Growth performance and haematology of broiler chickens fed enzyme supplemented neem leaf meal -based diets

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Abstract

The quest for sustainable poultry production using alternative feed ingredients is imperative. This study was conducted to assess the growth performance and blood profile of broiler chickens fed neem leaf meal (NLM) based diets with or without enzyme supplementation. Two hundred and eighty-eight, one-week old chicks (with an average weight of 98.44g) arranged in a completely randomized design with twelve birds/ replicate and three replicates/ treatment were fed NLM at 0, 1.5, 3 and 5% with or without Polyzyme® to form eight experimental treatments. Data on initial body weights, weekly body weights and feed intake were taken during the trial which lasted for eight weeks and feed conversion ratio calculated. Blood was also collected for haematological studies at the 4th and 8th week by humanely severing the jugular vein. Results indicated that total weight gain at starter phase decreased progressively with increase in NLM inclusion and addition of Polyzyme did not significantly ($P>0.05$) affect performance. At the finisher phase, growth performance was not significantly ($P>0.05$) influenced by NLM or enzyme addition. Furthermore, NLM with/without Polyzyme did not elicit any significant influence on the packed cell volume and haemoglobin concentration of the chickens. In conclusion, broiler chicks could tolerate up to 3% NLM and finishers up to 5% based on growth performance. The use of polyzyme at 400g/tonne in this study did not improve the utilization of NLM by the chickens.

Keywords: Alternative feed ingredients, Polyzyme, Blood profile, Broiler feed, Exogenous enzyme

Performances de croissance et hématologie des poulets à griller nourris avec des régimes à base de farine de feuilles de neem enrichis d'enzymes

Résumé

La recherche de la production de volaille durable utilisant des ingrédients d'alimentation alternatifs est impérative. Cette étude a été réalisée pour évaluer la performance de la croissance et le profil sanguin des régimes à base de feuilles neem (NLM) avec ou sans supplémentation en enzyme. Deux cent quatre-vingt-huit poussins âgés d'une semaine (avec un poids moyen de 98,44 g) disposés dans un design complètement randomisé avec douze oiseaux / répliqués et trois répliqués / traitement ont été nourris à 0, 1,5, 3 et 5% avec ou sans Polyzyme pour former huit traitements expérimentaux. Les données sur les poids corporels initiaux, les poids corporels hebdomadaires et l'apport alimentaire ont été pris au cours de l'essai qui a duré huit semaines et le ratio de conversion alimentaire calculé. Le sang a également été collecté pour des études hématologiques à la 4^{ème} et 8^{ème} semaine en coupant humainement la veine jugulaire. Les résultats ont indiqué que le gain de poids total à la phase de démarrage a diminué progressivement avec l'augmentation de l'inclusion de NLM et l'addition de polyzyme n'a pas significativement ($p>0,05$) affecter les performances. À la phase de finition, la performance de la croissance n'était pas significativement ($p>0,05$) influencée par l'addition NLM ou enzyme. De plus, la NLM avec / sans polyzyme n'a suscité

aucune influence significative sur le volume de la cellule emballée et la concentration en hémoglobine des poulets. En conclusion, les poussins de chair pourraient tolérer jusqu'à 3% de NLM et des finisseurs jusqu'à 5% sur la base de la performance de la croissance. L'utilisation de polyzyme à 400 g / tonne dans cette étude n'a pas amélioré l'utilisation de NLM par les poulets.

Mots-clés: Ingrédients d'alimentation alternatifs, Polyzyme, Profilé de sang, Flux de gril, Enzyme exogène

Introduction

When considering provision of affordable animal protein especially for the low income group, rearing of poultry would readily come to mind (Allouche *et al.*, 2015). And in order to ensure fast and optimum growth of chickens, giving them a well-balanced ration is highly essential. Albeit, scarcity of major feed ingredients (Adebayo and Adeola, 2005) like maize and soybeans, and increase in prices of commercial finished feeds due to unfavourable government policies and disease outbreaks have posed a serious challenge to the industry. The search for alternative feed ingredients such as agro-industrial byproducts (Ajila *et al.*, 2012; Fabunmi *et al.*, 2019) and leaf meals (Fasuyi, 2007; Hien *et al.*, 2017) have been intensified recently so as to keep the poultry industry from collapse. Furthermore, to cushion the high cost of feed ingredients, the addition of exogenous enzymes would be beneficial as it should lead to increased utilization of nutrients in compounded or finished feeds. Feed ingredients which contain considerable amounts of antinutrients can be better utilized as exogenous enzymes mitigate their adverse effects, one of which is inhibition of digestive enzymes (Alam *et al.*, 2003). Better utilization of nutrients in these feed ingredients results in better performance of the animals as shown by Costa *et al.*, 2008 and Allouche *et al.*, 2015, which invariably leads to increased profit for the farmer. Alam *et al.* (2003) tested the efficacy of three commercial enzymes in the feed of broiler chickens and the results showed that one of the enzymes did not improve the

utilization of feed as the other two enzymes. Also, according to Bedford and Schulze (1998), the response of birds to xylanase, β -glucanase and phytase vary. This suggests variations in responses of chickens to exogenous enzymes. Other researchers have also used enzymes successfully in animal feed (Allouche *et al.*, 2015). Ogunsipe *et al.*, 2015 concluded that when cassava plant meal was supplemented with Roxazyme G2G, it could be used up to 40% in broiler diet. Allouche *et al.* (2015) supplemented a low energy broiler diet with enzyme and the resulting effect was increased profit due to better feed utilization by the chickens which was the effect of concomitant reduction in feed intake by the birds. Neem leaves contains a good amount of protein, mineral and vitamins although, it is high in fibre which is a typical characteristic of leaves. The antibacterial, anticoccidial, antioxidant and hepatoprotective abilities of neem leaves are also well documented (Sithisarn *et al.*, 2005; Kumar *et al.*, 2010). Adesua (2019) noted that broilers were able to tolerate 5% neem leaf meal (NLM). Owing to the documented health benefits of NLM, this study was carried out to assess the response of broiler chickens to supplementation of diets which contained NLM at lower levels with Polyzyme using growth indices and haematology as response criteria.

Materials and methods

Experimental site

This trial was carried out at the Poultry Unit, Teaching and Research Farm, Federal University of Technology, Akure, Ondo State, Nigeria. It is geographically located

between latitude 7°5' N and longitude 5°15' E at an altitude of 370m above sea level (Oyinloye, 2013).

Experimental treatments

Neem leaf meal (NLM) was included in broiler rations at 0, 1.5, 3.0 and 5.0% with or without Polyzyme® supplementation to form 8 experimental treatments. The compositions of diets at starter and finisher phases are presented in Tables 1 and 2.

Experimental birds and management practices

Three hundred and fifty (350) day-old Marshal broiler chicks were purchased from a reputable farm at Ibadan, Oyo State,

Nigeria. The 1st week was a pre-experimental period after which 288 birds were randomly allocated to the treatments (3 replicates/ treatment; 12 birds/replicate). Data on initial body weights, weekly body weights and feed intake were taken appropriately during the trial. Feed conversion ratio was calculated as feed intake divided by weight gain. Two birds/replicate were humanely slaughtered at the end of starter phase and three at the end of finisher phase. Birds were put under good hygienic conditions throughout the experimental period. Experimental diets and water were supplied ad libitum and

Table 1: Compositions of neem leaf meal diets for broiler-chicken starters with/without polyzyme supplementation

Ingredients (kg)	Level of inclusion (%)			
	Control (0)	1.5	3.0	5.0
Maize	53.00	52.50	51.50	50.00
Neem Leaf Meal	0.00	1.50	3.00	5.00
Wheat offal	8.80	7.80	7.80	7.80
Soyabean meal	16.00	16.00	16.00	16.00
Groundnut cake	16.00	16.00	15.50	15.00
Fish meal (72%)	3.00	3.00	3.00	3.00
Bone meal	2.10	2.10	2.10	2.10
Oyster shell	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10
Methionine	0.25	0.25	0.25	0.25
Premix*	0.25	0.25	0.25	0.25
Table salt	0.30	0.30	0.30	0.30
Total	100	100	100	100
Calculated analysis				
ME (Kcal/Kg)	2923.72	2944.00	2952.61	2962.78
Crude protein (%)	22.57	22.69	22.71	22.80
Crude fibre (%)	3.68	3.80	3.97	4.20
Ether extract (%)	4.08	4.10	4.11	4.12
Calcium (%)	1.15	1.14	1.14	1.13
Phosphorus (%)	0.59	0.59	0.59	0.59
Analysed composition (%)				
Crude protein	22.23	22.30	22.50	22.62
Crude fibre	3.40	3.89	4.05	4.22
Ether extract	4.33	5.12	5.23	5.69
Ash	2.91	3.18	3.28	3.41

*2.5kg premix contains Vitamin A (8,000,000 I.U); Vitamin D3 (2,000,000 I.U); Vitamin E (5,000mg), Niacin (15,000mg); Vitamin B1 (1,500mg); Vitamin B2 (8,000mg); Vitamin B6 (1,500mg), Vitamin B12 (10mg); Vitamin K3 (2,000mg); Calpan (5,000mg); Biotin (20mg); Folic acid (500mg); Antioxidant (125,000mg); Choline chloride (200,000mg); Cobalt (200mg); Copper (5,000mg); Iodine (1,200mg); Iron (40,000mg); Manganese (80,000mg); Selenium (200mg); Zinc (60,000mg)

vaccination done as scheduled.

Haematological studies

Blood samples for haematological studies were collected as described by Lamb (1981) and were immediately put into bottles containing EDTA as coagulant, capped and inverted repeatedly for about a minute to prevent clotting of blood samples.

Statistical analysis

All data generated were subjected to one-way and factorial analysis of variance (ANOVA) as appropriate. Where significant differences were found, means were compared using Tukey test of the Minitab Statistical Package (v 17).

Table 2: Composition of neem leaf meal diets for broiler-chicken finishers with/without polyzyme supplementation

Ingredients (kg)	Level of inclusion (%)			
	Control (0)	1.5	3.0	5.0
Maize	52.50	52.00	51.50	51.30
Neem Leaf Meal	0.00	1.50	3.00	5.00
Wheat offal	13.00	12.00	11.40	8.00
Soyabean meal	15.00	15.00	15.00	15.00
Groundnut cake	13.90	13.90	13.50	15.10
Vegetable oil	2.00	2.00	2.00	2.00
Bone meal	2.40	2.40	2.40	2.40
Oyster shell	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20
Methionine	0.25	0.25	0.25	0.25
Premix*	0.25	0.25	0.25	0.25
Table salt	0.30	0.30	0.30	0.30
Total	100	100	100	100
Calculated analysis				
ME (Kcal/Kg)	2989.62	3009.90	3027.09	3073.74
Crude protein (%)	19.79	19.90	19.91	20.48
Crude fibre (%)	3.83	3.94	4.08	4.15
Ether extract (%)	4.11	4.14	4.15	4.22
Calcium (%)	1.03	1.05	1.06	1.08
Phosphorus (%)	0.50	0.53	0.53	0.57
Analysed composition (%)				
Crude protein	19.21	20.89	19.37	19.89
Crude fibre	4.23	5.16	5.98	6.05
Ether extract	6.05	5.62	5.64	4.69
Ash	2.91	4.81	4.28	4.41

*2.5kg premix contains Vitamin A (8,000,000 I.U); Vitamin D3 (2,000,000 I.U); Vitamin E (5,000mg), Niacin (15,000mg); Vitamin B1 (1,500mg); Vitamin B2 (8,000mg); Vitamin B6 (1,500mg), Vitamin B12 (10mg); Vitamin K3 (2,000mg); Calpan (5,000mg); Biotin (20mg); Folic acid (500mg); Antioxidant (125,000mg); Choline chloride (200,000mg); Cobalt (200mg); Copper (5,000mg); Iodine (1,200mg); Iron (40,000mg); Manganese (80, 000mg); Selenium (200mg); Zinc (60,000mg)

Results

Table 3 shows the growth performance parameters of broiler chicks fed diets containing neem leaf meal (NLM) with or without polyzyme supplementation. The final weight, weight gain and feed conversion ratio (FCR) were significantly

($P < 0.01$) influenced by levels of NLM inclusion. Final weight (g/bird) and total weight gain (g/bird) decreased progressively with increasing addition of NLM. Although values recorded for 1.5% NLM did not vary significantly from control. For FCR, control had the best value

which increased with increasing NLM inclusion. Although, control, 1.5% NLM and 3.0% NLM did not differ significantly. The effect of polyzyme supplementation was not significantly ($P>0.05$) different for the parameters measured.

Table 4 shows the growth performance parameters of broilers at the finisher phase. The initial weights recorded were as a result of the final weights of the birds at the starter phase. Significant differences were not also observed in all parameters based on level of NLM inclusion or polyzyme supplementation. The overall growth performance parameters of broiler chickens fed diets containing NLM with or without polyzyme supplementation are presented in Table 5. Based on the mean separation due to level of NLM included, all parameters were not significantly ($P>0.05$) different. Weight gain however seemed to decrease progressively with increasing level of NLM. No significant ($P>0.05$) difference was recorded in the growth performance parameters measured with or without polyzyme supplementation. Blood parameters of broiler chicks fed diets containing NLM with or without polyzyme supplementation are presented in Table 6.

Addition of NLM to broiler chicks' diets did not elicit any significant ($P>0.05$) influence on packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (Hb), erythrocyte sedimentation rate (ESR), mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, lymphocyte, heterophil, basophil and eosinophil values. Packed cell volume, RBC, Hb, ESR and basophil were significantly ($P<0.05$) influenced by polyzyme supplementation. Values recorded for these parameters were higher with polyzyme supplementation than without it. The PCV value for chicks fed polyzyme supplemented NLM diets was 27.92% while those without had 26.04%. Red blood cell count and Hb values were also $4.78 \times 10^6 \text{ mm}^3$; 9.31g/100mL and $3.79 \times 10^6 \text{ mm}^3$; 8.68g/100mL with or without polyzyme supplementation respectively. Table 7 shows the blood parameters of broiler finishers fed diets containing NLM with or without polyzyme supplementation. Only the lymphocyte values varied significantly ($P<0.05$) with NLM inclusion. However, no significant ($P>0.05$) difference was observed in all parameters based on polyzyme supplementation.

Table 3: Growth performance of broiler chicks fed diets containing neem leaf meal with or without polyzyme[®] supplementation

Diets	Level of inclusion (%)	Polyzyme supplementation	Initial weight (g/bird)	Final weight (g/bird)	Total weight gain (g/bird)	Average weight gain (g/bird/day)	Total feed intake (g/bird)	Average feed intake (g/bird/day)	Feed conversion ratio
Control	0	+	98.61	766.70	668.10	31.81	1295.30	61.68	1.94 ^a
Neem leaf meal based	1.5	+	98.61	745.80	647.20	30.82	1315.80	62.66	2.04 ^{ab}
		+	100.00	742.20	642.20	30.58	1381.11	65.77	2.16 ^{ab}
		-	95.83	729.20	633.30	30.16	1369.40	65.21	2.17 ^{ab}
	3	+	97.22	706.90	609.70	29.03	1281.70	61.03	2.11 ^{ab}
		-	100.00	613.90	513.90	24.47	1283.90	61.14	2.55 ^b
	5	+	98.61	629.20	530.60	25.27	1377.50	65.60	2.60 ^b
		-	98.61	621.00	522.30	24.87	1307.70	62.27	2.50 ^{ab}
<i>Pooled standard deviation</i>									
Level of neem leaf meal (NLM)			NS	**	**	**	NS	NS	**
Polyzyme supplementation			NS	NS	NS	NS	NS	NS	NS
Interaction between NLM and Polyzyme			NS	NS	NS	NS	NS	NS	NS
Mean separation									
Level of neem leaf meal									
	0		98.61	756.30 ^c	657.60 ^c	31.32 ^c	1305.60	62.17	1.99 ^c
	1.5		97.92	735.70 ^{cd}	637.80 ^{cd}	30.37 ^{cd}	1375.30	65.49	2.17 ^c
	3		98.61	660.40 ^{de}	561.80 ^{de}	26.75 ^{de}	1282.80	61.08	2.33 ^{cd}
	5		98.61	625.10 ^e	526.50 ^e	25.07 ^e	1342.60	63.93	2.55 ^d
<i>Pooled standard deviation</i>									
Polyzyme supplementation									
	+		2.16	57.67	57.58	2.74	70.52	3.36	0.22
	-		98.61	711.30	612.60	29.17	1333.90	63.52	2.20
			98.26	677.50	579.20	27.58	1319.20	62.82	2.31
<i>Pooled standard deviation</i>									
			2.10	76.46	76.55	3.65	76.31	3.63	0.30

NS = Not significant (P>0.05), **= P<0.01 + = With polyzyme supplementation - = Without polyzyme supplementation

^{a,b,c,d}Means with different superscripts (cde) are significant based on factorial analysis for effect of level of neem leaf meal (P<0.01)

Table 4: Growth performance of broiler finishers fed diets containing neem leaf meal with or without polyzyme® supplementation

Diets	Level of inclusion (%)	Polyzyme supplementation	Initial weight (g/bird)	Final weight (g/bird)	Total weight gain (g/bird)	Average weight gain (g/bird/day)	Total feed intake (g/bird)	Average feed intake (g/bird/day)	Feed conversion ratio
Control	0	+	754.00	1513.00	759.00	27.09	3247.20	115.97	4.51
Neem leaf meal based	1.5	-	766.70	1370.20	603.50	21.55	2892.60	103.31	4.85
		+	749.00	1333.30	584.30	20.87	3531.00	126.10	5.77
	3	-	746.70	1453.30	706.70	25.24	3122.00	111.50	4.48
		+	713.30	1366.50	653.10	23.33	3136.00	112.00	4.88
	5	-	585.00	1326.70	741.70	26.49	3118.00	111.37	4.31
		+	633.00	1189.20	556.00	19.86	3266.00	116.64	5.08
Pooled standard deviation		-	619.40	1274.10	654.60	23.38	3414.00	121.90	5.22
			68.43	135.69	125.31	4.48	322.50	11.52	0.98
Level of neem leaf meal (NLM)									
Polyzyme supplementation			NS	NS	NS	NS	NS	NS	NS
Interaction between NLM and Polyzyme									
Polyzyme			NS	NS	NS	NS	NS	NS	NS
Mean separation									
Level of neem leaf meal	0		760.30 ^a	1441.40	681.10	24.32	3069.90	109.64	4.68
	1.5		747.80 ^a	1393.30	645.50	23.05	3326.00	118.80	4.99
	3		649.20 ^{ab}	1346.60	697.40	24.91	3127.00	111.69	4.59
Pooled standard deviation	5		626.20 ^b	1231.60	605.40	21.62	3340.00	119.28	5.16
			70.77	134.12	129.62	4.63	326.84	11.67	0.95
Polyzyme supplementation	+		712.30	1350.40	638.10	22.79	3294.90	117.68	4.99
	-		679.40	1356.10	676.60	24.17	3137.00	112.02	4.72
Pooled standard deviation			151.49	89.69	127.41	4.55	325.20	11.61	0.92

NS = Not significant (P>0.05), * = P<0.05

^{ab}Means with different superscripts (ab) are significant based on factorial analysis for effect of level of neem leaf meal (P<0.05)

+ = With polyzyme supplementation

- = Without polyzyme supplementation

Table 5: Growth performance of broiler chickens fed diets containing neem leaf meal with or without polyzyme® supplementation

Diets	Level of inclusion (%)	Polyzyme supplementation	Initial weight (g/bird)	Final weight (g/bird)	Total weight gain (g/bird)	Average weight gain (g/bird/day)	Total feed intake (g/bird)	Average feed intake (g/bird/day)	Feed conversion ratio
Control	0	+	98.61	1513.00	1414.00	28.86	4542.40	92.70	3.28
		-	98.61	1370.20	1271.60	25.95	4208.40	85.89	3.31
Neem leaf meal based	1.5	+	100.00	1333.30	1233.30	25.17	4912.00	100.24	4.01
		-	95.83	1453.30	1357.50	27.70	4491.00	91.66	3.31
	3	+	97.22	1366.50	1269.30	25.91	4418.00	90.16	3.49
		-	100.00	1326.70	1226.70	25.03	4402.00	89.84	3.64
	5	+	98.61	1189.20	1090.60	22.26	4643.00	94.76	4.37
		-	98.61	1274.10	1175.50	23.99	4721.00	96.36	4.02
<i>Pooled standard deviation</i>									
			1.90	135.69	135.22	2.76	377.47	7.70	0.57
Level of neem leaf meal (NLM)			NS	NS	NS	NS	NS	NS	NS
Polyzyme supplementation			NS	NS	NS	NS	NS	NS	NS
Interaction between NLM and Polyzyme			NS	NS	NS	NS	NS	NS	NS
Mean separation									
Level of neem leaf meal									
	0		98.61	1441.40	1342.80	27.40	4375.40	89.30	3.29
	1.5		97.92	1393.30	1295.40	26.44	4702.00	95.95	3.66
	3		98.61	1346.60	1248.00	25.47	4410.00	90.00	3.57
	5		98.61	1231.60	1133.00	23.13	4682.00	95.56	4.20
<i>Pooled standard deviation</i>			2.19	134.12	134.09	2.74	368.89	7.53	0.55
Polyzyme supplementation									
	+		98.61	1350.40	1251.80	25.55	4628.80	94.47	3.79
	-		98.26	1356.10	1257.80	25.67	4456.00	90.94	3.57
<i>Pooled standard deviation</i>			2.10	151.49	151.51	3.09	374.43	7.64	0.62

NS = Not significant (P>0.05)

+ = With polyzyme supplementation

- = Without polyzyme supplementation

Table 6: Haematological variables of broiler chicks fed diets containing neem leaf meal with or without polyzyme® supplementation

Diets	Level of inclusion (%)	Polyzyme supplement	PCV (%)	RBC (10 ⁶ mm ³)	Hb (g/100ml)	ESR (mm/hr)	MCHC (%)	MCH (pg)	MCV (μ ³)	Lymphocyte (%)	Hetero phil (%)	Baso phil (%)	Eosinophil (%)
Control	0	+	27.67	4.63	9.22	2.67	33.31	22.75	68.30	60.50	26.00	2.17	1.00
Neem leaf meal based	1.5	+	25.67	3.57	8.55	3.50	33.32	27.91	83.70	59.17	24.33	2.00	1.00
		-	26.67	4.13	8.90	3.33	33.38	24.25	72.60	59.67	23.17	2.50	1.17
	3	+	26.83	4.04	8.95	3.50	33.35	25.32	75.90	58.67	27.00	2.00	1.17
		-	28.17	4.97	9.40	2.67	33.38	19.34	57.94	60.33	25.33	2.50	0.83
	5	+	27.00	4.36	9.00	3.33	33.33	21.02	63.05	60.33	23.67	2.00	1.00
		-	29.17	5.37	9.72	2.00	33.31	18.36	55.10	58.67	24.33	2.33	0.83
		-	24.67	3.22	8.22	4.17	33.31	29.30	87.90	60.17	24.17	2.33	0.67
<i>Pooled standard deviation</i>			2.63	1.33	0.87	1.35	0.10	8.36	25.07	1.39	2.69	0.40	0.41
Level of neem leaf meal (NLM)			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Polyzyme supplementation			*	*	*	*	NS	NS	NS	NS	NS	*	NS
Interaction between NLM and Polyzyme			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mean separation													
Level of neem leaf meal	0		26.67	4.10	8.88	3.08	33.31	25.33	76.02	59.83	25.17	2.08	1.00
	1.5		26.75	4.08	8.93	3.42	33.36	24.79	74.29	59.17	25.08	2.25	1.17
	3		27.58	4.66	9.20	3.00	33.35	20.18	60.50	60.33	24.50	2.25	0.97
	5		26.92	4.30	8.97	3.08	33.31	23.82	71.51	59.42	24.25	2.33	0.75
<i>Pooled standard deviation</i>			2.83	1.43	0.94	1.44	0.10	8.59	25.75	1.45	2.82	0.43	0.39
Polyzyme supplementation	+		27.92 ^a	4.78 ^a	9.31 ^a	2.67 ^a	33.34	21.17	63.50	59.79	24.71	2.38 ^a	0.96
	-		26.04 ^b	3.79 ^b	8.68 ^b	3.63 ^b	33.33	25.89	77.65	59.58	24.79	2.08 ^b	0.96
<i>Pooled standard deviation</i>			2.62	1.32	0.87	1.33	0.10	8.31	24.90	1.49	2.79	0.40	0.41

NS = Not significant (P>0.05), * = P<0.05

^{a,b}Means with different superscripts (ab) are significant based on factorial analysis for effect of Polyzyme supplementation (P<0.05)

PCV= Packed cell volume, RBC= Red blood cell, Hb= Haemoglobin, ESR= Erythrocyte sedimentation rate, MCHC= Mean cell haemoglobin concentration, MCH= Mean cell haemoglobin, MCV= Mean cell volume, + = With polyzyme supplementation, - = Without polyzyme supplementation

Table 7: Haematological variables of broiler finishers fed diets containing neem leaf meal with or without polyzyme[®] supplementation

Diets	Level of inclusion (%)	Polyzyme supplementa-tion	PCV (%)	RBC (10 ⁶ m ³)	Hb (g/100ml)	ESR (mm/hr)	MCHC (%)	MCH (pg)	MCV (μ ³)	Lymphocyte (%)	Hetero phil	Baso phil (%)	Eosinophil (%)
Control	0	With	23.56	2.63	7.86	5.44	33.35	32.48	97.35	60.56 ^{ab}	24.22	2.22	0.78
		Without	22.11	2.19	7.37	6.56	33.32	37.20	111.57	60.67 ^{ab}	25.89	2.11	1.00
Neem leaf	1.5	With	23.78	2.80	7.94	5.56	33.41	30.92	92.54	60.11 ^b	25.44	2.22	0.89
		Without	24.67	3.19	8.22	4.67	33.33	29.49	88.49	59.89 ^b	25.11	2.22	0.78
meal based	3	With	20.56	2.09	6.83	6.89	33.23	35.89	108.03	62.33 ^a	25.22	2.22	1.00
		Without	22.44	2.29	7.48	6.00	33.32	35.04	105.17	60.89 ^{ab}	23.22	2.00	1.00
	5	With	23.78	2.97	7.93	5.11	33.36	29.52	88.51	60.11 ^b	24.11	2.22	1.00
		Without	22.00	2.30	7.34	6.22	33.38	35.94	107.70	60.78 ^{ab}	24.89	2.33	0.78
Pooled standard deviation			3.31	1.07	1.10	2.38	0.12	8.76	26.23	1.48	2.84	0.41	0.29
Level of neem leaf meal (NLM)													
Polyzyme supplementation			NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
Interaction between NLM and Polyzyme			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mean separation													
Level of neem leaf meal			0	22.83	2.41	7.61	33.34	34.84	104.46	60.61 ^{cd}	25.06	2.17	0.89
		1.5	24.22	3.00	8.08	5.11	33.37	30.20	90.51	60.00 ^d	25.28	2.22	0.83
		3	21.50	2.19	7.16	6.44	33.27	35.47	106.60	61.61 ^c	24.22	2.11	1.00
		5	22.89	2.64	7.64	5.67	33.37	32.73	98.11	60.44 ^{cd}	24.50	2.28	0.89
Pooled standard deviation			3.31	1.07	1.10	2.37	0.12	8.75	26.21	1.49	2.84	0.40	0.30
Polyzyme supplementation													
		With	22.92	2.63	7.64	5.75	33.34	32.20	96.61	60.78	24.75	2.22	0.92
		Without	22.81	2.49	7.60	5.86	33.34	34.42	103.24	60.56	24.78	2.17	0.89
Pooled standard deviation			3.40	1.09	1.14	2.39	0.13	8.80	26.39	1.58	2.83	0.40	0.30

NS = Not significant (P>0.05), * = P<0.05

^{cd}Means with different superscripts (cd) are significant based on the factorial analysis for the effect of level of neem leaf meal (P<0.05)

PCV= Packed cell volume, RBC= Red blood cell, Hb= Haemoglobin, ESR= Erythrocyte sedimentation rate, MCHC= Mean cell haemoglobin concentration, MCH= Mean cell haemoglobin, MCV= Mean cell volume

Discussion

The observed effects of polyzyme® on the growth parameters of the chicks might infer that there was no improvement in the utilization of the feed at the level of 400g/tonne used in this study. However, no difference in level of NLM was observed when the assessment of final weight and total weight gain was done at the finisher phase and overall possibly because older birds have more developed digestive system. This suggests that values below 5% should be considered when NLM is to be used in broiler chickens diets.

Obikaonu (2012) also observed significant difference in final weights of finishers given control and 5% NLM diet. Onyimonyi *et al.* (2009) however suggested inclusion level of 0.5%. Polyzyme® as a multi-enzyme contains xylanase, phytase, cellulase, β -glucanase, pectinase, α -amylase, protease, α -galactosidase, β -galactosidase, lipase and mannanase. According to Manafi *et al.* (2011), the use of polyzyme® elicited a non-significant and marginal improvement in the final weights of broilers fed corn bran diets when reared up to 8 weeks. Also, when Fabunmi *et al.* (2019) used polyzyme® in diets which had maize partially replaced with kolanut husk, no significant improvement in broiler weight gain was observed. Furthermore, Sunmola *et al.* (2018) found no difference in final body weight and weight gain of broilers fed sweet orange peel meal diets with or without polyzyme® supplementation. So, the submission of Duru and Dafwang (2010) that maxigrain® supplementation did not improve an isocaloric and isonitrogenous diet but did improve the utilization of low energy diet might be applicable. Also, according to Bedford and Schulze (1998), factors like source of enzyme, variety of unconventional feedstuff, age and health status of the animal affect the response of such animal to feed supplemented with enzymes. As such, increase in the quantity

of Polyzyme to be incorporated in fibrous feedstuffs might be considered. Haematology is valuable in assessing the physiological responses of chickens (Khan and Zafar, 2005). And as noted earlier, packed cell volume (PCV) and haemoglobin concentration (Hb) are two parameters of high significance in determining their health status. The parameters were not affected by level of NLM up to 5% at the starter phase. Although, the values were significantly different based on polyzyme supplementation, both values were still within the ranges (PCV= 25- 45%; Hb= 7- 13g/100ml) described for healthy birds by Mitruka and Rowsley (1977) and Ross *et al.* (1978) and PCV range of 22-43% by Orawan and Aengwanich (2007). Fabunmi *et al.* (2019) however did not record significant effect of polyzyme supplementation on PCV and Hb of broilers fed kolanut husk meal. The significance seen in lymphocyte values based on levels of NLM were slight and did not follow any trend with increase/ decrease in inclusion level and the values fell within the reference range. In line with this result, Bonsu *et al.* (2012) did not record significance in Hb of birds fed NLM up to 2.5%. Hence, inclusion of up to 5% NLM in broiler diets did not predispose them to anaemia.

Conclusion

The study showed that broiler starters could tolerate up to 3% neem leaf meal and finishers up to 5% based on growth performance. The enzyme (Polyzyme®) used at 400g/tonne did not improve the utilization of neem leaf meal by the broiler-chickens. The two parameters of high significance in determining the health status of birds which are packed cell volume and haemoglobin concentration were not affected by level of neem leaf meal (up to 5%).

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