GROWTH PERFORMANCE AND HAEMATOLOGICAL INDICES OF BROILERS PLACED ON GRADED DIETORY INCLUSION LEVEL OF NEEM (AZADIRACTA INDICA) LEAF MEAL.

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Abstract

Different dietary inclusion levels D^n, D^1 (2%), D^2 (5%), D^3 (10%) and D^4 (20%), of Neem Leaf Meal (NLM) was fed to broilers, divided into four dietary groups in three replicates. The aim is to study the impact of NLM as protein substituent on broiler growth and haematological indices. Parameters evaluated were; Daily Feed Intake (DFI), Crude Protein (CP), Average Weight Gain (AWG), Feed Conversion Ratio (FCR), Packed Cell Volume (PCV), Haemoglobin (Hb) count. DFI and CP intakes of D^2, D^3 and D^4 were notably ($p< 0.05$) higher than the control. AWG differences were negligible ($p> 0.05$) for D^n, D^1 and D^2. There was significant difference in the haematological characteristics of dietary groups D^n and D^1 compared to D^2, D^3 and D^4. While the Hb and PCV levels of D^1 and D^2 compared favourably with the control, D^2 and D^3 showed significantly reduced levels than the control. Neem leaf meal can be further investigated as a substitute for conventional broiler protein sources.

KEY WORDS: Neem, Leaf Meal, Broiler, Haematology, Growth

INTRODUCTION

Beneficial effects of plant products in animal feed

Leaf meals are the dried and ground products of plant leaves. They have shown great promise as livestock feeds, including broilers (Abdulsalam et al (2015); Rama-Rao et al (2019); The specific areas of interest for plant leaf inclusions in poultry production include; Protein source; As partial or total replacements for conventional plant and animal protein feed ingredients. Due to the ever increasing price of feed materials, particularly the protein-rich feed ingredients, the incorporation of leaf meal in broiler diet is encouraged, to reduce feed cost. (Oloruntola et al (2019); Ubua et al (2019) had succesfully incorporated Cassava at 5% inclusion levels. Oloruntola et al (2018) performed inclusion of *Amaranthus cruentus* at levels of 5, 10, 15, 20 and 25%, and reported that 5% inclusion favoured growth performance, nitrogen utilization, muscle development and blood indices. As Growth Promoters, some plant leaves have been demonstrated to contain a number of bioactive compounds that are beneficial for the health of chickens Rama Rao et al (2019). These compounds include vitamins, phenolic acids, flavonoids, isothiocyanates, tannins as well as saponins, Obikaonu (2012). Some of these have been reported to improve feed intake and secretion of digestive juices, thus, they can act as natural growth promoters (NGPs) by; increasing the production and activities of digestive enzyme, Rahman and Yang, (2018), improving the intestinal morphology (villi development) of broilers resulting in improved nutrient digestibility

and utilization, Akintomide et al (2018). Antibiotic activity: In response to the emergence of antibiotic resistance, several European countries have restricted or banned the use of antibiotics as growth promoters. Plant-based antibacterial agents have been under investigation as potential replacement of antibiotic growth promoters Raman et al (2015); Onyimonyi et al., (20069); Ouyang et al (2016). Plant parts such as the leaf, roots, barks and fruits have been known to contain numerous active compounds. These bioactive components include phenols, flavonoids, alkaloids, saponins, tannins, terpenoids, steroids, polyphenols, and lignans which show pharmacological activities such as anti-bacterial, anti- inflammatory, anti-fungal, anti-oxidant and analgesic properties, Sood et al. (2012). Ouyang et al (2016) indicated that alfalfa flavonoids improved the antioxidative status and fatness in broiler chickens. *Polyalthia longifolia* leaf meal containing 0.51% alkaloids may be employed as an antibiotic substitute for improving the growth performance of broilers, Alagbe (2017) exhibited that saponin in Moringa leaf meal was responsible for protecting the erythrocytes from destruction (caused by the free radicals or reactive oxygen species) as well as stimulating the erythrocytes production in broiler chickens. In line with this, Zhang et al (2017) showed that saponins derived from mango leaf improved growth performance, meat characteristics and plasma lipid profile of broilers. None of these alternatives have yielded an efficient control equivalent to in-feed antibiotic supplementation. Consequently, there is an urgent need to develop effective approaches that would preclude changes to existing poultry production practices.

Deleterious effects of plant products in animal feed

High crude fibre content; Apart from their benefits, the use of leaf meals in broiler diets may be limited by their high content of crude fibre, (Oloruntola et al (2016), Ubua et al (2019). In general, broiler chickens showed low tolerance to dietary fibre, and therefore feeding diets containing high levels of leaf meal may result in compromised nutrient digestibility and thus depressed growth performance of broilers. Researchers have suggested different ways of ameliorating the effects of high plant fibre in poultry diet, Fasuyi and Akindahunsi (2009), Oloruntola et al (2016), suggested dietary supplementation of fibre-degrading enzymes. Santoso et al (2015), suggested that fermentation could degrades plant fibre, thus enabling higher inclusion levels of dietary leaf meal.

Anti-nutritional factors in leaf meals***;*** The presence of anti-nutritional factors (ANF's) such as tannin, alkaloids, saponins, flavonoids, cyanide, oxalate, phytate, etc. are important factors to consider when including leaf meals into broiler diets, they antagonize and reduce nutrient digestibility and utilization in broiler chicks Kagya-Agyemang et al (2007) Tannin reduces protein digestibility and utilization in poultry, by binding protein and digestive enzymes to be a complex form that are not readily digestible, Akintomide (2018). The presence of tannin in leaf meals may also damage the mucosa of enterocytes and interrupt the metabolic processes leading to compromised growth performance of broilers. Saponins in leaf meals act as anti-nutritional factors for broiler chickens, Peiretti (2018).

Chamorro et al (2019), suggested that the deleterious effects of these phytochemicals are linked to their dietary inclusion levels.

Moderate amounts of phenolic compounds and other secondary plant metabolites (such as tannin and saponins) may positively affect the health and production performance of broilers, whereas high levels of such compounds negatively affect the nutrient utilization of broilers. In this regard, study to optimize the level of leaf meals in broiler rations is necessary to obtain the functional properties of leaf meals without compromising the growth of broilers.

Different suggestions have been made to mitigate the effects of ANF's in leaf meal. Among the methods, sundrying has been reported to reduce the anti-nutritional factors content in leaf meal Fasuyi et al (2008). Other methods such as cooking, autoclaving, dehulling, soaking, toasting and using the anti-nutritional binding agents may also be carried out to reduce the contents of anti-nutritional factors in leaf meals Medugu et al (2012). Moreover, fermentation may also be conducted to reduce the anti-nutritional factors in leaf meals Santoso et al (2015)

The nutrient and phytochemical profile of neem leave as reported by Akintomide et al (2018), Onyimonyi et al (2009) gives protein content of up to 22.58, 180mg / g of vit. A, 287mg / g of vit C, calcium phosphorus, carotene etc. And as reported by Madaki et al, (2016), Akintomide et al (2018) they also contain glutamic acid, tyrosine, aspartic acid, alanine, praline, glutamine and cystine like amino acids. The Above nutrient and phytochemical profile of neem leave makes it attractive nutritionally for animal feed.

Neem leaves are now known to contain nimbin, nimbinene desacetylnimbinase, nimbandial, nimbolide and quercentin (a polyphenolic flavonoid). and several fatty acids (dodecanoic, tetradecanoic, elcosanic, etc and nimbosterol (ß- sitosterol) as well as number of liminoids (nimbin and its derivatives). Quercetin (a polyphenolic flavonoid Limonoids like nimocinolide and isonimocinolide

Previouly, researchers had conducted studies to ascertain the effects of inclusion of Neem leaf meals to poultry diet. Ansari et al (2012) had incorporated

Azadirachta indica as supplement in broiler rations at levels 1.25, 2.5 and 5.0 g /kg of feed Supplementation of 2.5 g/kg resulted in no negative effects on broiler performance, serum biochemical constituents and haematological indices. Wiryawan et al (2017), included Neem leaf meals in broiler rations at levels of 0, 0.1, 0.3, 0.5 and 0.7%, and suggested that NLM may be optimally included in broiler rations at the level of 0.3%, Kharde et al (2014) incorporated at levels of 0, 1 and 2 g/kg broiler diets treatment, elicited improved body weight gain and feed conversion ratio of broilers. Ubua et al (2019) on the other hand, included in broiler rations at levels of 0, 2.5, 5 and 7.5%, and posited that the leaf may be included in broiler rations at maximum 2.5% without impairing the growth and carcass traits.

While at low inclusion levels, beneficial effects had been noted, higher inclusion levels retarded growth. This study was therefore designed to incorporate the previous works, in our new idea, in order to determine and optimum inclusion inclusion level of neem leaf extract for desired yield.

Materials and Methods

Neem Leaf Meal (NLM): Neem (Azadiracta indica) leaves were harvested from Ufuma in Orumba North Local Government Area. The leaves were washed and dried under room temperature for 7 days. The dried leaves were then ground using a Phillips blender, to a fine powder. The latter product was subsequently

stored in sealed polythene bags, and kept in a refrigerator until required.

Experimental design: One hundred and twenty, Abor Acre day-old chicks were brooded and raised on commercial starter feed (Hybrid feeds), for three weeks. After this period, they were allotted to five treatments (D^n, D^1, D^2 and D^3) with 3 replicates per treatment and 10 birds per replicate in a completely randomized design. D^n (control), containing 0% neem leaf meal (NLM), treatments, D^1, D^2 and D^3 had NLM included at 2.0%, 3.5%, and 5.0% respectively.

All required vaccination and bird management protocols were strictly observed. The lighting program was 18 h per day for the duration of the experiment. Water and feed were available ad libitum. The entire treatment groups were fed an isocaloric and isoproteinic basal diet, which was formulated and compounded to meet or exceed the nutrient requirements of broiler chickens based on 1994 NRC guidelines

Chicks were weighed at the start of the trial (day 0) thereafter weekly weight were obtained. Feed intake was measured at 7days interval for 4 weeks (four times). Five birds were randomly selected for weighing. The trial lasted for four weeks, at the end 4birds per replicate group, (12 birds per treatment group) were randomly selected, slaughtered, and the blood was collected into two sets of Sample bottles per group. The first set of bottles contained ethylenediamine tetraacetic acid (EDTA) an anticoagulant. The blood samples in these bottles were used for the determination of haematological parameters. The second set of bottles were without anticoagulant; the samples were for serum assay. All bottles were tightly sealed and stored in a cupboard at room temperature until required. The bottles without EDTA were placed slanted in a test tube rack, until coagulation occurred, and serum was formed.

Data collected were subjected to one-way Analysis of Variance using ANOVA package (v.15). In instances where there was a significant difference among the experimental treatments, Duncan's multiple-range test was conducted. Least significance difference was used to separate treatments means whenever the F-value was significant. The 0.05 P-value was used to declare significance.

RESULTS AND DISCUSSION

The composition of basal feed formulated and used for the feeding trial is as in table 1 where T1, T2, T3 and T4 represents graded level of inclusion of neem leave meal of 0%, 2.0%, 3.5%, and 5.0%.

Table 1: Basal feed formulation and nutrient analysis.

	INGREDIENTS	**T1 0% NLM**	**T2 2.0% NLM**	**T3 3.5% NLM**	**T4 5.0%NLM**
1	Maize	58.00	56.30	54.70	53.20
2	Full fat Soybean meal	10.00	10.00	10,00	10,00
	NLM	00.00	01.50	3.50	5.00
3	Groundnut meal	22.70	21.50	20.00	19.00
4	Fishmeal	1.00	1.00	1.00	1.00

5	Wheat offal	5.00	5.00	5,00	5.00
5	Lysine	0.35	0.35	0.35	0.35
6	DL-Methionine	0.15	0.15	0.15	0.15
7	Vitamin Premix*	0.30	0.30	0.30	0.30
8	DiCalcium phosphate	1.40	1.40	1.40	1.40
9	Calcium carbonate	1.30	1,.30	1.30	1.30
10	Salt	0.30	0.30	0.30	0.30
	ME [Kcal/Kg]	**2,900**	**2,900**	**2,900**	**2,900**
	CP [%]	**21.60**	**21.60**	**21.60**	**21.60**
	Ether extract	**4.20**	**4.20**	**4.20**	**4.20**
	Dry matter	**83.10**	**83.20**	**83.10**	**83.10**
	Methionine + Cysteine	**0.80**	**0.80**	**0.80**	**0.80**

*The vitamin premix supplied the following per kilogram: vitamin A, 3,600,000 IU; vitamin D3, 800,000; vitamin E, 14,400 IU; vitamin K3, 800 mg; vitamin B1, 700 mg; vitamin B2, 2,640 mg; vitamin B3, 3,920 mg; biotin, 6 mg; choline chloride, 120,000 mg; antioxidant, 400 mg; manganese, 39,680 mg; iron 20,000 mg; zinc, 33,880 mg; iodine, 396 mg; selenium, 80 mg; copper, 4,000 mg; carrier and calcium carbonate, up to 1,000 g.

The proximate composition of neem leaf meal (NLM), as experimentally determined, is depicted in Table 2. It indicated a crude protein (CP) of 22.7%, and 12.4% crude fibre, with 5.8% ether extract. The result compares favourably to that obtained by Onyimonyi et al (2009), and Akintomide et al (2018). This is with the exception of the crude protein, which at 22.7%, was significantly lower than that obtained by and Akintomide et al (2018). This variation in protein content may be attributed to differences in soil composition.

Table: 2 **Experimental values of proximate composition of neem leaves**

PARAMETER	MOISTURE	Crude protein	Crude fibre	Ether Extract
PERCENTAGE	8.8	22.7	12.4	5.8

The impact of varied dietary inclusions of NLM on body weight (BW), feed intake and feed conversion ratio, can be seen in Table 3

Table 3 **Performance of broilers fed NLM supplemented diets at varied inclusion levels**

	EXPERIMENTAL GROUPS					
PARAMETERS	**D^n**	**D^1**	**D^2**	**D^3**	**SE**	**p value**
BW gain (g/bird)	718a	729b	704c	613b	25.00	0.20

Feed intake (g/bird)	1,125a	1,129	1,135	1,187b	4.15	<0.01
FCR	1.57	1.59	1.61	1.94	0.08	0.38

Where D^n = Control (0% NLM), D^1 = (2.0% NLM) D^2 = (3.5% NLM) D^3 = (5.0% NLM)
a, b, c and d Means in the same row with different letters show significant differences ($p<0.05$) among dietary treatments BW: body weight, FCR: feed conversion ratio, SE: standard error
D^n = Control (0% NLM), D^1 = (2.0% NLM) D^2 = (3.5% NLM) D^3 = (5.0% NLM)

For all parameters evaluated, no statistically significant differences were noted between dietary groups D^n and D^1 ($P > 0.05$). The variations in body weight, feed intake and calculated feed conversion ratio, was however, significant between D^n and D^1 on one hand, and D^3. Ansari et al (2012), had observed that supplementation of 2.5 g/kg of NLM resulted in no negative effects on broiler performance, serum biochemical constituents and haematological indices.
It is noteworthy, that at 2% inclusion level, there was a notable improvement on body weight gain, and feed conversion ratio, compared to the control. This may be attributable to several factors. Plant leaves and their phytochemicals can be used as natural growth promoters (NGPs) and as phytogenic feed additives (Vergara-Jimenez et al 2017, Rahman and Yang,2018, Rama Rao et al 2019). They improve feed intake and secretion of digestive juices, improving the intestinal morphology (villi development) of broilers resulting in improved nutrient digestibility and utilization (Mariana et al 2018; Mustafa 2019).

The haemoglobin and erythrocytes levels for D^3 dietary group was markedly depressed, compared to the control group, and the D^1 and D^2. There appears to be a correlation between depression of red blood cells and increased NLM intake as seen in table 4. Whether this is due to effect of Anti Nutritional factors (ANFs), or low protein content of the NLM was not determined.

Table 4: Haematological profile of the dietary groups D^n and D^1 D^2 D^3

Treatment groups **Items**	**D^n**	**D^1**	**D^2**	**D^3**	**SE**	**p value**
Hemoglobin (g/dL)	11.8a	11.8a	11.3a	9.44b	0.60	0.11
Erythrocytes (106/µL)	2.8	2.8	2.63	2.10	0.19	0.22
Hematocrit (%)	28.2	27.5	27.0	25.4	1.61	0.25
MCV (fl)	110	110	109	101	1.15	0.67
MCH (pg)	43.3a	43.0a	41.8a	40.2b	0.49	<0.01

MCHC (g/dL)	39.6a	39.6a	38.7a	34.3b	0.44	0.02
Leukocytes (103/µL)	66.5	73.4	74.5	70.2	5.69	0.65
Heterophils (103/µL)	7.37	8.31	8.03	6.52	1.05	0.22
Eosinophils (103/µL)	4.64	4.97	4.48	4.15	0.29	0.28
Lymphocytes (103/µL)	60.6	63.9	63.9	56.6	4.85	0.79
Thrombocytes (103/µL)	8.16	8.75	8.64	6.77	0.54	0.27

a, b, c, d Means in the same row with different letters show significant differences ($p<0.05$) among dietary treatments BW: body weight, FCR: feed conversion ratio, SE: standard error
D^n = Control (0% NLM), D^1 = (2.0% NLM) D^2 = (3.5% NLM) D^3 = (5.0% NLM)
MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, SE: standard error.

Comparatively, birds in the control, D^n, D^1 and D^2 experimental groups group showed haematological index values for MCH and MCHC, with no significant differences ($p<0.05$). The treatment group D^3, had reduced MCH and MCHC values.

The study showed that increase in dietary inclusion of NLM resulted in higher leucocytes values in broilers. The reason for this is unclear, but speculatively, NLM may contain a bioactive phytochemical that acts as immune boosters. Further study will need to be done in determining and isolating such a compound/ compounds, and the mechanism of their activity.

In conclusion, dietary inclusion of up to 3% NLM into broiler feed, has no negative effect on their growth performance. Blood quality parameters are unaffected at similar inclusion levels. More work will need to be done on the function of NLM, its extracts, or specific phytochemicals.

References

Abdulsalam S, Yahaya M S and Yakasai M A (2015) Performance of broiler chickens fed on Moringa oleifera leaf meal supplemented poultry feed. *Nigeria Agricultural Journal,* 46. 5.10

Adeyemi O A, Adekoya J A and Sobayo R A (2012) Performance of broiler chickens fed diets containing cassava leaf: blood meal mix as replacement for soybean meal. *Revista Cientifica UDO Agricola,* 12, 212-219.

Adu, O.A., (1997). Successful feeding in poultry. *Livestock News*, Volume 1, Number 1, Nigeria.

Akintomide A. A; Joseph O. G. and Onibi G. E. (2018) Haematological and Serum Biochemistry of Cockerels fed Diets containing Neem Leaf Meal. Nigerian Society of Animal Production – Conference Proceeding.

Alagbe J O (2017) Effect of dietary inclusion of Polyalthia longifolia leaf meal as phytobiotic compared with antibiotics on performance, carcass characteristics

and haematology of broiler chicken. *Scholarly Journal of Agricultural Science*, 7, 68-74.

American Journal of Food Technology, 4, 108-118.

Chamorro S, Romero C, Brenes A, Sánchez-Patán F, Bartolomé B, Viveros A and Arija I (2019) Impact of a sustained consumption of grape extract on digestion, gut microbial metabolism and intestinal barrier in broiler chickens. *Food & Function*, 10, 1444-1454.

Eevuri, T.R. and R. Putturu, (2013). Use of certain herbal preparations in broiler feeds-A review. *Vet. World*, 6: 172-179.

Fasuyi A O and Akindahunsi A. O (2009) Nutritional evaluation of Amaranthus cruentus leaf meal based broiler diets supplemented with cellulase/glucanase/xylanase enzymes.

Fasuyi A O, Dairo F. A. S and Adeniji A. O (2008) Tropical vegetable (Amaranthus cruentus) leaf meal as alternative protein supplement in broiler starter diets: bionutritional evaluation. *Journal of Central European Agriculture*, 9, 23-34.

Gunjan P, and Ashok K. S. (2006) Modeling of Azadirachtin production by Azadiracta Indica and Its Use toward Optimization Studies *biochemical engineering journal* volume 29 page 62 – 68.

Kagya-Agyemang J K, Takyi-Boampong G, Adjei M and Karikari-Bonsu F R (2007) A note on the effect of Gliricidia sepium leaf meal on the growth performance and carcass characteristics of broiler chickens. *Journal of Animal and Feed Science*, 16, 104-108.

Obikaonu H. O. (2012) Evaluation of thr Nutritional value of Neem (Azadilacta Indica) Leaf Meal on the Performance of Finisher Broilers, *International Journal of Agriculture and Rural development* vol. 15(3) 1230 – 1239.

Oloruntola O D (2018) Gliricidia leaf meal in broiler chickens diet: effects on performance, carcass, and haemato-biochemical parameters. *Journal of Applied Life Sciences International*, 18, 1-9.

Oloruntola O D, Agbede J O, Ayodele S O and Oloruntola D A (2018) Neem, pawpaw and bamboo leaf meal dietary supplementation in broiler chickens: Effect on performance and health status. *Journal of Food Biochemistry*, 43.

Oloruntola O D, Ayodele S O, Agbede J O and Oloruntola D A (2016) Effect of feeding broiler chickens with diets containing Alchornea cordifolia leaf meal and enzyme supplementation. *Archivos de Zootecnia*, 65, 489-498.

Onyimonyi A E and Ernest O (2009) An assessment of pawpaw leaf meal as protein ingredient for finishing broiler. *International Journal of Poultry Science*, 8, 995-998.

Ouyang K, Xu M, Jiang Y and Wang W (2016) Effects of alfalfa flavonoids on broiler performance, meat quality, and gene expression. *Canadian Journal of Animal Science*, 96, 331-340;

Peiretti P G (2018) Amaranth in animal nutrition: a review. Livestock Research for Rural Development, 30(5).

Rahman M A, Ali M A, Saha B K, Hasan M A A, Rahman M A and Mostofa M (2015) Use of neem leaf and ginger extracts for cost effective broiler production. *International Journal of Natural and Social Sciences*, 2, 11-16.

Rahman Md M and Yang D K (2018) Effects of Ananas comosus leaf powder on broiler performance, haematology, biochemistry, and gut microbial population. *Revista Brasileira de Zootecnia*, 47.

Rama Rao S V, Raju M V L N, Prakash B, Rajkumar U and Reddy E P K (2019) Effect of supplementing moringa (Moringa oleifera) leaf meal and pomegranate (Punica granatum) peel meal on performance, carcass attributes, immune and antioxidant responses in broiler chickens. *Animal Production Science*, 59, 288-294.

Santoso U (2018) The usefullness of Sauropus androgynus leaf as a feed supplement for poultry. 1. Its effect on chicken performances. *Jurnal Sain Peternakan Indonesia*, 13, 151-156.

Santoso U, Fenita Y and Kususiyah (2015) The effect of fermented Sauropus androgynus leaf on performance, fat deposition and carcass quality in broiler chicken. International Seminar on Promoting Local Resources for Food and Health, 12-13 October, 2015, Bengkulu, Indonesia

Sood, R., D. Swarup, S. Bhatia, D.D. Kulkarni, S. Dey, M. Saini and S.C. Dubey, (2012). Antiviral activity of crude extracts of Eugenia jambolana Lam. against highly pathogenic avian influenza (H5N1) virus. *Indian J. Exp. Biol.*, 50: 179-186.

Sugiharto S, Isroli I, Yudiarti T, Widiastuti E, Wahyuni H I and Sartono T A (2018b) Performance, physiological and microbiological responses of broiler chicks to Moringa oleifera leaf powder, garlic powder or their combination. *Livestock Research for Rural Development*, 30(12).

Ubua J A, Ozung P O and Inagu P G (2019) Dietary inclusion of neem (Azadirachta indica) leaf meal can influence growth performance and carcass characteristics of broiler chickens. *Asian Journal of Biological Science*, 12, 180-186.

Zhang Y. N; Wang J and Qi G. H. (2017) *Asian –Australia Journal of Animal Science* 30(8) 1143 – 1149 published online.