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DOES SPOTTED PUMPKIN (*LAGENARIA BREVIFLORA* R.) POSITIVELY AFFECT THE HEALTH STATUS OF BROILER CHICKEN?

***Deji A. Ekunseitan*[1], *Oluwabamise V. Odubela*[2], *Ridwan A. Ahmed*[3], *Oluwaseun S. Iyasere*[4], *Boluwatife A. Adeniran*[5]**

[1] ORCID: 0000-0002-9817-4505
[2] ORCID: 0000-0002-5144-5996
[3] ORCID: 0000-0003-0229-4359
[4] ORCID: 0000-0001-5987-9492
[5] ORCID: 0000-0002-1462-4899
[1–3,5] Department of Animal Production and Health
[4] Department of Animal Physiology
Federal University of Agriculture, Abeokuta, Nigeria
[1] Poultry Ethnomedicine Research Laboratory
Department of Animal Sciences
North Carolina Agricultural and Technical State University, North Carolina, USA

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Abstract

Coccidiosis poses a significant challenge in poultry production, necessitating the development of effective, natural, and health-promoting alternatives such as *Lagenaria breviflora* to conventional treatments. Two hundred and eight (208) day-old Cobb chicks hatchery-infected with *Eimeria* were divided into four treatment groups and administered: control (0), 50, 100 and 150 grams of *Lagenaria breviflora* R. (Lb) whole fruit per litre of water thrice a week for 6 weeks. Data were obtained on faecal count, haematological indices, serum metabolites and stress biomarkers. There was reduction in *Eimeria* count as Lb increased with the highest reduction (85.20%) in birds that received 150 g. The highest ($P < 0.05$) packed cell volume, haemoglobin and red blood cell count was observed in 150 g Lb while white blood cell count was highest ($P < 0.05$) in 50 and 150 g. Total protein was highest ($P < 0.05$) in 150 g, globulin was highest in 100 and 150 g LB while the serum stress biomarkers (SOD and GSH-Px) was least in 50 g. The administration of Lb up to 150 g/L of water can used in eradicating coccidiosis without compromise on health of birds.

Address: Deji A. Ekunseitan, Federal University of Agriculture, PMB. 2240, Abeokuta, Nigeria, e-mails: ekunseitanda@funaab.edu.ng, daekunseitan@ncat.edu

Introduction

The Nigerian poultry industry is faced by various problems most especially the quality of day-old chicks in terms of health conditions, which is prominent among the issues affecting its positive output apart from quality feed. Prominent among these diseases are salmonellosis, infectious bronchitis and coccidiosis. Salmonellosis and coccidiosis are currently the bane of the Nigerian poultry industry resulting in high mortality and morbidity in the first 5 days and later in life (BALARABE and OBETA 2015). Although, there is no existing evidence or science to suggest the likelihood of a vertical transmission of coccidiosis in birds but recent occurrence of deaths in first few days of chick life may point to the possible infection in the hatchery.

Coccidiosis is caused by coccidian parasites of the genus *Eimeria*, intracellular protozoan parasites of enterocytes resulting in high chicks' mortality. Most anticoccidial drugs currently used in abating the menace exhibit low efficacy and also result in deleterious side effects in birds (AL-MATHAL 2010). The consequential side-effect of exposure to conventional drugs and its overuse results in damages to internal organs invariably resulting to system breakdown and death. The consistent use of these conventional drugs has led to the development of resistant coccidian strains, mostly an offshoot of extensive use, under usage (underdose) and adulteration of drugs experienced in most developing countries.

The resistance mostly due to physical or genetic alterations, and the side effects of these drugs led to its consequent vertical shedding to offspring and the rise of transmission coupled with rearing of birds on floor system. Since most meat-type and eggers are reared on floor from first day with continuous access to litter, coccidiosis will continually be a menace to poultry rearers. Coccidiosis provides a good example of the relationship between the number of invading organisms and the severity of the resulting infection, because the morbidity and mortality of the host species are usually proportional to the number of sporulated coccidial oocysts ingested by the bird.

The inability of farmers to access constant veterinary services and effective cocci-vaccines has greatly affected the development of poultry in developing countries leaving the farmers with no choice than alternative options in combating the threat. The continent of Africa is blessed with natural reserves of plant resources of botanical importance for human and animal health management. One of such plant is Spotted pumpkin (SP). SP has been documented and used in rural poultry health management against coccidiosis and Newcastle disease (SONAIYA et al. 1999) likewise in

rural human health as herbal remedy for the treatment of some disorders such worm infestation, wound antiseptics and skin infections. The extracts of spotted pumpkin have been documented to contain important chemical constituents such as phenols, flavonoids carotenoids, saponins etc (EKUNSEITAN et al. 2016, ADEYEMI et al. 2017) capable of inducing positive health effect.

Numerous laboratory studies have demonstrated its ethnobotanical properties, such as miracicidal and cercaricidal activities (AJAYI et al. 2002), antioxidant activity (ONASANWO et al. 2011) and antibacterial activity (TOMORI et al. 2007). These findings underscore the importance of integrating these attributes into poultry production systems to evaluate their efficacy and potential for industry-wide application. Recent studies (EKUNSEITAN et al. 2017, 2019) revealed positive responses in orally administered dosages in poultry birds raised on modified housing systems, favouring its usage in animal health management especially with the growing drive for organic products in most developing countries. These positive outcomes include increased immune response to Newcastle disease vaccination in growing pullets (EKUNSEITAN et al. 2019), haematinic and therapeutic activity (ALADE 2000, EKUNSEITAN et al. 2017), antimicrobial activities (EKUNSEITAN et al. 2017), immunomodulatory effect (SABA et al. 2009) and worm control (EKUNSEITAN et al. 2017).

Materials and Methods

The experiment was carried out at the Poultry unit, Teaching and Research Farms, Federal University of Agriculture, Ogun state, Nigeria.

Lagenaria breviflora (spotted pumpkin) whole fruits was washed with clean water, weighed and cut into smaller parts to increase the surface area and then extracted according to the method of EKUNSEITAN et al. (2017) to give concentrations of 50, 100 and 150 grams per litter of water.

Two hundred and eight day-old Cobb broiler chicks, hatchery-infected with *Eimeria spp* were used for the experiment. The chicks exhibited clinical signs of ruffled feather and blood stained to brownish diarrhoea from day one of the experiment, birds were also clinically observed by personnel. Sample birds were selected from the pool, euthanized and accessed for identification and counts of *Eimeria* spp. (REHBEIN et al. 1999). The birds were then randomly assigned to four (4) treatment groups: 0 (control), 50, 100 and 150 grams of Lb per litre of water (weight/ volume) in a Completely Randomized Design (CRD), containing fifty-two (52) birds each. Each treatment was further divided into four replicates of thirteen (13)

birds each. *Lagenaria breviflora* was administered determined concentration three consecutive days in a week from day one till the 6th week (day 42) of the experiment via drinking water (EKUNSEITAN et al. (2017). Birds in the control group were immediately placed on Amprolium + Sulphaquinoxaline (+vit K). Conventional vaccination was given to all treatment groups while medication (antibiotics and coccidiostat) was administered only to the control group. The chicks were housed in a well-ventilated deep litter pen. The chicks were fed with commercial starter feed (Metabolizable Energy 2800 kcal/kg, Crude Protein: 21%) and finisher feed (Metabolizable Energy 2900 kcal/kg, Crude Protein: 19%).

Fresh faecal droppings were collected from each replicate group into a plain bottle weekly for six weeks starting from the first week of the experiment. The oocyte count and identification were carried out using McMaster egg counting technique. The sample was viewed under the compound microscope at 10 x 10 magnification. Oocytes were identified and counted within the engraved area of the counting chambers (REHBEIN et al. 1999).

At the 42nd day of the experiment, 10 ml of blood was collected from four pre-selected birds per replicate via the jugular vein with use of hypodermic syringe. Approximately 3.0 ml of blood was collected into EDTA bottles for haematological parameters while the 4 ml was collected into a plain bottle for the serum metabolites determination and the remaining into another labelled plain bottles for quantifying oxidative stress biomarker.

Haematological parameters such as Red blood cell (RBC) and Packed cell volume (PCV) was determined according to BRIAN et al. (2000), Haemoglobin as reported by DACE AND LEWIS (1984), White blood cell and differential counts via visual cell count using a haemocytometer.

Serum metabolites (Total protein, Albumin, Globulin, Asparte aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphate (ALP) was determined using Randox Laboratories Standardized Test Kit (Hitachi Model 917 Multichannel Analyser). Serum superoxide dismutase (SOD) was determined by the method of OBERLEY et al. (1984), glutathione peroxidase (GSH-Px) by REDDY et al. (2004) and Thiobarbituric Acid Reactive Substances (TBARs) by KELES et al. (2001).

Data on faecal analysis were transposed to base of log 10 and evaluated using repeated measures analysis (SPSS 2010). Haematological and serum data were subjected to one-way Analysis of Variance (ANOVA) in a completely randomized design (CRD). Significant means among other measured variables were separated using Tukey test at 95% probability.

Results and Discussion

Effect of *Lagenaria breviflora* (spotted pumpkin) fruit extract on faecal egg count of broiler chicken

The effect of *Lagenaria breviflora* (spotted pumpkin) fruit extract on faecal egg count of broiler chicken is presented in Figure 1. A continuous reduction of oocysts count was observed as the administration of *Lagenaria breviflora* increased. The highest oocysts count (3.49) in week 2 was observed in birds administered 150 g of *Lagenaria breviflora* while the least oocysts count (3.17) was observed in birds administered 100 g of *Lagenaria breviflora.* The lowest ($P < 0.05$) oocysts count (0.65) in week six was observed in birds administered 150 g of *Lagenaria breviflora* while the highest oocysts count (1.79) was observed in the control group. A 50.54% oocysts count reduction was observed in the control group, 62.51% in birds administered 50 g of *Lagenaria breviflora.* 70.85% in 100 g of *Lagenaria breviflora* group and 85.20% in 150 group. *Eimeria* is the causative agents of coccidiosis and a blight in poultry production causing significant commercial losses in the animal industry (WILLIAMS 2002).

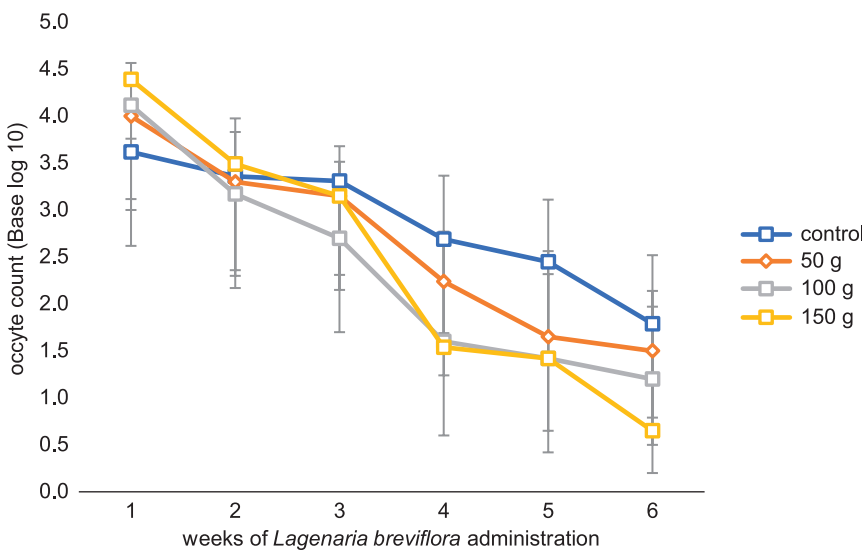

Fig. 1. Effect of *Lagenaria breviflora* (spotted pumpkin) fruit extract on faecal egg count of broiler chicken

The observed decrease in oocyte per gram as the level of the administration of *Lagenaria breviflora* increased may be due to various mechanisms of phytochemicals such as phenols, saponins, alkaloids acting in

synergy and capable of inhibiting the life cycle of Coccidia (MOLAN et al. 2009) and decimating *Eimeria* count in the birds. Plants contain immunoregulatory compounds capable of enhancing and inducing immune responses thereby maintaining bird's health (SUGIHARTO et al. 2017).

The phytochemical analysis of *Lagenaria breviflora* has been examined to contain considerable amount of metabolites like flavonoids, saponin, tannin, etc. (EKUNSEITAN et al. 2016, ADEYEMI et al. 2017) while comparable dose-associated responses in faecal count had been reported by researchers using phytobiotics plants as an alternative to conventional drugs (BIU et al. 2010, ELKHTAM et al. 2014, EKUNSEITAN et al. 2017, EKUNSEITAN et al. 2018) to safeguard the welfare of animals and consumers of products. The increase in dosage will invariably result in the amount of metabolites in the extract (ODULANA 2014, AREMU 2014) potentially enhancing its biological activity and therapeutic efficacy. Chemical metabolites present in *Lagenaria breviflora* R. fruit display various mechanisms of action such as inhibiting intestinal motility while some components have been shown to inhibit specific pathogens (AHMAD et al. 2006, EKUNSEITAN et al. 2016, ADEYEMI et al. 2017).

Tannins, has been reported to inhibit the life cycle of Coccidia (MOLAN et al. 2009), reduction in the rate of oocysts hatching and development (HUR et al. (2005) through the penetration of the wall of the oocyst and damage to the cytoplasm as tannins has been posited to weaken endogenous enzymes responsible for sporulation. In addition, tannins have been reported to improve the immune system, and exert bactericidal and coccidiostatic properties in birds (PERIN et al. 2019). *Lagenaria breviflora* whole fruit contains considerable amount of flavonoids (223.00 mg/100 g) and saponins (138.0 mg/100 g) (EKUNSEITAN et al. 2016), flavonoid have been reported to control coccidian infection by induction of oxidative stress through the conversion of hydroxyl groups into pro-oxidant when reacting oxidizing species (ROS) oxidize the lipids and proteins present in inner cell membrane of coccidian thereby resulting in necrosis and apoptosis of damaged cells (BAKKALI et al. 2008). NAIDOO et al. (2008) observed that the use of plant-derived antioxidants when compared to conventional coccidiostat exhibited a similar positive effect observed in the study. In addition, the reduction trend was also in tandem with report of RAKHMANI et al. (2014) which observed proportionally inverse relationship between saponins content and percentage of oocyte per gram count reduction as a consequence of penetration through micropyle cap on polar end of oocyst (WIEDMER et al. 2011).

Effect of extract of *Lagenaria breviflora* (spotted pumpkin) on the haematological indices of broiler chicken

The result of the haematological indices of broiler chicken administered *Lagenaria breviflora* is presented in Table 1. Oral administration of *Lagenaria breviflora* had significant ($P < 0.05$) increasing effect on Packed cell volume (PCV), Haemoglobin (Hb), White blood cells (WBC), Red blood cells (RBC), and Eosinophils. The highest RBC value (3.88) was observed in birds administered 150 g/L of *Lagenaria breviflora* while birds administered 50 g/L of *Lagenaria breviflora* had the least (3.16) value. The highest ($P < 0.05$) PCV value (36.00) and Hb (12.30) value was observed in birds administered 150 g/L of *Lagenaria breviflora* while the lowest value (27.50, 9.20) was observed in birds administered 50 g/L of *Lagenaria breviflora*. The levels of PCV, Hb and RBC count observed in the study might be linked with the *Eimeria* load observed in the treatment groups. Anaemic condition is characterized by decreased PCV, Hb and RBC in infected birds and conceivably due to loss of blood into the gastrointestinal tract with haemorrhages in the ceca and intestine. Lb has been proposed to induce haematinic and therapeutic values in periods of anaemia (OLORUNFEMI et al. 2014, EKUNSEITAN et al. 2017), an ability associated with cucurbitaceae family indicating increased production of reticulocytes and suggestive of stimulating erythropoiesis. The significant reduction in the *Eimeria* load correlated with the elevated erythrocytes component counts in Lb dosed groups with greatest counts in the 150 g per litre.

Table 1

Effect of extract of *Lagenaria breviflora* (spotted pumpkin) on the haematological indices of broiler chicken

Parameters	*Lagenaria breviflora* administration				P-Value
	0 g/L Lb	50 g/L Lb	100 g/L Lb	150 g/L Lb	
Packed cell volume [%]	29.00 ± 1.41^{c}	27.50 ± 0.71^{c}	34.50 ± 3.5^{b}	36.00 ± 1.41^{a}	0.036
Haemoglobin [g/L]	9.80 ± 0.57^{ab}	9.20 ± 0.14^{b}	11.55 ± 1.20^{ab}	12.30 ± 0.71^{a}	0.041
Red blood cells [10^{12}/L]	3.41 ± 0.19^{ab}	3.16 ± 0.06^{b}	3.78 ± 0.23^{ab}	3.88 ± 0.07^{a}	0.028
White blood cells [10^{3}/ul]	9.75 ± 0.92^{b}	22.20 ± 2.97^{a}	12.35 ± 1.91^{b}	24.15 ± 1.63^{a}	0.005
Heterophils [%]	44.00 ±9.90	47.00 ±16.97	36.00 ±38.18	12.50 ±4.95	0.464
Lymphocytes [%]	51.00 ±11.31	49.00 ±15.56	57.00 ±38.18	85.50 ±6.36	0.409
Basophils [%]	0.00 ±0.00	0.50 ±0.71	0.50 ±0.71	0.00 ±0.00	0.615
Eosinophils [%]	1.50 ± 0.71^{ab}	3.00 ± 1.41^{ab}	4.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.026
Monocytes [%]	3.50 ±0.71	0.50 ±0.71	2.50 ±0.71	2.00 ±1.41	0.125

^{abc}: Means in the same row with different alphabet differ significantly ($P < 0.05$), mean ±SD

White blood cell was statistically highest ($P < 0.05$) and similar in birds administered 50 g/L (22.20) and 150 g/L (24.15) of *Lagenaria breviflora*. The highest value of Eosinophils (4.00) was observed in birds administered 100 g of *Lagenaria breviflora* while the lowest value of Eosinophils (0.00) was observed in birds administered 150 g/L of *Lagenaria breviflora*. The white blood cell count obtained in the study indicate that oral administration of *Lagenaria breviflora* had a substantial effect on the immune system of the experimental birds. Increase in administration of *Lagenaria breviflora* led to an increase in lymphocytes count, *Lagenaria breviflora* and *Telfairia occidentalis* are part of the Cucurbitaceae family whereas *Telfairia occidentalis* has been reported to have a haematopoietic effect in rats (ALADE 2000). Although there was a non-significant increase in lymphocyte counts as the level of LB increased in birds, the increase observed which was dosage-dependent may be ascribed to the induction of immune response by LB in the infected birds (ADEYEMI et al. 2017, EKUNSEITAN et al. 2016). Lymphocytes participate in the first line of defence against many infections especially intra-intestinal epithelial lymphocytes which makeup a relatively large proportion of the total immune system (ROSE et al. 1996) thereby participating in resistance to the *Eimeria* infection.

Effect of extract of *Lagenaria breviflora* (spotted pumpkin) on the serum indices in broiler chicken

The effects of extract of Lb on the serum indices of experimental broiler chicken is presented in Table 2. It was observed that Lb extract had significant ($P < 0.05$) effect on Total protein, globulin and Uric acid. Increasing concentration of Lb extract from 0 to 150 g resulted in an increase in total protein. Globulin was statistically similar and highest in 100 and 150 g dosed group with the least values observed in the control and 50 g group. This affirms the immune-stimulating effect of Lb by influencing synthesis of blood proteins by hepatocytes as observed with the elevated values of serum total protein and globulin. The plant's hepatic influence implies that higher rate of protein synthesis occurs in the liver thereby elevating the synthesis of serum proteins (JALEEL and NAIR 2004) resulting in subsequent increase in the concentration of total protein in the blood (SZABÓ et al. 2005). The serum enzymes were similar ($P < 0.05$) in all treatment groups indicating no hepatic injuries in birds administered Lb up to 150 g/litre when compared with the control group. Although, PIEME et al. (2006) stated that AST, ALT and ALP enzymes produced by the liver can be influenced by phytochemicals present in plant extracts, only AST and ALT were observed to be numerically higher in birds administered *Lage-*

naria breviflora except ALP where it was numerically highest in the control group. The values obtained for AST, ALT and ALP fell within the range of values reported by (EKUNSEITAN et al. 2018, RUBIO et al. 2019), while the ALT values were higher than that reported CHUSKIT et al. (2024) in broiler chickens at 21 and 28th age of age. Serum electrolytes (Ca^+ and Na^+) are important substances for myriads of homeostatic and physiological functions: cell muscle contraction and bone formation, transfer of impulses in nerve cells and blood clotting. Although, a non-significant reduction ($P < 0.05$) was observed in serum calcium concentration as the level of Lb dosage increased similar to AJANI et al. (2015) thereby negating the possibility of plant toxicity.

Table 2

Effect of extract of *Lagenaria breviflora* (spotted pumpkin) on serum metabolites of broiler chicken

Parameter	*Lagenaria breviflora* administration				P-Value
	0 g/L Lb	50 g/L Lb	100 g/L Lb	150 g/L Lb	
Glucose [mg/dL]	140.45 ±2.33	143.95 ±2.05	138.95 ±9.12	152.90 ±8.49	0.271
Total protein [g/L]	56.90 ±1.41^{d}	62.00 ±0.42^{c}	67.70 ±1.70^{b}	70.85 ±0.21^{a}	<0.001
Albumin [g/L]	35.75 ±1.48	38.55 ±1.91	34.85 ±2.62	37.45 ±0.64	0.306
Globulin [g/L]	21.15 ±0.05^{b}	23.45 ±1.65^{b}	32.85 ±3.05^{a}	33.40 ±0.30^{a}	0.015
Aspartate aminotransaminase [U/L]	191.50 ±2.55	222.55 ±7.42	224.90 ±28.71	192.95 ±8.27	0.174
Alanine aminotransferase [U/L]	11.20 ±1.27	13.90 ±3.11	17.10 ±0.57	17.70 ±0.57	0.056
Alkaline Phosphatase [U/L]	295.35 ±7.14	195.00 ±7.78	245.65 ±93.13	209.75 ±12.94	0.289
Calcium [mg/dL]	9.35 ±0.49	9.00 ±0.14	8.80 ±0.14	7.70 ±1.13	0.190
Sodium [mmol/L]	121.20 ±13.01	133.20 ±6.79	136.50 ±8.77	129.65 ±10.82	0.536

abc: Means in the same row with different superscript differ significantly ($P < 0.05$), mean ±SD

Effect of administration of extract of *Lagenaria breviflora* (spotted pumpkin) on oxidative stress marker of broiler chicken

The effect of *Lagenaria breviflora* (spotted pumpkin) on oxidative stress marker of broiler chicken is presented on Table 3. Aqueous *Lagenaria breviflora* extract had significant effect ($P < 0.05$) on the oxidative stress makers except Malondialdehyde (MDA). Superoxide dismutase (SOD) and Glutathione peroxidase (GSH-Px) was lowest ($P < 0.05$) in birds administered 50 g *Lagenaria breviflora* but similar and highest in other treatment groups. Oxidative stress occurs in animals as a consequence of pathogenic occurrence therefore the animal's biological system subsequently promotes the production and expression of antioxidant enzymes as a defence mechanism.

Table 3
Effect of administration of *Lagenaria breviflora* (spotted pumpkin) on oxidative stress marker of broiler chicken

Parameter	Levels of oral administration				P-Value
	0 g/L Lb	50 g/Lb	100 g/Lb	150 g/Lb	
Superoxide dismutase [U/L · 10^{-2}]	1.62 ±0.14^{a}	1.09 ±0.07^{b}	1.23 ±0.00^{b}	1.33 ±0.12^{ab}	0.049
Malondialdehyde [U/L · 10^{-9}]	2.61 ±0.42	2.07 ±0.05	1.90 ±0.04	3.44 ±0.75	0.073
Glutathione peroxidase [U/L]	6.10 ±0.14^{a}	3.90 ±0.92^{b}	4.75 ±0.28^{ab}	5.25 ±0.64^{ab}	0.040

^{abc}: Means in the same row with different alphabet differ significantly ($P < 0.05$), mean ±SD

The significant ($P < 0.05$) increase in activities of antioxidant enzymes SOD and GSH-Px in birds administered with 100 and 150 g dosed group statistically *comparable* with the control group is suggestive of the plant capable of directly activating synthesis of antioxidant enzymes to combat the stress induced by Eimeria counts in the bird's system. Similar increment in GSH-Px activity was observed in male wistar rats as a defence response to oxidative stress (FOLORUNSHO et al. 2019), indicating the ability of the fruit of beneficially affecting the activities of some antioxidant enzymes such as Superoxide dismutase and Glutathione peroxidase. The use of natural antioxidants can improve the health status of birds infected with coccidiosis (QUIROZ-CASTANEDA and DANTAN-GONZALEZ 2015) as wide range of herbs and their extracts are capable of inducing potential antioxidant functions (BRENES et al. (2010). *Lagenaria breviflora* contain phenolic compound which increases the activity of antioxidant enzyme thus affecting the concentration of (O_2^- and H_2O_2) free radicals in the serum of the birds (KAURINOVI and VASTAG 2019).

Conclusion

The results of the study revealed that administration of LB at increasing levels demonstrated a dosage-dependent reduction in *Eimeria* counts, with the greatest reduction at 150 g. This dosage also resulted in the improved blood indices (Packed cell volume, haemoglobin, red blood cell count, total protein, and globulin levels), stress biomarkers (SOD and GSH-Px). These findings indicate that LB, administered at levels up to 150 g/L of water, effectively mitigates coccidiosis while supporting the overall health and physiological balance of the birds.

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