EFFECTS OF WATERLEAF VEGETABLE (*Talinum triangulare*) MEAL ON THE PERFORMANCE AND IMMUNITY OF NEWCASTLE DISEASE VACCINATED LAYERS

***SANDA, M. E. AND ABU, M. H.**

Department of Animal Production, Faculty of Agriculture, Kogi State University, Anyigba, Kogi State, Nigeria.
***Corresponding author: mary@davidsanda.com**
Article received: 14th Feb. 2015; Article accepted: 5th July, 2015.

ABSTRACT
Sixty four point of lay pullets were allotted to 4 groups. The groups, T_1, T_2, T_3 and T_4 were fed feed composed of 0%, 1%, 1.5% and 2% of Waterleaf Vegetable Meal (WLVM) respectively. The trial lasted for 8 weeks and each group had two replicates. The pullets were vaccinated with Newcastle disease vaccine (Komarov) and blood was collected from each pullet 10 days post vaccination and subsequently fortnightly for Haemagglutination Inhibition (HI) tests till the end of the study. The daily feed intake (DFI) and Feed Conversion Ratio (FCR) varied significantly and ranged from 103.72 – 106.56g and 2.49 – 2.90, respectively. The egg production also varied significantly ($P<0.05$) with the following ranges; average egg weight (49.11 -55.1), Egg mass (35.76-42.55g), Egg number (36.89 – 48.52), Hen day production (HDP) (65.88-86.64) and Hen housed production (HHP) (65.88-86.64). There was no significant difference ($P>0.05$) in initial weight and final weight of hens in different groups. Also, NDV antibody titre of T3 (2048.00) did not vary significantly ($P>0.05$) from 1280, 770 and 1536 of the groups fed feeds of 0, 1 and 2 % WLVM respectively. T_3 (1.5% WLVM inclusion) had the best FCR (2.49), HHP (86.64) and HDP (86.64) and so inclusion of WLVM at 1.5% is recommended
Keywords: Newcastle disease, Waterleaf Vegetable Meal, Vaccination, Immune Response, Egg Production, Layers

INTRODUCTION

Most developing countries of the world including Nigeria are faced with the challenge of inadequate animal protein consumption. An average Nigerian consumes only about 10g per day of the minimum recommended daily protein intake of 35g per person per day (FAO, 1997).

Poultry production can be used to ameliorate this shortage in animal protein intake due to its short generation interval and quick returns on investment. Poultry eggs are known to provide the most perfectly balanced food containing all essential amino acids, minerals and vitamins (Branckaert *et al.*, 2000). Eggs have been described as nature's complete food that contains the basic dietary needs of man. However, the reproductive efforts of birds can be influenced by disease processes either by acting directly and altering the ability of the lining cells to perform their specialized functions or by generally compromising the health of the bird (Solomon, 2002).

Newcastle Disease (ND) is a highly contagious and widespread disease of the avian species causing severe economic losses in domestic poultry, especially chickens (Alexander, 2001). The causative agent of the disease is *Newcastle Disease Virus* (NDV), also designated as Avian *Paramyxovirus* Serotype 1 (APMV-1) and belongs to the genus *Avulavirus* within family *Paramyxoviridae* (Mayo, 2002a, b).

It is believed that there is no chemotherapeutic agent for Newcastle disease (ND) and control is by vaccination and biosecurity measures but Ezeibe *et al*. (2011) reported cure for ND with synthethic aluminum – magnesium silicate. Despite vaccination, outbreaks of ND are still common (Sanda *et al.*, 2008). There is, therefore, the need for nutritional manipulation involving leafy vegetables which contains vitamins and minerals that have been reported to possess immune-stimulatin properties (Ezekwe *et al.*,2001).

Waterleaf (*Talinum triangulare*) is a non-conventional vegetable crop of the Protulacea family which originated from Tropical Africa and is widely grown in West Africa, Asia, and South America (Schippers, 2000). Ibeawuchi *et al.*(2007) noted that the leaves and young shoots are used to thicken sauce and is consumed in large quantities in Southern part of Nigeria. Waterleaf crude protein compares favorably with that of cowpea, peanut, millet and cashew nuts (Ofusor *et al.*, 2008). Akachukwu and Fawusi (1995) investigated the crude protein content of waterleaves and tender stems and found it to be as high as 29.4% and 13.4%, respectively. There is therefore the need for nutritional manipulations with leafy vegetables which contain vitamins and minerals that have been reported to possess immune-stimulating properties (Ezekwe *et al.*, 2001)

Several reports abound as to the immune-stimulating properties of certain vitamins which can boost immunity and this study is pertinent since waterleaf possesses essential nutrients like β-carotene which is a precursor of vitamin A, mineral (such as

magnesium, calcium and potassium), pectin, protein and other vitamins (Ezekwe *et al.*,2001).

This study, therefore, sets out to determine the effect of inclusion of waterleaf vegetable in diets of layer birds vaccinated with Newcastle Disease Komarov Vaccine (NDKV) with particular attention to immune and performance responses.

The rampant outbreak of Newcastle Disease (ND) despite vaccination justifies this study. The fact that the test feed ingredient (waterleaf) possesses some immune stimulating properties also justifies the study.

The objective of this study was to determine the effect of waterleaf vegetable meal on the performance and immune responses of Newcastle disease vaccinated layers.

MATERIALS AND METHODS

The study was carried out at the Poultry Unit of the Livestock Teaching and Research Farm of the Department of Animal Production, Kogi State University, Anyigba. Anyigba is located in the derived savanna zone of Nigeria on latitude 7^0 30` N and longitude $7^0$ 09`E.

Waterleaf Vegetable (WLV) was collected from the university farm, processed and subjected to proximate analysis. The processed Waterleaf vegetable meal (WLVM) was used with feed ingredients to formulate different diets. Four iso-nitrogenous and iso-caloric layer diets were formulated with the feed ingredients as shown on Table 1. Diet 1 was the control diet and served as the reference diet with which other diets were compared with 0% inclusion of WLVM. Diets 2, 3 and 4 were formulated such that WLVM was introduced into the diets at graduated levels of 1%, 1.5% and 2% respectively.

Fresh Waterleaf Vegetable (WLV) was collected from different places including the Kogi State University Teaching and Research Farm, Anyigba. The vegetable was cleaned thoroughly to remove contaminants (dust, dung, dirt and sand) and chopped into tiny pieces. The chopped vegetable was then dried in the green house located at the Faculty of Agriculture, Kogi State University, Anyigba at a temperature of about 43^0C. Thereafter, the dried vegetable was milled and a portion of it taken to the Ahmadu Bello University (ABU) Biochemical Laboratory, Department of Animal Sciences, Zaria for proximate analysis according to the AOAC (1995) Procedure.

A total of 64 layers at point of lay were used for the study. The study was carried out for 8 weeks. The birds were randomly assigned into 4 dietary treatments groups of T_1 (control), T_2, T_3 and T_4. Each of the treatment groups were replicated with 8 birds in each replicate.

The birds were vaccinated against Newcastle disease with a Mesogenic strain of *Newcastle disease virus* (Komarov vaccine) at the point of lay (Week 20).

Blood sample collection commenced 10 days post inoculation and subsequent collection thereafter was done every 2 weeks. The sera samples obtained was subjected to Haemagglutination Inhibition (HI) test to determine the immune response as described by OIE (2002).Sera samples were stored at -20^0C and were later taken for HI analysis at the Veterinary Medicine Laboratory of the Veterinary Teaching Hospital, University of Nigeria (UNN), Nsukka.

Data generated from the study (HI and performance characteristics) were subjected to one-way analysis of variance using SAS statistical package (SAS, 2002). Significant differences ($P<0.05$) among the means were separated using Duncan Multiple Range Test of the same software.

RESULTS AND DISCUSSION

The proximate composition of Waterleaf Vegetable Meal on Table 2 differed from the 88.94% DM, 19.89% CP, 8.10% CF, 58.16% NFE, 3.35% EE and 10% Ash (mineral) reported by Nworgu *et al.*(2014). The analyzed CP and EE obtained from this result (30.94% and 1.56% respectively) were higher than those reported by Aja *et al.* (2010) who reported CP and EE of 18.75% and 1.44% respectively for waterleaf vegetable. The proximate results obtained also disagree with those reported by Anselm and Ubokudom (2010). The percent Ash and percent CF reported by the workers above (33.98% and 11.12% respectively) were higher than those obtained in this result (7.84% and 5.88%). However, the percent CP and percent NFE reported by the workers (22.1% and 32.8%) were lower than those obtained in this study (30.94% and 53.78%). The percent CP obtained in the result of this study is also slightly higher than that reported by Akachukwu and Fawusi (1995) who reported a CP of 29.4%. The handling and processing technique (drying under room temperature) might have contributed to this percent CP and NFE values.

From the results on Table 3, feed intake increased with increase in WLVM. T_3 (106.00g) and T_4 (106.56g) were significantly higher ($P<0.05$) than the control T_1. The increase in feed intake of the pullets can be attributed to the palatability of the diet. There was no effect of the feed on weight changes because of the fact that the birds were in their early productive phase such that the feed they consumed was mobilized into egg production. The average daily feed intake was within the 111g upper limits of feed intake reported by Tion and Njoku (2001) but it disagrees with the 120g reported by Say (1992). The

effect of the treatment on egg number was significant and this can be attributed to the fact that the birds were in their early laying phase. Average daily egg number had values ranging from 36.89g to 48.52g. There was no significant difference (P>0.05) in the average egg weight which varied from 49.11 – 55.12. Feed conversion ratio (FCR) from this study was lower than the 2.96 to 3.27 reported by Akande *et al.* (2007) in a study on the response of laying chickens to graded levels of *Tephrosia bracteolate* leaf meal. The FCR obtained in this study was also slightly higher than 2.23 to 2.62 reported by Khan and Sardar (2005). T_3 (1.5% WLVM) had a FCR of 2.49 which is considered the best when compared with T_1, T_2 and T_4 which have FCR values of 2.90, 2.86 and 2.86 respectively. The FCR results can also be attributed to the rate of egg production of the

Table 1: Gross Composition (%) of Experimental Diets for Laying Pullets Fed Waterleaf Vegetable Meal (WLVM)

Ingredients	T1 (0%WLVM)	T2 (1%WLVM)	T3 (1.5%WLVM)	T4 (2%WLVM)
WLVM	0	1.00	1.50	2.00
Maize	34.00	34.00	34.00	34.0
BDG	13.00	12.00	11.50	11.00
FFSBM	24.00	24.00	24.00	24.00
Maize offal	10.25	10.25	10.25	10.25
Rice offal	8.00	8.00	8.00	8.00
Bone Meal	3.00	3.00	3.00	3.00
Limestone	7.00	7.00	7.00	7.00
Salt	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Vitamin/Mineral Premix	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**
Calculated nutrients				
Crude protein (%)	17.10	17.02	16.98	16.94
Crude fibre (%)	4.31	4.28	4.26	4.24
Ether extract (%)	7.25	7.23	7.21	7.19
Calcium (%)	3.88	3.89	3.89	3.90
Total phosphorus (%)	0.92	0.92	0.92	0.91
Energy (Kcal/kgME)	2594.4	2599.56	2602.14	2604.72

WLVM = Waterleaf., BDG = Brewer's dried grain, FFSBM = Full fat Soyabean meal. Premix: Vitamin A 1000 IU, Vitamin D 200 IU, Vitamin E 1.01 IU, Vitamin K 0.2mg, Thiamine(B1) 0.15mg, Riboflavin (B2) 0.4mg, Pyridoxine (B6) 0.15mg, Niacine 1.5mg, Vitamin B12 0.15mg, Pantothenic acid 0.5mg, Folic acid 0.05mg, Biotin 0.002mg, Choline chloride 0.02g, Antioxiant 0.0125g, Manganese 0.008g, Zinc 0.002g, Iron 0.005g, Copper 0.0005g, Iodine 0.00012, Selenium 0.002mg, Cobalt 0.02g.

Table 2: Proximate Composition of Waterleaf Vegetable Meal (WLVM) Fed to Laying Pullets.

Determined Analysis	WLVM
Dry Matter (%)	94.56
Crude Protein (%)	30.94
Crude Fibre (%)	5.88
Ether extract (%)	1.56
Ash (%)	7.84
NFE (%)	53.78
Energy (kcal/kgME)	3530.25

ME= Metabolizable Energy, NFE = Nitrogen Free Extract Metabolizable energy was calculated using the formula of Pauzenga (1985).

Table 3: Performance of Hens fed feeds supplemented with waterleaf vegetable meal.

Ingredients	T1 (0%WLVM)	T2 (1%WLVM)	T3 (1.5%WLVM)	T4 (2%WLVM)	SEM	LOS
Growth						
Initial weight (kg)	1.50	1.51	1.50	1.52	0.00	NS
Final weight (kg)	1.50	1.49	1.49	1.52	0.00	NS
Weight gain (kg)	0	-0.02	-0.01	0	0.00	NS
Daily feed intake (g)	103.72^{b}	105.08^{ab}	106.00^{a}	106.56^{a}	3.29	*
FCR	2.90^{b}	2.86^{ab}	2.49^{a}	2.86^{ab}	0.00	*
Mortality (%)	0	6.25	0	0	0.28	NS
Production						
Egg weight (g)	2002.32^{b}	2064.19^{b}	2382.82^{a}	2085.63^{b}	4.24	*
Average egg weight (g)	54.27	54.34	49.11	55.12	0.23	NS
Egg Mass (g)	35.76^{b}	36.86^{b}	42.55^{a}	37.24^{b}	0.08	*
Egg number	36.89^{b}	37.98^{b}	48.52^{a}	37.84^{b}	0.19	*
HDP (%)	65.88^{b}	72.63^{ab}	86.64^{a}	67.57^{b}	3.86	*
HHP (%)	65.88^{b}	67.82^{ab}	86.64^{a}	67.57^{ab}	0.34	*

abcd=means on same row with the same letters are not significantly different (P>0.05). * = significant. FCR = Feed conversion ratio, HDP = Hen-day egg production, HHP = Hen-house egg production, SEM = Standard error of mean, LOS = Level of significance, NS =No Significant difference.

Table 4: Mean Haemagglutination Inhibition (Hi) Titres of NDV of Laying Pullets Fed Waterleaf Vegetable Meal (WLVM).

Ingredients	T1 (0%WLVM)	T2 (1%WLVM)	T3 (1.5%WLVM)	T4 (2%WLVM)	SEM	LOS
Week 22	1088.00	1792.00	1408. 00	1664.00	42.01	NS
Week 24	928.00	1152.00	1536.00	2048.00	42.01	NS
Week 26	1153.00	1409.00	1216.00	961.00	42.0	NS
Week 28	1280.00	770.00	2048.00	1536.00	42.0	NS

birds since egg production was used to compute FCR. Hen-day production (HDP) value for T3 (1.5% WLVM) which was 86% was higher than the 61% to 70% reported by Chineke (2001) as compared to HDP values of T_1, T_2 and T_4. HDP values were generally above the range of 72.4% to 85.7% for layers in their 7th month of lay as reported by Ezieshi *et al.* (2001). Hen-house production (HHP) had values similar to the HDP except at T_2 where the HHP reduced due to the mortality recorded in that treatment group. The improved HDP and HHP values can also be further attributed to the appreciable egg mass and egg number recorded during the study. Mean total egg weight ranged between 2002.32g to 2382.82g. Average egg weight fell within the range of 53.23g to 62.54g reported by Orunmuyi *et al.* (2007) in a study on comparison of egg quality traits of four poultry species.

Table 4 shows that the four treatment groups (T_1, T_2, T_3 and T_4) which were vaccinated against Newcastle disease (ND) using Komarov vaccine all had HI titres (immunity) high enough to protect against Newcastle disease but there was no significant ($P>0.05$) difference in the immunity of the laying pullets served WLVM.

In the second collection period (week 24), T_4 had the best mean HI titre (immunity) when compared with T_1, T_2, and T_3. The HI titres for T_1, T_2, T_3 and T_4 in the 28th week were 1280, 770, 2048 and 1536 respectively ($P>0.05$) with T_3 having the best titre. There was, however, no significant effect of WLVM on the immunity of the laying hens statistically throughout the period of study.

CONCLUSION AND RECOMMENDATIONS

Conclusively, Hen-day production (HDP) and hen-house egg production (HHP) were best at 1.5% inclusion level of WLVM. Waterleaf vegetable meal caused appreciable increase in egg weight, egg mass, number of eggs laid, feed intake and FCR at 1.5% level of inclusion. It did not however cause significant increase in average egg weight at this inclusion level also, feeding the pullets with WLVM did not have any adverse effect on the immunity of the pullets.

Based on the findings in this study, it is recommended that farmers should include WLVM in diets of laying pullets at 1.5% level of inclusion for better egg production, laying pullets should be vaccinated with Newcastle Disease (ND) vaccine (Komarov) as this will go a long way to provide protective antibody against Newcastle disease and the birds' immunity should be monitored through HI test. Further studies should be carried out with WLVM to validate the immune-stimulating properties of Waterleaf in flocks challenged with the velogenic strain of NDV.

REFERENCES:

Aja, P.M., Okaka, A.N.C., Ibiam, U.A., Uraku, A.J. and Onu, P.N. (2010). Proximate Composition of Talinum triangulare (Waterleaf) and its Softening Principle. Pakistan Journal of Nutrition 9(6): Pp 524.

Akachukwu, C.O and Fawusi, M.A.O. (1995). Growth characteristics, yield and nutritional value of waterleaf, Talinum triangulare (Jacq) wild in a semi-wild environment; Discovery and Innovation; 7: 163-172.

Akande, T.O. Adeyeri, M.K., Longe, O.G. and Odunsi, A.A. (2007). Response of laying chickens to graded levels of Tephrosia bracteolate leaf meal fed with soybean meal or full fat soybean meal. Livestock Research for Rural Development 19 (8) 2007.

Alexander, D.J. (2001). Newcastle disease - The Gordon Memorial Lecture. British Journal of Poultry Science. 42: 5-22.

Anselm, A. E. and Ubokudom, E.O. (2010). Economics of Waterleaf (Talinum triangulare) in Akwa Ibom State, Nigeria. Journal of Field Actions Research 2: 16-22

AOAC (1995). Official Methods of Analysis. 16th edn. Association of Official Analytical Chemists. Washington D.C.

Branckaert, R.D.S., Gaiviria, I., Jallade, J., and Seiders, R.W. (2000). Transfer of technology in poultry production for developing countries. FAO, Rome; http//:www.fao.org/sd/cddirect/cdre0054.htm.

Chineke, C.A. (2001). Inter-relationship existing between body weight and egg production traits in Olympia Black layers. Nigerian Journal of Animal Production 28: 1-8.

Ezeibe, M.C.O., Ijabo, O., Uzopuo, C., Okoroafor, O.N., Eze, J.I., Mbuko, I.J., Sanda, M.E., Animoke, P.C. and Ngene, A.A. (2011). Effects of Aluminium-Magnesium Silicate on Newcastle Disease Virus and on Recovery of Infected Chicks. International Journal of Biological and Chemical Sciences. 5: 825-829. http://dx.doi.org/10.4314/ijbcs.v5i2.72160

Ezekwe, M.O., Besong, S.A. and Igbokwe, P.E. (2001). Beneficial benefits of purslane and waterleaf supplements to human, FASEBJ. 16: A639.

Ezieshi, E.U., Omoregie, A. and Olomu, J.M. (2001). Performance and some physical and internal qualities of laying chickens fed palm kernel

cake-based diets. Proceedings of the 26th Annual Conference of the Nigerian Society for Animal Production (NSAP), pp. 199-201.

FAO (1997). FAO Statistical database. Food and Agricultural Organization of the United Nations, Rome. World watchlist for domestic animal diversity. Pp 716.

Ibeawuchi, I.I., Nwufo, M.I., Oti, N.N., Opara, C.C. and Eshet, E.T. (2007). Productivity of intercropped Green (Amaranthus cruentus)/waterleaf (Talinum triangulare) with Poultry Manure Rates in Southeastern Nigeria. Journal of Plant sciences. 2(2): 222 -227.

Khan, S.H and Sardar, R. (2005). Effects of Vitamin C supplementation on the performance of Desi-Fayoumi and Commercial White Leghorn Chicken exposed to heat stress. Pakistan Veterinary Journal 25(4): 163 – 166.

Mayo, M.A. (2002a). Virus taxonomy-Huston 2002. Archives of Virology, 147: 1071-1079.

Mayo, M.A. (2002b). A summary of taxonomic changes recently approved by ICTV. Archives of Virology 147: 1655- 1663.

Nworgu, F.C., Philip, C.N.A., Gabriel N.E. and Elijah, I.O. (2014). Performance and Haematological Indices of Broilers Fed Waterleaf (Talinum triangulare) Meal Supplement. Journal of Agriculture and Ecology Research. 2(1):223-228.

Ofusor, D.A., Adelakun, A.E., Ayoka, A.O., Oluwayinka, O.P., Omotoso, E.O., Odukossya, S.A., and Adeyemi, D.O. (2008). Waterleaf (Talinum triangulare) enhances cerebral functions in swiss albino mice. Journal of Neurological science. (Turk) 25: 239-246.

Orunmuyi, M., Okezie, O.I., Bawa, G.S. and Ojo, O.A. (2007). A comparison of egg quality traits of four poultry species. Proceedings of the 41st Annual conference of the Agricultural Society of Nigeria (ASN), pp. 345 – 347.

Pauzenga, U. (1985). Feeding Parent Stock. Journal of Zootechnica International, December, 1985. Pp 22-24.

Sanda, M.E.; Anene, B.M. and Owoade, A. (2008). Effects of Levamisole as an Immunomodulator in Cockerels vaccinated with Newcastle Disease Vaccine. International Journal of Poultry Science 7(11) 1042 -1044, ISSN 1682 – 8356.

SAS (2002). Statistical Analysis System, Computer Software, Version 9. Statistics SAS Institute Inc. Cary, NC 27513. USA.

Say, R.R. (1992). Manual of Poultry Production in the Tropics, English Edn. Published by CB International. Page 118.

Schippers, R.R. (2000). African Indigenous Vegetables – An overview of the cultivated species, NRI/ACP, EU, Chatten, UK.

Solomon, S.E. (2002). The Oviduct in Chaos. World Poultry Science Journal, 58:41-48.

Tion, M.A. and Njoku, P.C. (2001). The effect of calcium sources and limestone deposit on laying hens' performance and egg shell quality. Proceedings of the 26th Annual Conference of the Nigerian Society for Animal Production (NSAP), 26: 276-278.