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Response of birds to aqueous administration of spotted pumpkin extract

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SUMMARY

This study was conducted to evaluate growth response, lipoprotein content of blood, liver and kidney indices and humoral response of pullet administered aqueous extracts of *Lagenaria breviflora* Robert. A total of ninety (90) fourteen (14) days old Yaffa brown chicks were used for this experiment. The birds were randomly allotted into three (3) treatment groups, containing three (3) replicates of ten (10) birds each. Aqueous *Lagenaria breviflora* extracts was administered at control (0), 100 and 150g/litre respectively. Aqueous of administration of *Lagenaria breviflora* Robert had no effect (P>0.05) on growth indices (final weight, weigh gain, total feed intake and feed conversion ratio) considered. High density lipoprotein (HDL) was significantly (P<0.05) influenced in 100 and 150g/litre groups only at 16 weeks of age. Liver indices (Alanine Aminotransferase and Aspartate Aminotransferase) were (P<0.05) highest in birds administered 150g/litre group while the serum calcium content was highest in 100g group at 16 and 8weeks of age respectively. The highest antibody titre value was observed at 100g/litre and 150g/litre *Lagenaria breviflora*.

Resposta das aves à administração aquosa de extrato de abóbora manchada

Palavras chave adicionais
Imunidade.
Lipídios e proteínas.
Titulação de anticorpos.

RESUMO

Este estudo foi conduzido para avaliar a resposta de crescimento, o conteúdo de lipoproteínas do sangue, os índices hepáticos e renais e a resposta humoral de extratos aquosos administrados com franga de *Lagenaria breviflora* Robert. Um total de noventa (90) pintos marrons Yaffa de quatorze (14) dias de idade foram usados para este experimento. As aves foram alocadas aleatoriamente em três (3) grupos de tratamento, contendo três (3) réplicas de dez (10) aves cada. Extratos aquosos de *Lagenaria breviflora* foram administrados no controle, 100 e 150g/litro, respectivamente. Aquoso de administração de Lagenaria breviflora Robert não teve efeito (P>0,05) sobre os índices de crescimento (peso final, ganho de peso, consumo total de ração e conversão alimentar) considerados. A lipoproteína de alta densidade (HDL) foi significativamente (P<0,05) influenciada nos grupos de 100 e 150g/litro apenas às 16 semanas de idade. Índices hepáticos (alanina aminotransferase e aspartato aminotransferase) foram (P<0,05) mais elevados em aves que receberam 150g/litro de grupo, enquanto o conteúdo de cálcio sérico foi maior no grupo de 100g em 16 e 8 semanas de idade, respectivamente. O maior título de anticorpos foi observado em 100g/litro e 150g/litro de *Lagenaria breviflora*. Pode ser recomendado que extratos aquosos possam ser administrados até 150g/litro para melhorar o estado de saúde e o estado imunológico das aves.

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INTRODUCTION

Plants play significant role in the health management system of local African communities and serve as the main source of therapy for the majority of the rural population (Adewunmi *et al.*, 2001, p. 19). Herbs, shrubs and trees are natural sources of organic chemical on earth, valuable in part or in whole in the treatment and management of diseases and health disorders. Their usage as primeval herbal remedies in Africa

is as a result of abundance of green resources. In recent years, there has been an increased awareness of the potentials that natural plant compounds have in the prevention and treatment of poultry diseases (Cross *et al.* 2007, p. 496; Ekunseitan *et al.*, 2017, 156). Plants produce variety of secondary metabolites, which are of great importance when used in animals and capable of inducing interesting biological and pharmacological actions (Ekunseitan *et al.*, 2016, p. 109).

However, the continued increase in the cost of drugs and crave by the populace for food of animal origin completely free from trace of drugs or chemicals necessitated the need to provide a viable alternative. Over the past few decades, emphasis has been made on enhancing the growth and production performances in the poultry industry which badly resulted in an adverse effect on the immunological parameters of poultry, thereby damaging the natural defensive mechanism against various microorganisms including viruses, bacteria, pathogenic fungi, ecto and endoparasites, various toxins etc. Due to incidence and development of antibiotic resistance by the bacteria and pathogenic microbes, researchers are now thinking towards immunomodulation.

Despite the abundant information on the beneficial effect of leaf and fruit extract of *Lagenaria breviflora* Robert and in-vitro antimicrobial properties in terms of resistance to disease from studies on white rats, no information exists pertaining to the effect of the fruit on lipoprotein content of blood and humoral responses of bird. Although, phytogenic sources have been documented to comprise compounds renowned to have cholesterol and lipoprotein decreasing effects (Ponte *et al.*, 2004, p. 810-814; Bolukbasi *et al.*, 2006, p. 190; Dehkordi *et al.*, 2010, p. 363-365), the component and use of ethno-medicine has long been ignored by many biomedical practitioners for various reasons: the chemical composition of plants, dosages and toxicity of plants are not clearly defined (Lowe *et al.*, 2000). It suffices to say that its broad-spectrum ability finds its continuous usage in health management of poultry by local rearers inevitable. Thus, it was the purpose of the experiment reported herein to explore the effect of aqueous spotted pumpkin extract (of *Lagenaria breviflora* Robert) source on a macro (performance) and micro-level (immune response).

MATERIAL AND METHODS

The experiment was carried out at the Teaching and Research Farms Directorate (TREFAD), Federal University of Agriculture, Abeokuta, Ogun State. The farm lies within latitude $7^{0}10^{I}$ N Longitude 3^{0} 2E and altitude 76mm (Google Earth, 2016).

A total of ninety (90) 14-days old Yaffa Brown chicks were used for the experiment. The birds were randomly allotted into three treatments containing thirty (30) birds each. Each treatment was further divided into three replicates of ten birds each.

Fresh weight of *L. breviflora* whole fruit was determined (100 and 150 g each), cut into bits, and soaked in a litre of fresh clean water for 24 hours (w/v) (Ekunseitan *et al.*, 2017, p. 155). Extracts were then administered to birds once a week over the duration of the experiment. *Lagenaria breviflora* extract was administered at 3 levels control (0), 100 and 150 g fresh weight. In the control *Lagenaria breviflora* was not given to the birds, but other conventional vaccinations and medication were given.

The experiment was divided into two phases: Chicks' phase: 14 – 56 days, Growers' phase: 57 - 112 days.

Performance indices such as feed intake, weight gain were measured and recorded on weekly basis while Feed:Gain ratio was calculated.

Birds were vaccinated with Newcastle Disease (ND) vaccine on day 28 and 70 (Minimum virus content per dose of vaccine is 105 EID50). Sera samples were collected on the 42^{nd} and 84^{th} day of age from three (3) birds per replicate (selected and tagged) for determination of antibody titre against ND vaccinations in birds. The test procedure was carried out as described by Allan and Gough (1974, p. 120-123).

Serum samples were harvested and used immediately for determination of serum metabolites following standard procedures. Three (3) ml of blood sample from three birds (selected and tagged) per replicate was collected with the aid of sterilised needle and syringes on the 56^{th} and 112^{th} days of age.

Serum calcium was determined using Moore *et. al.* (1974, p. 1458); potassium was evaluated according to description of Tietz *et. al* (1994). Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were determined spectrophotometrically according to the method of Reitman and Frankel (1957, p. 60) using Randox Kit. Serum biochemical analysis of blood was carried out to determine: Serum total cholesterol, triacylglycerol and High Density Lipoprotein (HDL) Cholesterol, Low Density Lipoprotein (LDL) Cholesterol and Very Low Density Lipoprotein (VLDL) Cholesterol were determined using Sigma enzymatic kits.

STATISTICAL ANALYSIS

Data collected were subjected to One-Way Analysis of Variance (ANOVA) in completely randomized design. Significant differences ($P<0.05$) among treatment means was separated using Duncan Multiple Range Test as contained in SAS (2009).

RESULTS AND DISCUSSION

The effect of *Lagenaria breviflora* Robert levels of administration on the performance indices of pullets is presented in **Table I**. No significant differences ($P>0.05$) was observed for all indices of performance measured in both phases of growth. However, Final weight gain and Weight gain per bird was numerically highest in 100 and 150g/L group, while feed conversion ratio was numerically lowest in pullet administered 100g/L LB at the chick phase. This effect may be a consequence of bioactive substances present in *Lagenaria breviflora* capable of actively influencing gut flora within the

Table I. Effect of Aqueous Dosages of *Lagenaria breviflora* R on Performance Indices of Birds (Efeito das dosagens aquosas de *Lagenaria breviflora* R sobre índices de desempenho de aves).

Parameters	Chicks' Phase			
	0/L	100g/L	150g/L	SEM
Initial Weight (g/bird)	106.67	106.67	106.67	0.83
Final Weight(g/bird)	561.67	586.67	575.00	5.74
Weight Gain(g/bird)	455.00	480.00	468.33	5.78
Total Feed Per Bird	1454.17	1416.33	1437.50	15.54
Feed Conversion Ratio(FCR)	3.20	3.00	3.07	0.06
Mortality	0.00	0.00	0.00	0.00
Parameters	Growers' Phase			
	0/L	100g/L	150g/L	SEM
Initial Weight (g/bird)	561.67	586.67	575.00	5.74
Final Weight(g/bird)	1219.33	1228.00	1214.00	6.81
Weight Gain(g/bird)	657.67	641.67	639.00	9.62
Total Feed Per Bird	3986.67	3936.67	3938.33	19.31
Feed Conversion Ratio(FCR)	6.08	6.14	6.17	0.09
Mortality	0.00	0.00	0.00	0.00

GIT resulting in statistically similar growth rate and performance in birds as observed in the study when compared to those in the control group. Extracts of phytobiotic plants have been studied and documented to positively affect nutrient digestibility, stimulating properties and improved growth performance of animals (Windisch *et al*., 2007). This result agrees with the findings of (Chakraborty *et al*., 2013, p. 15) who attributed the positive effects of extracts on nutrient digestibility, stimulating properties and improved growth performance of animals offered phytobiotics

The effect of *Lagenaria breviflora* Robert levels of administration on serum lipid profile of pullets is presented in **Table II.** The lipid lowering effect exhibited by the extract was as a result of the presence of phytochemical in the plant extract such as saponin as reported by (Jahan *et al*., 2011, p. 1003; Adeyemi *et al*., 2017, p. 1048) to have a lipid-lowering attributes by binding with bile salt and cholesterol in the intestinal wall causing a reduction of blood cholesterol and preventing its re-absorption into the bloodstream.

Increase in the level of administration extract of *Lagenaria breviflora* was accompanied with elevated serum triglyceride of the pullet administered extract at 100g/litre (P<0.05) while the highest dosage of the extract (150g/litre) resulted in a lowered triglyceride level all through the phases of growth. This decrease in the level of triglyceride might be due to a decrease in the activity of lipoprotein lipase in the muscle of the heart resulting in fewer uptakes of triglycerides from the circulation as reported by Sivakumar *et al.* (2007, p.72-75) and Kareem *et al.* (2009, p. 340). The action of extract also showed a high lipid effect believed to have arisen from the high triacylglycerol and fatty acids constituents of the fruit (Oshodi, 1996, p. 297, Esuoso and Bayer, 1998).

The significant increase in high density lipoprotein observed throughout the phase at 100 and 150g/Litre

Table II. Effect of Aqueous *Lagenaria breviflora* R. Fruit Extract Administration on Serum Lipid Profile of Pullets (Efeito da administração aquosa de extrato de frutas Lagenaria breviflora R. no perfil lipídico sérico de frangas).

Parameters	Weeks	Control	100g/litre	150g/litre	SEM
Total Cholesterol (mg/dl)	8	96.00	89.00	84.33	6.44
	16	86.67	84.00	83.00	5.15
Triglycerides (mg/dl)	8	87.00	96.33	88.67	4.29
	16	85.00^{b}	100.67^{a}	84.67^{b}	5.83
High-Density lipoprotein (mg/dl)	8	59.67	52.67	49.67	6.88
	16	43.33^{b}	51.00^{a}	53.33^{a}	3.90
Low-Density Lipoprotein (mg/dl)	8	18.93	17.07	16.93	2.25
	16	16.33	14.87	13.73	1.02
Very-Low-Density Lipoprotein (mg/dl)	8	17.40	19.27	17.73	1.20
	16	17.00	20.13	16.93	1.16

^{a,b}means the superscript differs significantly at (P<0.05) within the same row.

of the extract maybe due to the potential of the extracts as a repairing and maintenance of the membrane as a result of the presence of antioxidant, and polyphenols, capable of preventing the secretion of enzymes attributed to its free radical scavenging potential of the extract as observed by Karthikeyan *et al.* (2007, p. 298-299). These observations affirms the role HDL plays in the transport and metabolism of lipid hydro-peroxides in vivo resulting in its cardio-protective properties.

The level of low density lipoprotein declined at 100 and 150g/litre of the extract. Oladele *et al.* (1995) reported the presence of flavonoids, alkaloids to be the most potent lowering effect in many medicinal plant. Flavonoids directly scavenge some radical species and help in uptake of oxidatively modify LDL through scavenger receptors (Jahan *et al.*, 2011, Sharma *et al.*, 2007, p. 95-97) and likewise documented to inhibit lipid peroxidation formation in tissues and also inhibit the free radical production in the cells at various stages in rats (Karthikeyan *et al*., 2007, p. 297). Since continuous increase in HDL has the potential to limit oxidative modification of low density lipoprotein (LDL).

The rise in Total cholesterol and Triglycerides is associated with the increase in VLDL and decrease in HDL of pullets administered 100g/Litre *Lagenaria breviflora* extracts. The reduction in the value of VLDL was as a result of the presence of saponins which may be responsible for strong hypocholesterolemic effect. Saponins may interfere with cholesterol biosynthesis in the liver which may stop cholesterol absorption in the intestine (Jahan *et al*., 2011, p. 1004). Very Low density lipoprotein is an important indicator in determining the degree of fat in pullets or chickens (Whitehead and Griffin, 1984; Guo *et al*., 2011, p. 2030) thus, reduction in VLDL-C level at (150g/litre) may cause a decrease in the abdominal fat in pullet. Legrand and Hermier (1992) and Lee *et al.* (2003) indicate that the growth of adipose fat in birds depends directly on the VLDL-TG level.

The decrease in serum Triglyceride and low density lipoprotein and increase in high density lipoprotein level at 150g/litre treatment proved that its administration as a natural remedy may be the basis for lower triglyceride in the blood. Oladele *et al.* (1995) revealed that flavonoids and alkaloids are active phytochemical agents capable of reducing the level of triglyceride down significantly to that which is normal; hence, they proved a strong lipid lowering phytochemical agents. Sharma *et al.* (2007, p. 97) reported that there is a positive correlation between the quantity of flavonoids and increase in reduction potential of lipid profile.

The effect of aqueous extract of LB on liver and kidney indices of birds at 8 and 16 weeks of age is presented in **Table III**. All indices measured were not significantly ($P<0.05$) influenced except calcium at 8 weeks; ALT, Albumin and AST at 16 weeks of age. The measurement of these indices most especially ALT, AST and albumin gives clinical information of the effect and possible pathological damage to organs when herbs are administered (Ajani *et al.*, 2015, p. 210).

Calcium is an important electrolytes involved in maintenance of homeostasis and in the generation of electrical signals. Calcium level was significantly ($P<0.05$) influenced at 100 g/L LB administration compared to other groups at 8 weeks of age. This high value may not be an indication that the kidney was unable to clear excess electrolytes from serum since lowest value was obtained at highest dosage (150g/L LB). This falls in line with report of Saba *et al*. (2009, p 760-761) who observed no negative effect of *Lagenaria breviflora* on serum electrolytes; this therefore, invariably negates the impression of nephrotoxic effect of the extract.

Table III. Effect of Aqueous *Lagenaria breviflora* R. Fruit Extract Administration on liver and kidney indices of Pullets (Efeito da administração aquosa de Lagenaria breviflora R. extrato de frutas em índices hepáticos e renais de frangas).

Parameters	Control	100 g/l	150 g/l	SEM
Potassium (mg/dL)				
8 weeks	4.64	4.57	3.57	0.26
16 weeks	4.47	4.43	3.83	4.42
Calcium (mg/dL)				
8 weeks	9.03^{ab}	10.63^{a}	8.77^{b}	0.33
16 weeks	9.04	10.37	8.37	0.50
Albumin (g/L)				
8 weeks	1.97	2.17	1.87	0.13
16 weeks	2.67^{b}	3.43^{a}	2.90^{ab}	0.15
Alanine Aminotransferase (U/L)				
8 weeks	29.67	28.00	30.67	1.00
16 weeks	25.67^{b}	24.33^{b}	33.67^{a}	1.77
Aspartate Aminotransferase (U/L)				
8 weeks	61.33	59.67	60.67	1.81
16 weeks	55.00^{b}	51.00^{b}	67.00^{a}	3.00

^{a,b}means the superscript differs significantly at ($P<0.05$) within the same row.

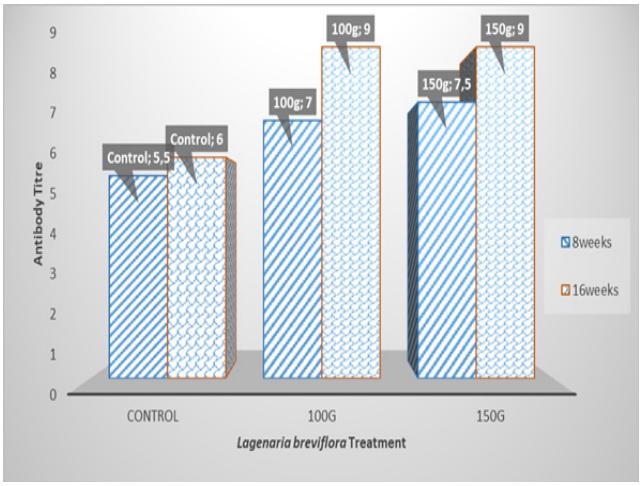

Figure 1. Effect of Aqueous Lagenaria breviflora R. Fruit Extract Administration on humoral antibody titre of Birds (Efeito da administração de extrato de frutos aquoso *Lagenaria breviflora* R. em títre de anticorpos humoral de aves).

The non-significant effect of LB at lower dosages of the extract on serum AST and ALT suggest no disruption in the biological function of liver and a clear indication it will not alter the metabolic and regulatory role of the liver compared to 150g/L since they both serve as the diagnostic markers for possible leak out from impaired tissue to blood stream when cell membrane becomes compromised. Values of AST and ALT observed at 150g/L at 16 weeks though significantly higher compared to other groups at both phases of growth could indicate possible damage to the plasma membrane and subsequent disruption of the integrity of the membrane (Yakubu *et al.,* 2003) if dosage is maintained over a long period of time.

The effect of *Lagenaria breviflora* R fruit extract administration on humoral antibody titre of pullets is presented in **Figure 1**. Extracts at 100 and 150g/litre were found to be substantially and significantly ($P<0.05$) effective in enhancing the humoral immune response of pullets. The treatment of 100 and 150g/litre of the extract increased the antibody production and enhanced the ability of the macrophages. Result of the study presented in **Figure I** revealed that LB possesses promising immunomodulatory activity as it increased antibody titre against Newcastle disease. Its flavonoids and phenol content (Ekunseitan *et al.*, 2017, p. 162) are important bioactive molecules that possess the immuno-modulatory potentials (Sharma *et al.*, 2007*)* and also capable of significantly increasing haemagglutination antibody titre as observed in the study.

Macrophages play an important role in innate and adaptive immunity against disease (Jahan, 2011, p. 1001-1002). Okwor *et al.* (2011) reported that vaccination of bird is practiced to provide some degree of protection against disease. The antigen will stimulate the bird's immune system which response effectively in controlling losses due to infections. Cornax *et al*. (2012, p. 465-466), Dortmans *et al.*(2012) and Kapczynski and King (2005) suggested that if the vaccines are viable and administered correctly to healthy birds and appropriate time is allow for immune response to developed prior to exposure to the challenge virus the bird will develop an active immunity.

The increase in antibodies production could be due to the immuno-stimulatory effect of *Lagenaria breviflora*. Antibodies are specific immune proteins and their production is the major function of immune system as they have the capacity to combine with antigens that can activate immune response, this correspond with the report of Saba *et al*. (2009, p. 760) that *Lagenaria breviflora* enhanced immunity especially the cell-mediated immunity thereby effective in enhancing the stimulation of antibody production.

CONCLUSION

In conclusion, present study demonstrated that administration of *Lagenaria breviflora* R whole fruit at 100g/L dosage is safe on serum liver, kidney and protein indices of birds. In addition, the 100 and 150g/litre of the extract increase dose dependently in the entire treatment group eliciting immune response to ND vaccination as shown in their mean HI antibody titre values.

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