence of the chyme in these sections (Rodríguez *et al.*, 2006). This did not affect this experiment because all diets were formulated with low levels of crude fiber (2.00 to 2.5%; Tables 1, 2 and 3).

On the other hand, many birds under different stressful conditions suffer from bacterial dysbiosis, which causes intestinal inflammation and nutrient malabsorption, which impacts broiler health (Prado-Rebolledo *et al.*, 2017). Therefore, the use of functional food rich in lactic acid bacteria and secondary metabolites can promote the growth of selective beneficial bacteria in the gut, which has been linked to better anti-inflammatory, antioxidant, and immunological activity. Authors such as Martínez *et al.* (2021) reported a higher quantification of cecal lactic acid bacteria when they used up to 0.75% of *Anacardium occidentale* (rich in tannic, alkaloids and coumarins) on broiler diets. However, despite the fact that the *Ganoderma lucidum* mushroom has beneficial secondary metabolites, its dietary inclusion at 0.25% was not enough to modify the growth of cecal lactic acid bacteria (Table 7). The effect of this natural product is apparently more decisive in the small intestine than in the cecum (which has the largest bacterial population). Supporting this argument, Molina *et al.* (2019) did not find a relationship between the dietary use of 0.5% of *G. lucidum* powder and the growth of cecal lactic acid bacteria in broilers.

CONCLUSIONS

The dietary use of *Ganoderma lucidum* powder and Zn-Bacitracin did not modify broiler's growth performance. However, this medicinal mushroom increased carcass and breast yields and decreased abdominal fat. Moisture, protein, and *a** content increased with both additives and these treatments reduced fat and *L** in breast meat. Furthermore, *Ganoderma lucidum* powder modified the relative weight of bursa of Fabricius and the morphometry of the small intestine; although without changes for the relative weight, morphometry, and cecal lactic acid bacteria.

REFERENCES

Aguilar YM, Yero OM, Liu G, Ren W, Bertot RR, Jiménez YF, *et al.* Effect of dietary supplementation with Anacardium occidentale on growth performance and immune and visceral organ weights in replacement laying pullets. Journal of Food, Agriculture and Environment 2013;11(3-4):1352-1357.

Al-Khalaifa H, Al-Nasser A, Al-Surayee T, Al-Kandari S, Al-Enzi, N, Al-Sharrah T, *et al.* Effect of dietary probiotics and prebiotics on the performance of broiler chickens. Poultry Science 2019;98(10):4465-4479.

Ang CYW, Young LL. Wilson R. Interrelationships of protein, fat and moisture content of broiler meat. Journal of Food Science 1984;49(2):359-362.

AOAC - Association of Official Analytical Chemists. Official methods of analysis. 18th ed. Gaithersburg; 2006.

Aroche R, Martínez Y, Ruan Z, Guan G, Waititu S, Nyachoti CM, *et al.* Dietary inclusion of a mixed powder of medicinal plant leaves enhances the feed efficiency and immune function in broiler chickens. Journal of Chemistry 2018;2018:1-6.

Azad MKA, Kikusato M, Zulkifi I, Toyomizu M. Electrolysed reduced water decreases reactive oxygen species-induced oxidative damage to skeletal muscle and improves performance in broiler chickens exposed to medium- term chronic heat stress. British Poultry Science 2013;54(4):503-509.

Bederska-Łojewska D, Świątkiewicz S, Muszyńska B. The use of *Basidiomycota mushrooms* in poultry nutrition – A review. Animal Feed Science and Technology 2017; 230:59-69.

Bidegain MA, Palma SD, Cubitto MA. Formulation and evaluation of a lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (*Agaricomycetes*), nutraceutical hydroalcoholic suspension. International Journal of Medicinal Mushrooms 2020;22(8):719-730.

Butt MS, Pasha I, Sultan MT, Randhawa MA, Saeed F, Ahmed W. Black pepper and health claims: a comprehensive treatise. Critical Reviews in Food Science and Nutrition 2013;53(9):875-886.

Chen BR, Du LJ, He HQ, Kim JJ, Zhao Y, Zhang YW, Dai N. Fructo-oligosaccharide intensifies visceral hypersensitivity and intestinal inflammation in a stress-induced irritable bowel syndrome mouse model. World Journal of Gastroenterology 2017; 23(47):8321-8333.

Chen HW, Yu YH. Effect of *Ganoderma lucidum* extract on growth performance, fecal microbiota, and bursal transcriptome of broilers. Animal Feed Science and Technology 2020;267:114551.

Cobb-Vantress. Suplemento informativo sobre rendimiento y nutricion de pollos de engorde Cobb-500. 2018 [cited 2020 Feb 10]. Available from: https://cobbstorage.blob.core.windows.net/guides/ee5706d0-5d14-11e8-9602-256ac3ce03b1

El-Tahawy AAS, Taha AE, Adel SA. Effect of flock size on the productive and economic efficiency of Ross 308 and Cobb 500 broilers. European Poultry Science 2017;81:1-10.

Emami NK, Jung,U, Voy B, Dridi S. Radical Response: Effects of heat stress-induced oxidative stress on lipid metabolism in the avian liver. Antioxidants 2021;10(1):1-15.

Farag SA, El-Rayes TK. Effect of bee-pollen supplementation on performance, carcass traits and blood parameters of broiler chickens. Asian Journal of Animal and Veterinary Advances 2016;11(3):168-177.

Fletcher DL, Qiao M, Smith DP. The relationship of raw broiler breast meat color and pH to cooked meat color and pH. Poultry Science2000;79(5):784-788.

Fouad AM, El-Senousey, HK. Nutritional factors affecting abdominal fat deposition in poultry:a review. Asian-Australasian Journal of Animal Sciences 2014;27(7):1057-1068.

Gong P, Wang S, Liu M, Chen F, Yang W, Chang X, *et al.* Extraction methods, chemical characterizations and biological activities of mushroom polysaccharides: a mini-review. Carbohydrate Research 2020;494:108037.

Grashorn MA. Use of phytobiotics in broiler nutrition–an alternative to infeed antibiotics. Journal of Animal and Feed Sciences 2010;19(3):338-347.

Guo WL, Pan YY, Li L, Li TT, Liu B, Lv XC. Ethanol extract of Ganoderma lucidum ameliorates lipid metabolic disorders and modulates the gut microbiota composition in high-fat diet fed rats. Food & Function 2018;9(6):3419-3431.

Hahn TW, Lohakare JD, Lee SL, Moon WK, Chae BJ. Effects of supplementation of β-glucans on growth performance, nutrient digestibility, and immunity in weanling pigs. Journal of Animal Science 2006;84:1422-1428.

Hajjaj H, Macé C, Roberts M, Niederberger P, Fay LB. Effect of 26-oxygenosterols from *Ganoderma lucidum* and their activity as cholesterol synthesis inhibitors. Applied and Environmental Microbiology 2005;71(7):3653-3658.

Huang SQ, Li JW, Wang Z, Pan HX, Chen JX, Ning ZX. Optimization of alkaline extraction of polysaccharides from Ganoderma lucidum and their effect on immune function in mice. Molecules 2010;15(5):3694-3708.

Jayachandran M, Xiao J, Xu B. A critical review on health promoting benefits of edible mushrooms through gut microbiota. International Journal Molecular Science 2017;18:1-12.

Jenkins DJ, Kendall CW, Hamidi M, Vidgen E, Faulkner D, Parker T, *et al.* Effect of antibiotics as cholesterol-lowering agents. Metabolism 2005;54(1):103-112.

Jung S, Son H, Hwang CE, Cho KM, Park SW, Kim HJ. *Ganoderma lucidum* ameliorates non-alcoholic steatosis by upregulating energy metabolizing enzymes in the liver. Journal of Clinical Medicine 2018;7(6):152.

Khadem A, Soler L, Everaert N, Niewold TA. Growth promotion in broilers by both oxytetracycline and Macleaya cordata extract is based on their anti-inflammatory properties. British Journal of Nutrition 2014;112(7):1110-1118.

Li J, Gu F, Cai C, Hu M, Fan L, Hao J, *et al.* Purification, structural characterization, and immunomodulatory activity of the polysaccharides from *Ganoderma lucidum.* International Journal of Biological Macromolecules 2020;143:806-813.

Liu T, Ma Q, Zhao L, Jia R, Zhang J, Ji C, *et al.* Protective effects of sporoderm-broken spores of *Ganoderma lucidum* on growth performance, antioxidant capacity and immune function of broiler chickens exposed to low level of aflatoxin B1. Toxins 2016;8(10):278-290.

Liu T, Zhou J, Li W, Rong X, Gao Y, Zhao L, *et al.* Effects of sporoderm-broken spores of Ganoderma lucidum on growth performance, antioxidant function and immune response of broilers. Animal Nutrition 2020;6(1):39-46.

Lu J, He R, Sun P, Zhang F, Linhardt RJ, Zhang A. Molecular mechanisms of bioactive polysaccharides from *Ganoderma lucidum* (Lingzhi), a review. International Journal of Biological Macromolecules 2020;150:765-774.

Martínez Y, Rodríguez R, Borrero G. Análisis de los metabolitos secundarios del hongo *Ganoderma lucidum.* Revista de Producción Animal 2021a;33(1):1-5.

Martínez Y, Tobar LA, Parrado A, Urquia AM, Valdivié M. Phytobiotic effect of cashew (*Anacardium occidentale* L.) leaves powder on growth performance, carcass traits, lymphoid organ weights and cecal acid-lactic acid bacteria in broilers. Brazilian Journal of Poultry Science 2021b;23(1):1-10.

Mashayekhi H, Mazhari M, Esmaeilipour O. Eucalyptus leaves powder, antibiotic and probiotic addition to broiler diets: effect on growth performance, immune response, blood components and carcass traits. Animal 2018;12(10):2049-2055.

Mir NA. Determinants of broiler chicken meat quality and factors affecting them:a review. Journal of Food Science and Technology 2017;54(10):2997-3009.

Molina DS, Bernardo J, Machado OD, Más D, Martínez, Y. Nutraceutical effect of *Ganoderma lucidum* fungus on neonatal broilers diet. International Journal of Poultry Science 2019;18(12):641-647.

Mourão JL, Pinheiro VM, Prates JAM, Bessa RJB, Ferreira LMA, *et al.* Effect of dietary dehydrated pasture and citrus pulp on the performance and meat quality of broiler chickens. Poultry Science 2008;87(4):733-743.

Nasr J, Kheiri F. Effects of lysine levels of diets formulated based on total or digestible amino acids on broiler carcass composition. Brazilian Journal of Poultry Science 2012;14(4):249-258.

Ni H, Martínez Y, Guan G., Rodríguez R, Más D, Liu G. Analysis of the impact of isoquinoline alkaloids, derived from *Macleaya cordata* extract, on the development and innate immune reaction in swine and poultry. Biomed Research International 2016;2016:1352146 .

Niewold TA. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poultry Science 2007;86(4):605-609.

Ogbe A, Affiku JP. Effect of polyherbal aqueous extracts (*Moringa oleifera, Gum arabic* and wild *Ganoderma lucidum*) in comparison with antibiotic on growth performance and haematological parameters of broiler chickens. Research Journal of Recent Sciences. 2012;1:10-18.

Ogbe AO, Atawodi SE, Abdu PA, Sannusi A, Itodo AE. Changes in weight gain, faecal oocyst count and packed cell volume of *Eimeria tenella*-infected broilers treated with a wild mushroom (*Ganoderma lucidum*) aqueous extract. Journal of the South African Veterinary Association 2009;80(2):97-102.

Ogbe RJ, Ochalefu DO, Mafulul SG, Olaniru OB. A review on dietary phytosterols:Their occurrence, metabolism and health benefits. Asian Journal of Plant Science and Research 2015;5(4):10-21.

OIE - Organización Mundial de Sanidad Animal. Nuevo informe muestra un cambio a nivel mundial en el uso de antibióticos en animales. Paris; 2019. [cited 2020 April 12] Available from: https://www.oie.int/es/para-los-periodistas/comunicados-de-prensa/detalle/article/new-report-shows-global-shift-in-use-of-antibiotics-in-animals/

Perozo F, Nava J, Mavárez Y, Arenas E, Serje P, Briceño M. Morphometric characterization of Ross line broiler chickens lymphoid organs reared under field conditions in Zulia state. Revista Científica 2004;14(3):1–18.

Prado-Rebolledo OF, Delgado-Machuca JDJ, Macedo-Barragan RJ, Garcia-Márquez LJ, Morales-Barrera JE, Latorre JD, *et al.* Evaluation of a selected lactic acid bacteria-based probiotic on Salmonella enterica serovar Enteritidis colonization and intestinal permeability in broiler chickens. Avian Pathology 2017;46(1):90-94.

Qiao M FD. The effect of broiler breast meat color on pH, moisture, water-holding capacity, and emulsification capacity. Poultry Science 2001;80(5):676–680.

Rafiq S, Kaul R, Sofi SA, Bashir N, Nazir F, Nayik GA. Citrus peel as a source of functional ingredient:a review. Journal of the Saudi Society of Agricultural Sciences 2018;17(4):351-358.

Rodríguez R, Martínez M, Valdivié M, Cisneros M, Cárdenas M, Sarduy L. Morfometría del tracto gastrointestinal y sus órganos accesorios en gallinas ponedoras alimentadas con piensos que contienen harina de caña proteica. Revista Cubana de Ciencia Agrícola2006;40(3):361-365.

Saelin SW. Evaluation of water holding capacity in broiler breast meat by electrical conductivity. International Food Research Journal 2017;24(6):2593-2598.

Sang T, Guo C, Guo D, Wu J, Wang Y, Wang Y, *et al.* Suppression of obesity and inflammation by polysaccharide from sporoderm-broken spore of Ganoderma lucidum via gut microbiota regulation. Carbohydrate Polymers 2021;256:117594.

Shen HS, Shao S, Chen JC, Zhou T. Antimicrobials from mushrooms for assuring food safety. Comprehensive Reviews in Food Science and Food Safety 2017;16(2):316-329.

Soler L, Hermes R, Niewold TA. Macleaya cordata extract reduces inflammatory responses of intestinal epithelial cells in vitro. American Journal of Plant Sciences 2016;7(11):1531-1537.

Sultana N, Amin, T, Afrose M, Aqter RS, Rafiq K. Effects of dexamethasone on the morphometry and biometry of immune organs of broiler chicken. International Journal of Morphology 2020;38(4):1031-1038.

Tashla T, Puvača N, Pelić DL, Prodanović R, Bošković J, Ivanišević, D, *et al.* Dietary medicinal plants enhance the chemical composition and quality of broiler chicken meat. Journal of the Hellenic Veterinary Medical Society 2019;70(40):1823-1832.

Toghyani M, Tohidi M, Gheisari A, Tabeidian A, Toghyani M. Evaluation of oyster mushroom (*Pleurotus ostreatus*) as a biological growth promoter on performance, humoral immunity, and blood characteristics of broiler chicks. The Journal of Poultry Science 2012;49(3):183-190.

Torki M, Schokker D, Duijster-Lensing M, Van Krimpen MM. Effect of nutritional interventions with quercetin, oat hulls, β-glucans, lysozyme and fish oil on performance and health status related parameters of broilers chickens. British Poultry Science 2018;59(5):579-590.

Van Den Borne JJGC, Heetkamp MJW, Buyse J, Niewold TA. Fat coating of Ca butyrate results in extended butyrate release in the gastrointestinal tract of broilers. Livestock Science 2015;175:196–200.

Wang J, Wang Y, Liu X, Yuan Y, Yue T. Free radical scavenging and immunomodulatory activities of Ganoderma lucidum polysaccharides derivatives. Carbohydrate Polymers2013;91(1):33-38.

Xiong W, Wang Y, Sun Y, Ma L, Zeng Q, Jiang X, *et al.* Antibiotic-mediated changes in the fecal microbiome of broiler chickens define the incidence of antibiotic resistance genes. Microbiome 2018;6(1):1-11.

Xu Z, Chen X, Zhong Z, Chen L, Wang Y. Ganoderma lucidum polysac charides:immunomodulation and potential anti-tumor activities. The American journal of Chinese Medicine2011;39(01):15-27.

Zampiga M, Soglia F, Petracci M, Meluzzi A, Sirri F. Effect of different arginine-to-lysine ratios in broiler chicken diets on the occurrence of breast myopathies and meat quality attributes. Poultry Science 2019;98(6):2691-2697.

Zhang B, Zhang X, Schilling MW, Tabler GT, Peebles ED, Zhai W. Effects of broiler genetic strain and dietary amino acid reduction on (part I) growth performance and internal organ development. Poultry Science2020;99(6):3266-3279.

Zhu J, Xu J, Jiang LL, Huang JQ, Yan JY, Chen YW, Yang Q. Improved antitumor activity of cisplatin combined with Ganoderma lucidum polysaccharides in U14 cervical carcinoma-bearing mice. The Kaohsiung Journal of Medical Sciences 2019;35(4):222-229.

Zhu XL, Chen AF, Lin ZB. *Ganoderma lucidum* polysaccharides enhance the function of immunological effector cells in immunosuppressed mice. Journal of Ethnopharmacology 2007;111(2):219-226.

Zolj S, Smith MP, Goines JC, Ali TS, Huff MO, *et al.* Antiproliferative effects of a triterpene-enriched extract from lingzhi or reishi medicinal mushroom, Ganoderma lucidum (Agaricomycetes), on human lung cancer cells. International Journal of Medicinal Mushrooms 2018;20:1173-1183.