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Serum biochemical assay of broiler chickens administered water containing various medicinal plant leaf methanol extract

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Abstract

Bans on the use of antibiotics as feed additives have accelerated and led to investigations of alternative feed additives in animal production. To this end, the response of broiler chickens fed various medicinal plants methanol extract as a replacement for antibiotics was investigated. A total of 180 unsexed Ross strain broiler chickens were randomly assigned to four available plant leaf extract namely, Gercinia kola (Bitter Kola), Alcornea cordifolia (Christmas bush), Pterocarpus santalinoides (Red scandal wood) and Chromolaena odorata (Hagony or Siam weed). Each treatment group had 30 birds each. The treatments were replicated thrice with 10 birds per replicate in a completely randomized design. Feed and water were provided ad libitum throughout the experiment which lasted for 56 days. Serum biochemical indices of the broiler chickens were evaluated. Significant differences ($p<0.05$) were observed in the mean values of all the parameters measured with the exception of total protein and globulin. However, the values (4.75 – 6.65g/dl) obtained did not reveal any health problem. In conclusion, the findings of this study showed that the medicinal plant methanol extracts have considerable potentials as component of broiler chicken diet. Alcornea cordifolia plant methanol extract can successfully be used to replace antibiotics for broiler production. Further research should be carried out on Alcornea cordifolia and other medicinal plants to examine their potentials and inhibitory characteristics.

Keywords: Serum biochemical indices, medicinal plant, methanol extract, *Gercinia kola, Alcornea cordifolia, Pterocarpus santalinoides, Chromolaena odorata*

Dosage biochimique sérique des poulets à griller administrés de l'eau contenant divers extrait de méthanol de feuille de plante médicinale

Résumé

Les interdictions sur l'utilisation d'antibiotiques sous forme d'additifs alimentaires ont été accélérées et ont conduit à des enquêtes d'additifs alternatifs d'alimentation dans la production animale. À cette fin, la réponse des poulets à griller nourria divers extrait de méthanol de plantes médicinales en remplacement des antibiotiques a été étudiée. Un total de 180 poulets de gril à Ross de Ross nonxé ont été attribués au hasard à quatre extraits de feuilles plantes disponibles, à savoir Gercinia Kola (Kola amère), Alcorna cordifolia (Bush de Noël), Pterocarpus santalinoides (bois de scandale rouge) et Chromolaena odorata (Hagonie ou mauvaise herbe d'Siam). Chaque groupe de traitement comportait 30 poulets chacun. Les traitements ont été reproduits trois fois avec 10 oiseaux par réplication dans une conception complètement randomisée. L'alimentation et l'eau ont été fournies publicitaires

dans l'expérience qui a duré 56 jours. Des indices biochimiques sériques des poulets de poulets à griller ont été évalués. Des différences significatives (p <0,05) ont été observées dans les valeurs moyennes de tous les paramètres mesurés à l'exception de la protéine totale et de la globuline. Cependant, les valeurs (4,75 - 6,65 g / dl) obtenues n'ont révélé aucun problème de santé. En conclusion, les conclusions de cette étude ont montré que les extraits de méthanol de méthanol de l'usine médicinale ont des potentiels considérables en tant que composant du régime de poulet au poulet. L'extrait de méthanol d'usine d'Alcorna cordifolia peut être utilisé avec succès pour remplacer les antibiotiques pour la production de poulets à gril. Des recherches supplémentaires devraient être menées sur Alcorna cordifolia et d'autres plantes médicinales pour examiner leurs potentiels et leurs caractéristiques inhibitrices.

Mots-clés: indices biochimiques sériques, plante médicinale, extrait de méthanol, gercinia kola, alcorna cordifolia, pterocarpus santalinois, chromolaena odorata

Introduction

The use of antibiotics as growth promoters in animal nutrition is facing reduced social acceptance due to the appearance of residues and resistant strains of bacteria; antibiotic use has been banned in the European Union since January 2006. Natural feed additives of plant origin are generally believed to be safer, healthier, and less subject to hazards for humans and animals. Many herbs and plant extracts have antimicrobial activities and antioxidant properties which make them useful as natural animal feed additives (Faixova and Faix, 2008). Nutrition has been a major determinant of profit in livestock business as feeding accounts for about 70-80% of the costs of producing livestock, especially, poultry (Onunkwo *et al*., 2018). High cost of feed has been one of the key factors militating against the development of poultry, resulting from a cumulative effect of cost of feed ingredient used in feed production. In Nigeria, concerted efforts have been given to alternative macro feed ingredients especially from agro-industrial by-products and feedstuffs to solve this costly feed problem. These ingredients have been used mainly to resolve the high cost of energy and protein ingredient in livestock production. Medicinal plant aqueous extract, an important additive which contribute substantially to animal well-being in terms of providing essential micro-nutrients and improved gut performance have however not received same attention.

Many of the medicinal plants commonly available have not been scientifically studied to validate the efficacy and to identify the phytochemical constituents that may be responsible for their medicinal values. Only limited studies have been conducted to investigate the biochemical profile of broiler chickens administered water containing medicinal plant (*Gercinia kola, Alcornea cordifolia*, *Pterocarpus santalinoides* and *Chromolera odorata*) methanol extract. In consequence present study was arranged to evaluatethe serum biochemical assay of broiler chickens administered four medicinal plant (*Gercinia kola, Alcornea cordifolia*, *Pterocarpus santalinoides* and *Chromolera odorata*) methanol extract.

Materials and methods

This study was carried out in the Poultry unit of the Teaching and Research farm, Michael Okpara university of Agriculture, Umudike, Abia State. Umudike is located on latitude 05^{o} $21^{'}$N and longitude 07^{o} $33^{'}$E, with an elevation of about 112m above sea level. The location has an annual rainfall of

177 - 2,000mm per annum, (April to October) and a short period of dry season (November to March) with a relative humidity of about 50-90% and monthly temperature range of 17°C – 36°C (NRCRI, 2019).

The fresh leaves of the medicinal plants investigated (*A. cordifolia*, *C. odorata*, *P. Santalinoides* and *G. kola*) were collected within Michael Okpara University of Agriculture, Umudike and air dried for two weeks under shade. The dried parts were pulverized to fine powder using a mechanical grinder, sieved,and weighed.A total of 500g of each of the powdered plant materials was soaked in 1500ml of methanol for 24h at room temperature. The extracts were filtered using non-adsorbent muslin cloth into a clean beaker. The filtrate was dried by evaporating off the solvent at 50°C in a hot air oven over a period of one to two days. The experimental design for the experiment was completely randomized design with medicinal plant methanol extract as the only factor of interest. The study lasted for 56 days.

A total of 180, one-day-old Ross strained unsexed chicks were weighed and randomly allotted to six equal treatment groups (T1, T2, T3, T4, T5 and T6) each having 30 chicks. Each treatment was replicated three times of 10 chicks per replicate. T1 was the positive control, T2(Negative control), T3 (*Chromolaena odorata)*, T4 (*Pterocarpus santalinoides*, T5 (*Alchornea cordifolia)*and T6 (*Garcinia kola)*. Administration of medicinal plant methanol extract in their drinking water (1g/l of water) commenced the first day of the experiment. Feed and water were given *ad libitum* throughout the experiment. The gross composition of the experimental diet is presented in Table 1. Blood samples were collected from one bird randomly selected from each replicate per treatment for the evaluation of serum biochemical profile. Blood collection was carried out by using a sterile needle to puncture the right jugular vein, and blood drawn into the syringe. Serum was separated for determination of albumin globulin ratio, total cholesterol, creatinine, urea, ALT and AST using commercial kits. The SOD activity was determined using the Bio Vision-Superoxide Dismutase Activity Assay Kit. This sensitive SOD assay kit utilizes WST-1 to produce a water-soluble Formosan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to xanthine oxidase (XO) activity and is inhibited by SOD. Therefore, the inhibitory activity of SOD can be determined via a colorimetric method. The results were expressed as the inhibition rate (%). Other biochemical parameters were measured using a Roche Cobas Integra 400 Plus autoanalyzer (Roche Diagnostics, GmbH, Mannheim Germany). Data generated were subjected to analysis of variance (ANOVA) and treatment means were separated using Duncan's Multiple Range Test (Duncan, 1955) at $\alpha_{0.05}$, according to Steel and Torrie (1980) using computer software IBM SPSS Statistic version 20 (SPSS, 2012).

Table 1: Percent ingredients and nutrient composition of experimental diet

Ingredients	Starter	Finisher
Maize	48.00	57.00
Soyabean meal	31.00	23.00
Fishmeal	3.00	3.00
Palm kernel meal	10.20	9.30
Wheat offal	4.00	4.00
Bone meal	3.00	3.00
Salt	0.25	0.25
Lysine	0.20	0.10
Methionine	0.10	0.10
Vit/Min premix	0.25	0.25
Total	100	100
Nutrient composition		
Crude protein (CP) (%)	22.98	19.63
Metabolizable energy (kcal/kg)	2883.22	2940.96

Results and discussion

The serum biochemical profile of broiler chickens fed various plants methanol extract is presented in Table 2. Significant ($P<0.05$) differences were observed in albumin, urea, creatinine glucose, aspartate transference and alanine triphosphatase while in total protein, globulin and total cholesterol no significant differences were recorded ($p>0.05$). Albumin count was within the range of 2.23 – 3.24 g/dL obtained from treatments 1 and 4, respectively. Though significantly differences were not observed in treatments 2, 3, 4, 5 and 6 ($p>0.05$) but significantly different from treatment 1 ($P<0.05$). Except for treatments 3 and 6, which had a value slightly higher than the normal range of values, other treatment groups' fell within the range of 1.3 – 2.3 g/dL (Aiello and Mays, 1998). When protein intake exceeds the amount required for growth and maintenance, these probably results in increased level of serum albumin in indigenous chicken (Aiello and Mays, 1998). The level of urea in the serum is an excellent indicator of kidney function (Sakas, 2002). Significant differences were not observed ($p>0.05$) in treatments 1, 2, 3, 5 and 6 but significantly different ($p<0.05$) from treatment 4. However, the values obtained ranged from 22.73 – 31.33 mg/dl were above the normal range (2.5 – 8.1 mg/dl) reported (Aiello and Mays,1998). In all the treatment groups, it shows that this effect cannot be attributed to the test feedstuff. However, Ajagbonna *et al.* (1999) stated that high urea level suggests increase in urea enzymes activities (ominthine, carbonyl transferase and originase) which results in prerenal azotemia (observed in dehydrated birds). Serum creatinine and urea are waste products used to assess kidney function. The level of creatine and blood protein depends on the quality of dietary protein (Awosanya *et al*., 1999). However, significant differences were not observed ($p>0.05$) in treatment groups of 1 and 4 but significantly different ($p<0.05$) from treatments 2, 3, 5 and 6 which in turn did not record any significant difference ($p>0.05$) within them. Furthermore, the values obtained were within the range of 0.34 – 0.89 mg/dL which was slightly lower than the normal range for a healthy chicken of 0.9 – 1.8 mg/dl (Aiello and Mays, 1998). Campbell (2013) reported that lower value could also indicate good absorption and utilization of protein in the diet.

It was observed that glucose value obtained in this study (9.7.30 - 221.93 mg/dL) were significantly different ($p<0.05$) though treatments 1, 2 and 4 did not have any

significance ($p>0.05$) but significantly different ($p<0.05$) from treatments 3, 5 and 6 which in turn did not differ significantly ($p>0.05$) within them. The values obtained in this result except for Treatment 1 were within the range of126-204 mg/dL for a healthy bird (Campbell, 2013). Treatment 1 which had an increased level of glucose above the normal range could be due to stress (Hassan *et al*., 2017). *Aspartate transferase* is an enzyme present in very high amounts in the liver. It is one of the more reliable indicators of liver disease in birds raised in an enclosure (Agbede *et al*., 2011). The reduction in *Aspartate transferase* activities when compared to the positive and negative control groups is an indication that the antibiotics in medicinal plant methanol extracts had no toxic effect on the liver of the birds as elevation in the activity of these enzymes is associated with liver disease (Agbede *et al*., 2011) which agrees with the report of Hassan *et al*. (2017).

Cholesterol serves as an intermediate in the biosynthesis of all steroids and this is essential to life. In as much as that total cholesterol did not record any significant difference ($p>0.05$), the values obtained were ranged from 114-28 – 342.35 for treatments 2 and 3, respectively. The values obtained in this present study were within the range of values reported by Aiello and Mays (1998). Among the treatment groups fed various medicinal plant extract, treatment 5 recorded the least value when compared to the positive control group (treatment 1). This observation agreed with the report of Hassan *et al*. (2017) that reduction in cholesterol level is associated *Alchornea cordifolia* inclusion in the diet. Increased activities of AST and ALT in the serum are well known diagnostic indicators of liver injury. However significant differences did not exist ($p>0.05$) among the treatment groups except for Treatment 1 which was significantly different ($p<0.05$) from the other treatment groups. Furthermore, values obtained fell within the range of 126.17 – 211.80 U/l for Treatments 1 and 5, respectively. Result revealed that treatments were not significantly affected by the plant methanol extracts administration, suggesting that the test material did not pose any serious deleterious health challenges to the animals.

Table 2: Serum biochemistry of broiler chicken fed medicinal plant methanol extract

Parameters	Treatments						
	T1	T2	T3	T4	T5	T6	SEM
TP (g/dl)	5.45	4.75	6.65	5.40	4.65	5.20	0.35
ALB (g/dl)	2.23^{b}	2.42^{ab}	2.77^{ab}	3.24^{a}	2.89^{ab}	3.03^{ab}	0.13
GLO (g/dl)	3.22	2.33	3.88	2.16	1.90	2.17	0.32
TCHL (g/dl)	276.18	114.28	342.85	285.71	142.85	257.14	33.53
UREA (mg/dl)	28.20^{ab}	26.63^{ab}	29.80^{ab}	22.73^{b}	31.33^{a}	26.63^{ab}	1.07
CREA (mg/dl)	$0.78a^{b}$	0.54^{abc}	0.34^{c}	0.89^{a}	0.45^{bc}	0.46^{bc}	0.63
GLU (mg/dl)	221.93^{a}	194.10^{ab}	97.70^{b}	141.63^{ab}	98.43^{b}	96.20^{b}	15.72
AST (U/L)	163.75^{a}	182.31^{a}	55.83^{b}	50.89^{bc}	32.27^{bc}	22.48^{c}	15.95
ALT (U/L)	26.17^{b}	121.18^{ab}	99.93^{ab}	197.47^{a}	211.80^{a}	182.64^{a}	20.19

$^{a\text{-}b\text{-}c}$:Means along the same row with different superscripts are significantly ($p<0.05$) different. S.E.M= Standard Error of Mean. TP=Total protein; ALB=Albumin; GLO=Globulin; CREA=Creatinine; GLU=Glucose; AST= Aspartate Transferase; ALT= Alanine Triphosphatase; T CHL=Total Cholesterol

Conclusion

Findings of this study suggested that medicinal plant methanol extracts possess considerable potentials as component of broiler chicken diet. *Alchornea cordifolia* plant methanol extract could successfully replace antibiotics in broiler chicken production. Further research should be carried out on *Alchornea cordifolia* and other medicinal plants to assess their potentials and inhibitory characteristics.

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