DIETARY EFFECTS OF NEEM (*Azadirachta*indica) LEAF MEAL ON BROILERS PERFORMANCE AND SERUM BIOCHEMICAL INDICES

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Abstract
One hundred Cobb unsexed day-old broiler chicks managed and reared to finisher stage were used to evaluate the dietary effect of Neem leaf meal (NLM) on broilers performance and serum biochemical indices. A total of five dietary treatments designated T1, T2, T3, T4 and T5 which constitute the control (0%), 1%, 2%, 3% and 4% neem leaf meal inclusions respectively were utilized, and ten birds were randomly assigned to each replicate in the five treatments comprising of four replicates. Results showed that initial body weight, weight gain and feed efficiency were not significantly different ($P>0.05$). Mortality value was significantly higher ($P<0.05$) in treatment 5, than other treatments. Aspartate aminotransferases (AST) values in neem leaf meal treated groups were significantly different ($P<0.05$)from the control, whereas no significant difference ($P>0.05$) was observed for Alanine aminotransferases (ALT). Treatment 2 was significantly dissimilar ($P<0.05$). in the Alkaline Phosphatase level, when compared to the other treatments Total cholesterol and triglyceride levels increased with higher NLM inclusion levels, and were significantly different ($P<0.05$) from the control. High Density Lipoprotein levels reduced significantly ($P<0.05$) with increased levels of NLM. Low Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL) values also showed significant difference ($P<0.05$) amongst the treatment groups. The results, generally shows that Neem Leaf Meal (NLM) did not significantly improve broiler performance, and HDL levels reduced significantly in NLM treated groups, whereas Very Low-Density Lipoprotein (VLDL), a precursor of LDL cholesterol increased significantly. However, the serum enzymes (AST and ALT) levels reduced significantly with increased NLM in this study. It is therefore, recommended to utilize NLM with caution in broiler diets.

Keywords: Broilers, Neem Leaf Meal,Serum Biochemicals & Performance.

1. INTRODUCTION

Human and livestock population which has continued to increase and has resulted in, increase demand for food and feed in developing countries suggests that alternative feed resources be identified and evaluated (Nworgu &Fasogbon, 2007). In the course of assessing such unconventional feedstuffs, it is necessary that the health implications of such feedstuffs to the animal be considered.

Neem which is also known as *Azadirachtaindica*is a plant normally cultivated in the tropical regions, possesses a bitter bark, seeds and green leaves which are highly medicinal. Neem leaf has high medicinal qualities such as antibacterial, antiviral, antiprotozoal, hepatoprotective, immunomodulator and various other properties without showing any adverse effects (Kale *etal*., 2003; Sadekar*etal*., 1998). Neem leaf promotes growth and feed efficiency of birds due to its antibacterial and hepatoprotective properties (Padalwar, 1994). The result of using neem leaf as a supplement is that, it has been proven to show improvement in relation to weight gain, feed efficiency, lowered mortality, increased immunity and livability of poultry birds (Kumar, 1991).

Analysis and evaluation of serum biochemical indices of livestock and poultry is a vital part of researching as it provides information which is important for evaluating the health status of the animal, diagnosis of pathogens and understanding disease processes. WHO has recommended that blood parameters be used for medical and nutritional assessments (WHO, 1963).

The search for phyto-organic or natural additives which are economically viable as substitutes for the synthetic growth promoters is necessary as they have the potential to improve digestibility and strengthen the immune system, as well as growth of livestock and poultry yet, void of adverse effect on the animals. Furthermore, the use of neem leaf meal as an additive is more economical, easily available and safer to utilize, than synthetic growth promoters. This study was therefore, specifically designed to evaluate the effects of neem leaf meal on serum lipids,enzymes and performanceof broilers.

2. MATERIALS AND METHODS

The experiment was carried out in a Private farm (Pet Peter's Farm) located in Port Harcourt, Rivers State. A total of 100 broilers of Ross 308 strains were used and raised from day old to table size for 8 weeks. The birds were reared in an open sided poultry house, with feed and water, given ad-libitum. All required drugs and vaccinations were given to all the birds, as at due dates. Feed intake was recorded daily whilst weight gain, feed efficiency, and mortality rate were recorded weekly.

Neem leaves were harvested fresh from neem trees within the premises of the University of Port Harcourt. It was sun-dried and air-dried until it became crispy, before it was milled into powder to form neem leaf meal. Samples of the neem leaf meal were analyzed chemically using proximate analysis to determine its nutrient composition.

Broiler starters' diet containing 23%CP was fed to the chicks within the first 4 weeks of the experiment while broiler finishers' diet containing 20%CP was given to the birds within the 5^{th} – 8^{th} week of the experiment. Both diets were formulated and produced using locally available feed ingredients. Five treatments designated as T_1, T_2, T_3, T_4 and T_5 were used and birds assigned

randomly to each treatment in a Completely Randomized Design (CRD). Each treatment was replicated 4 times, with 5 birds per replicate. The prepared neem leaf meal was incorporated into the experimental diet at an inclusion rate of 0.00% for T_1 which was the control treatment, 1.0%, 2.0%, .3.0% and 4.0% for T_2, T_3, T_4 and T_5 respectively.

At the end of the feeding trial, blood samples were collected from one bird in each replicate in all the treatments, which was taken to the laboratory for analysis of serum lipids (Cholesterol, High density lipoprotein (HDL), Low density lipoprotein (LDL) and Triglycerides) and enzymes (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphate (ALP)).Data obtained were subjected to One-way Analysis of Variance (ANOVA) using SPSS Statistics (2008) software.

Table 1: Broilers' starter diet

INGREDIENT	TRT 1 (CONTROL)	(TRT 2)	(TRT 3)	(TRT 4)	(TRT 5)
Maize	40	40	40	40	40
PKC (Mech.)	7.5	7	6.5	6	5
Soya bean meal	14	14	14	14	14
Groundnut cake	14	14	14	14	14
Fish meal	7.75	7.25	7.25	7.25	7.25
Wheat bran	7	7	6.5	6	6
Soya oil	3	3	3	3	3
Bone meal	3	3	3	3	3
D-L Methionine	0.5	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5	0.5
Vit/MinPMX	2.5	2.5	2.5	2.5	2.5
Neem leaf meal	0	1	2	3	4
Salt	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**
Calculated nutrient composition					
Crude Protein	23.41	23.22	23.26	23.31	23.33
Energy	2797	2778	2767	2756	2741
Crude Fiber	4.80	4.86	4.87	4.88	4.87
Oil	6.62	6.57	6.52	6.47	6.41
Lysine	1.15	1.12	1.11	1.11	1.10
Methionine	0.45	0.44	0.43	0.43	0.43
Calcium	1.66	1.66	1.70	1.74	1.77
Phosphorous	1.10	1.09	1.07	1.07	1.07

Table 2: Broilers' finisher diet

INGREDIENT	TRT 1 (CONTROL)	(TRT 2)	(TRT 3)	(TRT 4)	(TRT 5)
Maize	45	45	45	45	45
PKC (Mech.)	9	8.5	8	7	7
Soya bean meal	10	10	10	10	10
Groundnut cake	10	10	10	10	10
Fish meal	7.75	7.25	7.25	7.25	7.25
Wheat bran	7.5	7.5	7	7	6
Soya oil	4	4	4	4	4
Bone meal	3	3	3	3	3
D-L Methionine	0.5	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5	0.5
Vitamin/Mineral					
PMX	2.5	2.5	2.5	2.5	2.5
Neem leaf meal	0	1	2	3	4
Salt	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**
Calculated nutrient composition					
Crude Protein	20.56	20.46	20.51	20.53	20.59
Energy	2897	2878	2867	2852	2845
Crude Fiber	4.71	4.77	4.78	4.77	4.80
Oil	7.65	7.60	7.55	7.49	7.45
Lysine	1.01	0.99	0.98	0.97	0.97
Methionine	0.41	0.40	0.40	0.40	0.39
Calcium	1.64	1.65	1.69	1.72	1.76
Phosphorous	1.07	1.06	1.05	1.05	1.04

Table 3: Proximate composition of neem leaf (*Azadirachtaindica*) meal

Proximate composition	Percentages
Moisture	10.50
Ash	9.30
Nitrogen free extract	15.63
Ether extract	8.10
Crude protein	21.75
Crude fiber	34.72

3. RESULTS

Table 4 below shows the dietary effect of Neem leaf meal on broilers performance. It indicates that initial weight, body weight gain and feed efficiency of birds were not significantly influenced ($P>0.05$) amongst the five treatments. However, average feed intake and mortality rates amongst treatments were significantly ($P<0.05$) affected.The average feed intake was significantly higher ($P<0.05$) in treatment 1, but showed similarity with treatments 3 and 5.

Treatment 2 had the least value but was also similarto treatments 3 and 4. Mortality rate for treatment 5 was significantly (P<0.05) higher than the other treatments.

Table 4: Dietary effect of Neem leaf meal on Broilers' Performance

	Treatments				
Parameters	**1(Control)**	**2(1%)**	**3(2%)**	**4(3%)**	**5(4%)**
Initial body weight (g)	41.50 ± 0.65	42.25 ± 0.85	42.75 ± 0.85	42.5 ± 1.19	42.5 ± 1.04
Average feed intake (g/bird)	620.28 ± 1.94^{a}	611.18 ± 2.26^{c}	614.65 ± 2.39^{abc}	611.63 ± 2.35^{bc}	618.60 ± 2.25^{ab}
Body weight gain (g/bird)	338.58±10.91	350.98±8.02	344.68±8.91	347.83±3.06	357.20±7.33
Feed efficiency	0.55±0.02	0.57±0.01	0.56±0.02	0.57±0.01	0.58±0.01
Mortality	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.50 ± 0.29^{a}

Means ^{abc} in the same row with different superscript differs significantly (P<0.05)

Table 5, indicatesthat, dietary treatment 4 showed highest AST value amongst the treatments while the least was exhibited by treatment 5. Although, the Control Treatment was significantly different (P<0.05) from the other Treatments. ALT value amongst treatments was not significantly (P>0.05) affected. The table further revealed that, thevalues of ALP in treatment 2was significantlydifference (P<0.05) from the other Treatments.

Total cholesterol and triglyceride levels were significantly different (P<0.05) from the control. HDL levels were significantly lower in the NLM supplemented diets than the control, and LDL values for treatment 2 and 5 were significantly different (P<0.05) from treatment 1, 3 and 4. VLDL levels in the NLM supplemented diets were significantly different (P<0.05) from the control group.

Table 5: Dietary effect of Neem leaf meal on serum biochemical indices of broilers

Dietary Treatment					
Parameters	**1(Control)**	**2(1%)**	**3(2%)**	**4(3%)**	**5(4%)**
AST (U/L)	80.0 ± 1.08^{a}	61.75 ± 1.75^{b}	62.0 ± 0.91^{b}	64.50 ± 5.12^{b}	61.00 ± 6.49^{b}
ALT (U/L)	9.20 ± 0.18	6.65 ± 0.55	6.80 ± 0.47	6.48 ± 1.95	6.65 ± 0.43
ALP (U/L)	31.50 ± 1.56^{b}	39.00 ± 0.91^{a}	34.50 ± 0.65^{b}	30.75 ± 0.85^{b}	32.00 ± 2.12^{b}
TC (mmol/L)	2.65 ± 0.07^{c}	2.70 ± 0.04^{c}	2.93 ± 0.05^{ab}	3.25 ± 0.13^{a}	4.25 ± 0.30^{b}
TG (mmol/L)	0.57 ± 0.01^{d}	1.12 ± 0.06^{b}	0.85 ± 0.05^{c}	1.41 ± 0.08^{a}	1.43 ± 0.09^{a}
HDL (mmol/L)	2.30 ± 0.11^{a}	1.46 ± 0.18^{b}	1.70 ± 0.20^{b}	1.36 ± 0.06^{b}	1.43 ± 0.1^{b}
LDL (mmol/L)	1.06 ± 0.09^{b}	0.58 ± 0.15^{c}	1.11 ± 0.14^{b}	0.71 ± 0.19^{bc}	1.55 ± 0.11^{a}
VLDL (mmol/L)	0.26 ± 0.00^{d}	0.51 ± 0.03^{b}	0.39 ± 0.02^{c}	0.64 ± 0.04^{a}	0.65 ± 0.04^{a}

Means ^{abc} in the same row with different superscript differs significantly (P<0.05),
AST – Aspartate aminotransferases, ALT – Alanine aminotransferases, ALP –Alkaline phosphatase, TC – total cholesterol, TG – Triglycerides, HDL – High density lipoprotein, LDL – low density lipoprotein, VLDL – Very low density lipoprotein.

4. DISCUSSION

From the study, the similarity of initial weight, body weight gain and feed efficiency in treated birds as compared with the control, showed that NLM maintained performance as reported by

Sadekar*etal*. (1998); and Kale *etal*. (2003), that NLM has medicinal qualities such as antibacterial, antiviral, antiprotozoal, hepatoprotective, immunomodulator and various other properties without showing any adverse effects. The similarity of treatment 3 and 5 with the control group on average feed intake indicates that NLM stimulates appetite and enhances digestion (Wankar*et al*., 2009). However, average feed intake reduced significantly in NLM treated group as compared to the control. This may be due to, high fibre content and presence of anti-nutritional factors such as tannins in the meal as reported by Esonu*et al*. (2006). More so, mortality observed in treatment 5 was minimal and may be due to inherent biological or environmental factor as Obun (2013) reported that broiler birds could tolerate 15% dietary level of NLM and Obikaonu*et al*. (2012) reported 10% tolerance level in starter broilers. However, the crude fiber content of the NLM is high as shown by the proximate composition, but this did not induce the mortality observed as it was not reflected in the feed intake and weight gain of the birds in the treatment group, as both parameters (feed intake and weight gain) showed no significant difference as the control group and treatment 5 numerically showed the highest weight gain.

From the study, the significant decrease in serum AST and ALT values among birds fed NLM from the control groups, suggests the hepatoprotective activity of NLM (Chattopadhyay *et al*., 2000). This result corresponds to the findings of Obikaonu*et al.* (2012) who reported that AST and ALT levels significantly reduced in concentration when neem leaf powder was added in broilers diet at 2.5, 7.5 and 10% rate. The ALP values observed amongst all treatments were within the normal range 568-8831U/L as reported by Meluzzi*et al*. (1992).

The result observed for total cholesterol and triglycerides levels across the treatment groups were within the normal reference value of 87-192mg/100ml and 45.7-172mg/100ml respectively for broiler (Meluzzi*et al.,* 1992). The variation of total cholesterol amongst the treatment groups may be due to the effect of heredity, nutrition, sex and environmental conditions on total cholesterol (Strukie, 1986). The increase in total cholesterol and triglyceride levels across treatment groups aligns with the findings of Shihab *et al.* (2017), which showed significant increase in the concentration of cholesterol and triglycerides levels following the supplementation of NLM in broilers' diet.

The decrease in HDL levels could be as a result of increase in triglycerides level as Barrett *et al.* (1998) reported that elevated triglyceride concentrations have generally been associated with low HDL levels. The reduction of serum LDL level in the treatment 2 and 4 may be due to the presence of phyto-chemicals in NLM, such as resin which has hypolipidaemic impact (Maghembe*et al*., 2017) causing a decrease in lipoprotein lipase function, which further reduces the synthesis of LDL as reported by Friestas*et al*. (2008). The increase in VLDL levels across treatment groups may be attributed to increase in total cholesterol, as VLDL is a major transport system of this serum lipid, from the liver to tissues (Grundy 1986).

5. CONCLUSION

This study has shown that the dietary inclusion of NLM in broiler diets influenced feed intake but showed no significant effect on feed efficiency and body weight gain of the birds. It was further observed that the treatment significantly reduced serum enzymes such as Aspartate aminotransferases (AST) and Alanine aminotransferasees (ALT) but increased Alkaline

phosphatase (ALP), serum lipids such as total cholesterol and triglycerides and VLDL amongst treatment groups. However, LDL reduced in birds treated with 1% and 3% neem leaf meal whereas HDL levels significantly reduced as the NLM levels increased. The study generally, suggests thatNLM did not significantly improve broilers performance as weight gain was not significantly affected and should be utilized by farmers with caution, as it reduces HDL levels and increases Very low density lipoprotein (VLDL), Triglycerides, total cholesterol levels in birds fed with the dietary treatment. However, neem leaf meal can be utilized in birds to prevent hepatic disease as serum enzyme (AST and ALT) levels reduced significantly in this study.

REFERENCES

Barret P.H.R. (1998) Kinetics of triglyceride rich lipoproteins:chylomicrons and very low density lipoproteins. *Atherosclerosis*, 141:35-40

Chattopadhyay R.R., Chattopaphyay R.N and Maitras S.K. (2000) Effects of Neem on hepatic glycogen in rats. *Indian Journal of Pharmacology* 25:174-175

Esonu, B.O., Opara, M.N., Okoli, I.C., Obikaonu, H.O., Udedibie, C, and Iheshiulor, O.O.M. (2006). Physiological Response of Laying Birds to Neem (*Azadiractain dica*) Leaf Meal-Based Diets: Body Weight, Organ Characteristics and Haematology. *Online J. Health Allied Scs.*, 2:4, www.ojihas.org/issue 18/2006-2-4.htm.

Freitas F.L.C.I., Almeida K.S.I.I., Machado R.Z.I., Machado C.R.I.I. (2008) Lipid and glucose metabolism of broilers (*Gallus gallusdomesticus*) experimentally infected with *Eimeria acervulinaTyzzer*, 1929 oocysts. *Brazilian Journal of Poultry Science* vol 10(3).

Grundy S.M. (1986) Cholesterol and Coronary heart disease: A new era. *J. Am. Med. Assoc.*, 256:2849-2859

Kale B.P., Kothekar M.A., Tayade H.P., Jaju J.B. and Mateeddin M. (2003). Effect of aqueous extract of *Azadirachtaindica*leaves on hepatotoxicity induced by antitubercular drugs in rats. *Indian Journal of Pharmacology* 35:177

Kumar O.M. (1991) Effect of Liv-52 syrup on broiler performance in North Eastern region. *Indian Poultry Review* April 22, pp. 37-38

Maghembe R.S., Bakari G.G., Max. R.A., Balthazary S.T. (2017) Effect of *Commiphoraswynnertonii*resin on liver functions and levels of plasma glucose and lipids in carbon-tetrachloride treated chickens. *International Journal of Nutrition and Metabolism*, 9(7):58-66.

Meluzzi A., Primiceri G., Giordani R., Fabris G.(1992) determination of blood constituents reference values in broilers. *Poultry Sci.* 71:337-345.

Nworgu F.C., Fasogbon F.O. (2007) Centrosema (*Centrosemapubescens*) leaf meal as protein supplement for pullet chicks and growing pullets. *Int. J. Poult. Sci.* 6(4):255-260

Obikaonu H.O.O., Koli L.C., Opara M.N., Okoro V.M.O., Ogbuewu L.P., Etuk E.B., Udebible A.B.I (2012) Haematological and sserum biochemical indices of starter broilers fed leaf meal of neem (*Azadirachtaindica*). *Journal of Agricultural Technology* 8(1):71-79.

Obun C.O. (2013) Utilization of sun-cured neem leaf meal (*AzadirachtaindicaA.Juss*) Based biets by finisher broiler chickens. http://engormix.com/poultry-industry/articles/utilization-sun-cured-neem-t35610.htm.

Padalwar R.V. (1994) Neem (*Azadirachtaindica*) leaves as a feed additive in broiler ration. PunjabraoKrishividyapeeth; Akola, pp. 84

Sadekar R.D., Kolte A.Y., Barmase B.S., Desai V.F. (1998) Immunopotentiating effects of *A. indica*(Neem) dry leaves in broilers naturally infected with infectious bursal disease virus. *Indian Journal of Experimental Biology*, 36(11):1151-1153

Shihab I.M., Al-Zuhariy M.T., Abdullah S.M., Mutar S.S. (2017) Impact of supplementation Neem powder (*Azadirachtaindica)* to diet broiler in immunological, physiological and productive traits. *Advances in Environmental Biology* 11(3):44-51.

Strukie P.D. (1986) Lipid metabolism In: Avian Physiology. 4th Edu. Springer-Vilag. New York, pp:345-358.

Wankar, Alok K, Shirbhate RN, Bahiram KB, Dhenge SA and Jasutkar RA (2009), Effect of neem (*Azadiractaindica*) leaf powder supplementation on growth in broilers. Vertinary world, 2 (10): 396-397.

WHO (1963): Annual report of the Director-General to the World Health Assembly and to the United Nations. World Health Organization. http://www.who.int/iris/handle/10665/85765,