APRW -61

Haematological and Serum Biochemical Indices of Finisher Broilers Fed Varying Dietary Levels of *Artemisia annua* (Sweet Wormwood)

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Abstract
A 4-week study was conducted to evaluate the haematological and serum biochemical indices of finisher broilers fed varying dietary levels of *Artemisia annua* (sweet wormwood). A total of ninety 28- day old finisher broiler were randomly allocated to five groups and fed diets containing five levels of *A. annua* (0, 50, 100, 150 and 200 g per kg of diet, respectively). Each group was replicated three times with six birds per replicate in a completely randomized design (CRD). The results showed that birds fed diet 1 (control) had significantly ($p<0.05$) lower WBC value ($33.67x10^3/\mu l$) than those fed other diets. The RBC, PCV and Hb values for birds fed diet 2 (diet containing 50 g of *A. annua* per kg of diet) were significantly ($p<0.05$) higher than the RBC, PCV and Hb values for birds fed diet 5 (diet containing 200 g of *A. annua* per kg of diet). There were no significant ($p>0.05$) differences among treatments in the MCHC, MCH and MCV of the birds. The total protein (TP) value (4.50 g/dl) for broilers fed diet 1 (control) was significantly ($p<0.05$) higher than that (3.70 g/dl) of birds fed diet 5. The albumin, serum globulin, urea, cholesterol, low-density lipoprotein and high-density lipoprotein values for birds fed the different diets did not differ significantly ($p>0.05$). It was concluded that *A. annua* leaf meal can be included in the diet of finisher broilers at 200 g per kg of diet without any deleterious effect on their haematological indices and at 150 g per kg of diet without any adverse effect on the serum TP of birds.

Keywords: Broiler, Sweet Wormwood, *Artemisia annua,* Haematology

Introduction

Poultry production is one of the fastest means of achieving appreciable improvement in the nutritional standard of the populace because poultry has short generation interval and its production has quick turnover rate and relatively low capital investment (Smith, 2001; Ani and Okeke, 2011). To increase productivity, large commercial holdings use feed additives such as pre-, pro- and phytobiotics. Pre- and probiotics are commercial biological agents that improve gut health and enhance the digestion, absorption and utilization of nutrients (Patterson and Burkholder, 2003; Ricke, 2003). Phytogenic feed additives (often called phytobiotics or botanicals) are plant-derived compounds, which are incorporated into diets of livestock in order to improve the feed properties, productivity, reproduction, and quality of animal products (Windisch *et al.,* 2008).

Commercial phytobiotics are however not available to the small-scale poultry farmers especially in the rural areas. Besides, the ban on antibiotic feed additives (Yang *et al.,* 2009), necessitated the search for alternative growth promoters that are readily available and affordable to the farmers. Such materials would perform similar functions as the commercial feed additives without adverse effects associated with indiscriminate use of antibiotics, namely the development of antibiotic-resistant microbial organisms. Herbs and plant spices have been used by mankind for various purposes. They not only provide food but also prevent and cure different ailments. In modern animal feeding, they can be used as natural antimicrobial agents and growth promoters as well as vitamin/mineral supplements (Charis, 2000; Tipu *et al.,* 2006).

The present study was therefore conducted to evaluate the haematological and serum biochemical indices of finisher broilers fed varying dietary levels of *Artemisia annua* (sweet wormwood).

Materials and Methods

The study was carried out at the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. Five broiler starter experimental diets were formulated to contain 0, 50, 100, 150 and 200 g, respectively of *A. annua* per kg of diet. The percentage composition of the diets is shown in Table 1, while the proximate composition of broiler finisher diets is shown in table 2. A total of 90 two weeks old broiler chicks (Arbor Acre strain) were used for the study which lasted eight weeks. The birds were randomly divided into five treatment groups of 18 birds each. The groups were randomly assigned to the five experimental diets containing 0, 50, 100, 150 and 200 g, respectively of *A. annua* per kg of diet in a completely randomized design (CRD). Each treatment was replicated three times with 6 birds per replicate and housed in 2.6 x 3 m deep litter pens of fresh wood shavings. The birds were properly vaccinated as and when due. Feed and water were offered *ad libitum* every morning, from7.00 to 8.00 am.

At the 8th week of the experiment, three birds per treatment were randomly selected and blood samples were collected from the wing veins of each bird using a sterilized syringe and emptied into sterilized bottles containing EDTA (Ethylene diamine tetracetic acid) for haematological analysis, and into the bottles without EDTA for serum biochemical analysis. Haematological parameters that were determined included haemoglobin concentration (packed cell volume (PCV), white blood cell (WBC) count, and red blood cell (RBC) count .The PCV was determined by the micro haematocrit method described by Schalm *et al.* (1975), and Mitruka and Rawnsely (1977) using a micro haematocrit centrifuge and reader (Hawksley and Sons Ltd, England). The Hb was determined using a haemoglobin meter (Marienfeld, Germany), while the WBC counts were carried out by the haemocytometer method using an improved Neubauer counting chamber (Hawksley, England) and avian RBC and WBC diluting fluids as described by Campbell and Coles (1986) and Lamb (1991). Serum metabolites (total protein (TP), albumin, globulin, urea and cholesterol) were also determined according to the methods described by Campbell and Coles (1986).

Data collected were subjected to analysis of variance (ANOVA) for completely randomized design (CRD) as described by Steel and Torrie (1980) and according to the procedure for a one-way analysis in a completely randomized design using a Stat Graphic Computer Package (SPSS, 2007) Model. Significantly different means were separated using Duncan's New Multiple Range Test (Duncan, 1955).

Table 1: Percentage composition of broiler finisher diets

	Diets				
Ingredients	1	2	3	4	5
Maize	38.00	38.00	38.00	38.00	38.00
Wheat offal	16.00	15.95	15.90	15.85	15.80
Soya bean meal	10.00	10.00	10.00	10.00	10.00
Groundnut cake	12.00	12.00	12.00	12.00	12.00
Palm kernel cake	17.00	17.00	17.00	17.00	17.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Artemisia annua	0.00	0.05	0.10	0.15	0.20
Bone meal	4.00	4.00	4.00	4.00	4.00
Salt	0.25	0.25	0.25	0.25	0.25
Methiomine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Vit. M. P.	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis:					
Crude Protein (%)	20.02	20.02	20.03	20.04	20.04
Crude fibre (%)	5.90	5.90	5.89	5.89	5.89
Energy (Kcal of ME/kg)	2,900	2,900	2,982	3,031	3,079

*Vit A – 10,000.00 iu., D_3-2,000 iu., B_1-0.75 g., B_2-5 g., Nicotinic acid – 25 g., Calcium pantothenate 12.5 g., B_{12}-0.015 g., K_3-2.5g., E-25 g., Biotin – 0.050 g., Folic acid –1.0 g., Manganese 64 g., Choline chloride 250 g., Cobalt-0.8 g., Copper 8 g., Manganese 64 g., Iron –32 g., Zn-40 g., Iodine-0.8 g., Flavomycin-100 g., Spiramycin 5 g., DL-methionie-50 g, Selenium 0.6 g., Lysine 120 g., BA

Table 2: Proximate composition of broiler finisher diets

	Diets				
Components (%)	1	2	3	4	5
Dry matter	90.00	91.50	90.5	91.50	90.00
Crude protein	20.08	20.02	20.05	20.03	20.05
Crude fibre	5.50	6.00	6.50	6.80	6.90
Ash	7.80	8.30	8.30	8.30	7.30
Ether extract	3.50	2.50	3.50	3.50	3.50
Nitrogen-free extract	53.12	63.18	61.65	61.37	62.25

Results and Discussion

Data on the haematological indices of birds fed varying dietary levels of *Artemisia annua* leaf meal are shown in Table 3. The inclusion of varying levels of *A. annua* in the diets of birds had significant ($p < 0.05$) effects on the Hb, RBC, PCV and

WBC of birds. Birds fed diet 1 (control) had significantly ($p<0.05$) lower WBC value ($33.67x10^3/\mu l$), than those fed other diets (treatments 2 to 5) which had similar WBC values. The RBC, PCV and Hb values (5.20×10^6/ul, 31.20% and 10.40g/dl, respectively) of Birds fed diet 2 (diet containing 50 g of *A. annua* per kg diet) were significantly ($p<0.05$) higher than the RBC, PCV and Hb values ($27 \times 10^6/\mu l$, 25.63% and 8.54 g/dl, respectively) of birds fed diet 5 (diet containing 200 g of *A. annua* per kg of diet). Howevre, these were similar to those of birds fed diets 1 (control), 3 (diet containing 100 g of *A. annua* per kg of diet) and 4 (diet containing 150 g of *A. annua* per kg of diet). Bird fed the control diet (treatment 1) had similar RBC, PCV and Hb values with those fed diets 3, 4 and 5.

Table 3: Haematological indices of finisher broilers fed varying dietary levels of *Artemisia annua*

Parameters/Treatments	1	2	3	4	5	SEM
WBC($10^3/\mu l$)	33.67^b	78.00^a	76.00^a	66.00^a	67.00^a	4.73
RBC($10^6/\mu l$)	4.87^{ab}	5.20^a	4.71^{ab}	4.83^{ab}	4.27^b	0.11
PCV (%)	29.17^{ab}	31.20^a	28.23^{ab}	28.57^{ab}	25.63^b	0.68
HB (g/dl)	9.56^{ab}	10.40^a	9.41^{ab}	9.67^{ab}	8.54^b	0.22
MCV	60.00	60.01	60.01	60.01	59.99	0.03
MCH	20.01	20.01	20.01	20.01	19.99	0.00
MCHC	33.33	33.34	33.34	33.33	33.33	0.00

^{ab}Means on the same row with different superscripts are significantly ($p<0.05$) different. SEM = Standard error PCV= Packed Cell Volume, HB = Haemoglobin, RBC = Red blood cell, WBC = White blood cells MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration

There were no significant differences ($p>0.05$) among treatments in the MCHC, MCH and MCV of the birds. Table 4 shows data on serum biochemical indices of birds fed varying dietary levels of *Artemisia annua* leaf meal. The mean total protein (TP) values for broilers fed diets 2, 3 and 4 (4.33, 4.07 and 3.77 g/dl, respectively) were similar ($p > 0.05$) to those of birds fed diet 1 (control) and diet 5. The TP value (4.53 g/dl) for birds fed diet 1 (control treatment) was significantly ($p<0.05$) higher than that (3.70 g/dl) of birds fed diet 5 (diet containing 200 g of *A. annua* per kg of diet). The albumin, serum globulin, urea, cholesterol, low-density lipoprotein and high- density lipoprotein values for birds fed the different diets did not differ significantly ($p>0.05$). As shown in Table 3, the PCV, RBC, WBC and Hb were significantly affected by dietary treatments. The inclusion of *A. Annua* in the birds' diets resulted in significant increase in the WBC of birds. The WBC of the birds might have increased as a defensive mechanism. White blood cells or leucocytes are the cells of the immune system. They defend the body against pathogens, infection and foreign materials (Schalm *et al*., 1975). The lymphocytes in particular are the immunological competent cells that assist the phagocytes in defence of the body against infection and other foreign invasion (Hoffbrand *et al*., 2006).

Table 4: Serum biochemical indices of finisher broilers fed varying dietary levels of Artemisia *annua*

Parameters/Treatments	1	2	3	4	5	SEM
PROT(g/dl)	4.53^a	4.33^{ab}	4.07^{ab}	3.77^{ab}	3.70^b	0.12
ALB (g/dl)	1.87	1.77	2.10	1.80	2.13	0.13
GLO (g/dl)	2.73	2.47	1.70	1.93	2.83	0.18
URE (mg/dl)	8.33	8.00	6.00	8.67	7.33	0.64
CHOL (mg/dl)	125.67	114.33	126.00	139.00	169.00	50.62
LDL (mg/dl)	56.67	52.00	51.00	59.00	53.33	3.38
HDL (mg/dl)	57.67	62.33	85.00	88.67	65.00	5.64

^{ab}Means on the same row with different superscripts are significantly($p<0.05$) different. SEM = Standard error of the mean, PROT = Protein, ALB = Albumin, GLO = Globulin, URE = Urea, CHOL = Cholesterol, LDL = Low density lipoprotein, HDL = High density lipoprotein.

The PCV, RBC, WBC and Hb of birds were not affected adversely by the inclusion of varying levels of A. *Annua* in the birds' diets (table 3). These blood parameters are the major indices of physiological, pathological, and nutritional status of an organism and changes in the constituents of blood when compared to normal values could be used to interpret the metabolic state of an animal (Babatunde *et al*., 1992; Maxwell *et al*., 1990). The RBC in particular are the most sensitive targets for toxic compounds and constitute an important index of physiological and pathological status in man and lower animals (Mukinda and Syce, 2007).

Conclusion

It is evident from the results obtained in the present study that *A. annua* leaf meal can be included in the diet of finisher broilers at 200 g per kg of diet without any deleterious effect on their haematological indices and at 150 g per kg of diet without any adverse effect on the serum total protein value of birds.

References

Ani, A.O and Okeke, G.C. (2011). The performance of broiler birds fed varying dietary levels of roasted pigeon pea (*Cajanus cajan* seed meal. Pakistan Journal of Nutrition. 10(11): 1036-1040.

Babatunde, G.M., Fajimi, A.O. and Oyejide, A.O. (1992). Rubber seed oil versus palm oil in broiler chicken diets. Effects on performance, nutrient digestibility, haematology and carcass characteristics. *Animal Feed Science and Technology*, 35: 133–146.

Campbell, T.W. and Coles, E.H. (1986). Avian clinical pathology. In: Coles E.H (ed). *Veterinary clinical pathology,* 4th edn. Saunders, Philadelphia. Pp: 279-291.

Charis, K. (2000). A novel looks at a classical approach of plant powder. *Feed Mix (special issue on Nutraceuticals),* 19-21.

Duncan D.B. (1955). Multiple Range and Multiple F-test. *Biometrics,* 11:1-42.

Hoffbrand, A.V., Moss, P.A.H. and Pettit, J.E. (2006). *Essential haematology*, 5th ed.

Lamb, G.N. (1991). *Manual of veterinary laboratory technique*. CIBA – GEIGY, Kenya.

Maxwell, M.H. Robertson, W., Spener, S. and Maclorquodale, C.C. (1990) "Comparison of haematological parameters in restricted and *ad libitum* fed domestic fowls. *British Poultry Science*, 31: 407–413.

Mitruka, B.M. and Rawnsley, H.M. (1977). *Clinical biochemical and haematological reference values in normal experimental animals.* Masson Pub. USA Inc., N.Y.

Mukinda, J.T. and Syce, J.A. (2007). Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. *Journal of Ethnopharmacology*, 112: 138-44.

Patterson, J.A. and Burkholder, K.M. (2003). Application of prebiotics and probiotics in poultry production. *Poult. Sci.,* 82: 627–631.

Ricke, S.C. (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult. Sci. 82:632–639.

Schalm, O.W., Jain, N.C. and Caroll, E.J. (1975). *Veterinary haematology*, 3rd edn. Lea and Febiger, Philadelphia.

Smith, A.J. (2001). *Poultry. The Tropical Agriculture, 2*nd (revised) ed. Macmillan education ltd., UK.

SPSS.Com. (2007).IBM(R) SPSS(R) Advantage for Microsoft (R) IBM. Corporation 2010, IBM Corporation Route, 100, Somers, N4. 10589.

Steel, R.G.D. and Torrie, J.H. (1980). *Principles and procedures of statistics. A biometric approach,* (2nd ed.). McGraw-Hill Publishers, New York.

Tipu, M.A.., Akhtar, M.S.., Anjum, M.I. and Raja, M.L. (2006). New dimension of medicinal plants As Animal feed. *Pakistan Vet. J.,* 26(3): 144-148.

Windisch, W., Schedle, K., Plitzner, C. and Kroismayr, A. (2008). *Use of phytogenic products as feed additives for swine and poultry. J. Anim. Sci.,* 86 (E. Suppl.): E140–E148

Yang, Y., Iji, P.A. and Choct, M. (2009). Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World Poult. Sci. J.,* 65: 97–114.