Effect of Naturally Fermented Sweet Orange (*Citrus sinensis*) Peel Meal on Egg Quality and Blood Constituents of Nera Black Layers

Oyewole[1], B.O., Oluremi[2], O.I.A., Aribido[1], S.O. and Ayoade[3], J.A.

[1]Department of Animal Production, Faculty of Agriculture, Kogi State University, Anyigba, Nigeria
[2]Department of Animal Nutrition, College of Animal Science, University of Agriculture, Makurdi, Nigeria
[3]Department of Animal Production, University of Agriculture, Makurdi, Nigeria
Email:benjowole@yahoo.com

ABSTRACT

One hundred and twenty point of lay pullets were used in a fifteen week feeding trial to evaluate the effect of 24-hour naturally fermented sweet orange (*Citrus sinensis*) peel meal (SOPM) as substitute for maize on their egg quality and blood constituents. Observed result did not indicate significant differences ($p>0.05$) in all egg quality parameters measured. Egg weight, shell weight, shell thickness, yolk height, yolk diameter, yolk index, haugh unit and yolk colour ranged from 58.07g (T_{30}) - 59.67g, 6.40cm – 6.57cm (T_0), 0.33mm – 0.34mm, 1.96cm - 1.99cm, 4.00cm – 4.05cm, 0.48 - 0.50, 83.48 – 83.52 and 560.40 – 567.00 respectively. Haematology parameter ranges for PCV (32.70% - 33.62%), Hb (10.88 - 11.17g/dl), RBC ($4.28x10^6$µl - $4.80x10^6$µl), WBC ($5.39x10^3$µl – $5.88x10^3$µl), MCH (23.10pg – 25.55pg), MCV (69.47fl - 76.39fl) and MCHC (33.22% - 33.32%) indicated that fermented SOPM in the diets did not affect ($p>0.05$) haematology constituents adversely. Serum indices evaluated showed that substitution of maize with SOPM had no significant effect ($p>0.05$) on total protein, globulin, albumin, uric acid, SGOT and SGPT but had a significant effect ($p<0.05$) on urea. The obtained values were total protein 5.26g/dl - 5.56g/dl, globulin 3.28g/dl - 3.50g/dl, albumin 1.98g/dl - 2.10g/dl, uric acid 3.73mg/dl - 4.05mg/dl, SGOT 138.00µ/ml to 141.33µl/ml, SGPT 37.00µl/ml to 40.00µl/ml and urea 16.22mg/dl - 17.62mg/dl. All values fell within reference ranges for healthy birds. SOPM was capable of sustaining laying pullets and at the same time furnished adequate nutrients for good quality egg production even at 30% maize substitution.

Keywords: Egg quality, blood constituents, sweet orange peel meal, maize substitution, Nera black layers.

INTRODUCTION

Egg quality traits determine the acceptability and market value of table eggs (Oluyemi and Roberts, 2000). Ojedapo *et al.* (2009) posited that egg production enterprise is of great economic importance, whose success depends on the total number of quality eggs produced. The quality of table eggs is in turn influenced to a large extent by the quality of nutrition received by the bird (Roland, 1980; Quereshi, 1985). For long, the industry had subsisted on conventional feed ingredients such as maize, whose availability for poultry feed is being hampered by low production, other competitive uses and cost. Consequently, Garba *et al.* (2010) suggested partial or complete replacement of expensive and conventional feed ingredients with cheaper non-conventional substitutes. According to Yusuf *et al.* (2010), the use of non-conventional feedstuff may compromise the quality of the feed, which may necessitate the evaluation of the health status of the bird and the quality of its products.

Objective of the study

The study was designed to evaluate the effect of partial substitution of maize with fermented sweet orange peel meal (SOPM) on egg quality, haematology and serum biochemistry of layers.

MATERIALS AND METHODS

The feeding trial was conducted in the Poultry Unit of the Teaching and Research Farm of Kogi State University, Anyigba, Nigeria. Anyigba is located on latitude 7^0 06' N and longitude 6^0 43' E (Amhakhian, 2010). Fresh sweet orange (*Citrus sinensis*) peels of mixed varieties were collected from orange sellers and fermented for 24 hours and thereafter sun-dried (Oluremi *et al.,* 2010). The sun dried fermented orange peels were ground to obtain sweet orange peel

meal (SOPM). Four diets were formulated as shown in Table 1. Fermented SOPM substituted maize at 0%, 10%, 20% and 30% in the experimental diets coded as T_0 (control), T_{10}, T_{20} and T_{30}.

Table 1: Gross composition of experimental diets for layers fed 24-hour fermented SOPM (kg)

Ingredients	24 hour fermented SOPM			
	T_0	T_{10}	T_{20}	T_{30}
Maize	48.00	43.20	38.40	33.60
SOPM[1]	0	4.80	9.60	14.40
FFSBM[2]	23.00	23.00	23.00	23.00
BDG[3]	19.00	19.00	19.00	19.00
Bone ash	4.00	4.00	4.00	4.00
Limestone	5.20	5.20	5.20	5.20
Methionine	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10
Common salt	0.25	0.25	0.25	0.25
Vitamin/mineral premix[4]	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Analyzed nutrients				
Dry matter (%)	90.51	90.23	90.20	90.25
Crude protein (%)	20.05	19.63	19.74	19.91
Crude fibre (%)	3.80	3.68	3.64	3.59
Ether extract (%)	4.13	3.88	3.82	3.78
Ash (%)	9.52	8.77	8.73	8.71
Nitrogen free extract (%)	53.02	54.29	54.28	54.26
Energy (kcal/kgME)[5]	2935.38	2943.84	2942.66	2944.97

[1]SOPM=Sweet orange peel meal

[2]FFSBM=Full fat soyabean meal

[3]BDG=Brewer's dried grain

[4]Real agro-mix [(R)] (layers) supply /kg of feed

Vitamin A 1000 IU, Vitamin D 200IU, Vitamin E 1.0IU, Vitamin K 0.2mg, Thiamine (B1) 0.15mg, Riboflavin (B2) 0.4mg, Pyridoxine(B6) 0.15mg, Niacin 1.5mg, Vitamin B12 0.001mg, Pantothenic acid 0.5mg, Folic acid 0.05mg, Biotin 0.002mg, Choline chloride 0.02g, Antioxidant 0.0125g, Manganese 0.008g, Zinc 0.002g, Iron 0.005g, Copper 0.0005g, Iodine 0.00012g, Selenium 0.02mg, Cobalt 0.02g.
Calculated ME[5]= 37 x %CP + 81 x %EE + 35.5 x %NFE (Pauzenga, 1985)

Experimental house, birds and management
The study was conducted in an open sided deep litter poultry house. The building was partitioned into individual units of about 1.8m x 1.2m dimension. One hundred and twenty Nera black point of lay pullets were used for the feeding trial which lasted fifteen (15) weeks. Standard management practices were observed. Feed and drinking water were given *ad libitum*. The birds had earlier been fed diets containing same proportions of SOPM at both pullet chick and grower phases. The birds were randomly assigned to the experimental diets in a Completely Randomized Design. Each diet group had 30 birds and 3 replicates with each replicate having 10 birds. The same arrangement and position of birds were maintained as in the chick and grower phases.

Chemical analysis
The proximate composition of each experimental diet was analyzed as described by AOAC (1995). Nitrogen free extract (NFE) was determined by difference. The gross energy (GE) values of the samples were determined using the Adiabatic oxygen Bomb calorimeter and converted to metabolizable energy (ME) as outlined by Pauzenga (1985).

Parameters measured

Egg weight was taken with the aid of V-600 mettler electronic balance (Accula 3®). This was usually after the egg had been cleaned with tissue paper in order to remove any faecal material or litter. For egg quality data, two crack-free eggs per replicate were randomly sampled when the birds were 29, 30, 31, 32 and 33 weeks for egg quality determination. All eggs sampled for egg quality determination were collected on the same day and before 12 noon. The eggs were carefully cleaned with tissue paper to remove any faecal or litter materials prior to weighing. Egg quality determination was carried out within 12 hours of egg collection. Each egg after weighing was broken at the equatorial region and the content gently poured into a flat plate for internal egg quality determination. Egg yolk diameter was measured in centimetres (cm) with vernier calipers, as the widest circumference of the yolk, while yolk height (cm) was measured from the highest point of the yolk to the base on the flat plate, with the aid of vernier calipers. Yolk index was computed by dividing yolk height by yolk diameter. Egg yolk colour was measured using Sennelier Artists Egg Tempera (Sennelier, 2009), by visual matching of the yolk colour with the corresponding colour and code on the egg chart. Haugh unit (HU) was calculated using the formula HU = 100log [H + 7.37 – 1.7 x 0.37 logW]. Albumen height was taken with the aid of vernier calipers as the highest point just close to the edge of the yolk to the base on the flat plate. Egg shells were carefully cleaned using tissue paper and then air-dried for 24 hours before weighing. Shell weight was measured using mettler electronic balance (Accula 3®). Shell thickness was measured with the aid of micrometer screw gauge at 2 different locations, the equatorial region and the narrow tip of the egg (Fayeye *et al.*, 2005). The average value was taken as the shell thickness in mm.

Haematology and serum biochemistry

Blood samples were collected from the wing vein at the end of the feeding trial from two birds per replicate and six birds per treatment. Haematology specimens were collected into EDTA treated tubes, while serum biochemistry samples were collected in separate tubes without anticoagulant. The haematology indices determined were PCV, RBC, WBC, Hb, MCV, MCH and MCHC. PCV was determined using Wintrobe's microhaematocrit method (Dacie and Lewis, 1991). Hb was determined by cyanomethaemoglobin method (Kelly, 1979). The improved Neubaer haemocytometer was used to determine WBC (Jain, 1986). MCV, MCH and MCHC were computed as outlined by Jain (1986). Serum protein, globulin, albumin, urea and uric acid were analyzed using Sigma kits. SGOT and SGPT were assessed using the procedure of Baker and Silverton (1985).

Statistical analysis

All data collected were statistically analyzed using the Analysis of Variance (ANOVA) outlined in the Minitab statistical software for completely randomized design (Minitab, 1991). Where significant effects of the experimental diets were obtained, means were separated using Fisher's Least Significant Difference (LSD) as outlined by Steel and Torrie (1980).

RESULTS

Effect of 24-hour fermented SOPM on egg quality of laying pullets

The effect of fermented SOPM on egg quality of laying pullets is shown in Table 2. Egg weight, shell weight, shell thickness, yolk height, yolk diameter, yolk index, haugh unit and yolk colour were not significantly affected ($p>0.05$) by the incorporation of SOPM in the diets of the birds. Egg weight ranged from 58.07g - 59.67g, egg shell weight 6.40g -6.57g, egg shell thickness 0.33mm - 0.34mm, yolk height 1.95cm - 1.99cm, yolk diameter 4.00cm - 4.05cm, yolk index 0.48 - 0.50, haugh unit 83.48 - 83.52 and yolk colour 560.40 -567.00. There was no definite trend of variation in any of these traits attributable to the treatments.

Table 2: Effect of 24-hour fermented sweet orange (*Citrus sinensis*) peel meal on egg quality of laying pullets

Egg quality indices	Experimental diets				
	T_0	T_{10}	T_{20}	T_{30}	SEM
Egg weight (g)	59.63	59.67	59.67	58.07	0.46^{ns}
Shell weight (g)	6.57	6.43	6.46	6.40	0.06^{ns}
Shell thickness (mm)	0.33	0.33	0.34	0.34	0.00^{ns}
Yolk height (cm)	1.99	1.95	1.99	1.96	0.01^{ns}
Yolk diameter (cm)	4.05	4.01	4.00	4.01	0.03^{ns}
Yolk index	0.48	0.49	0.50	0.49	0.01^{ns}

Haugh unit	83.50	83.50	83.48	83.52	0.02^{ns}
Yolk colour	562.60	562.60	560.40	567.00	3.16^{ns}

SEM = Standard error of mean
^{ns} = Not significant ($p > 0.05$)

Effect of 24-hour fermented SOPM on haematology constituents of laying pullets

The effect of fermented SOPM on haematology of laying pullets is presented in Table 3. There were no significant effects ($p>0.05$) on PCV, Hb, RBC, WBC, MCH, MCV and MCHC values of laying pullets. Observed values were 32.70% - 33.62%, 10.88g/dl -11.17g/dl, 4.28 - 4.80x10^6µl, 5.39 - 5.88x10^3µl, 23.10pg - 25.55pg, 69.47flto 76.84fl and 33.22% - 33.32%, for PCV, Hb, RBC, WBC, MCH, MCV and MCHC respectively.

Table 3: Effect of 24-hour fermented sweet orange (*Citrus sinensis*) peel meal on haematology of laying pullets

Haematology indices	Experimental diets				
	T_0	T_{10}	T_{20}	T_{30}	SEM
PCV (%)	33.62	33.20	32.73	32.70	1.13^{ns}
Hb (g/dl)	11.17	11.04	10.88	10.89	0.37^{ns}
RBC (x10^6 µl)	4.41	4.80	4.28	4.31	0.20^{ns}
WBC (x10^3 µl)	5.88	5.46	5.39	5.70	0.19^{ns}
MCH (pg)	25.38	23.10	25.55	25.37	1.00^{ns}
MCV (fl)	76.39	69.47	76.84	76.17	3.02^{ns}
MCHC (%)	33.22	33.26	33.32	33.30	0.05^{ns}

Means on the same row with different superscripts are significantly different ($p < 0.05$)
^{ns} = Not significant ($p > 0.05$)
SEM = Standard error of mean

Effect of 24-hour fermented SOPM on serum constituents of laying pullets

The effect of fermented SOPM on serum biochemistry is shown in Table 4. The substitution of maize with SOPM had no significant effects ($p>0.05$) on total protein, globulin, albumin, uric acid, SGOT and SGPT but had a significant effect ($p<0.05$) on urea. The obtained values were total protein 5.26g/dl - 5.56g/dl, globulin 3.28g/dl -.50g/dl, albumin 1.98g/dl - 2.10g/dl, uric acid 3.73mg/dl - 4.05mg/dl, SGOT 138.00µ//ml - 141.33µl/ml, SGPT 37.00µl/ml to 40.00µl/ml and urea 16.22mg/dl - 17.62mg/dl. The utilization of SOPM in the diet caused a significant increase in serum urea.

Table 4: Effect of 24-hour fermented sweet orange (*Citrus sinensis*) peel meal on serum biochemistry of laying pullets

Serum biochemical indices	Experimental diets				
	T_0	T_{10}	T_{20}	T_{30}	SEM
Total protein (g/dl)	5.56	5.42	5.26	5.48	0.37^{ns}
Globulin (g/dl)	3.50	3.32	3.28	3.41	0.23^{ns}
Albumin (g/dl)	2.06	2.10	1.98	2.07	0.16^{ns}
Uric acid (mg/dl)	3.73	4.01	4.05	4.04	0.09^{ns}
Urea (mg/dl)	16.22^{c}	16.88^{ab}	16.94^{ab}	17.62^{a}	0.17
SGOT[1] (µl/ml)	138.00	141.33	139.67	141.00	1.76^{ns}
SGPT[2] (µl/ml)	37.70	39.67	38.67	40.00	1.23^{ns}

Means on the same row with different superscripts are significantly different ($p < 0.05$)
^{ns} = Not significant ($p > 0.05$)
SEM = Standard error of mean
[1]Serum glutamic oxaloacetic transaminase
[2]Serum glutamic pyruvic trans

DISCUSSION

Effect of 24-hour fermented SOPM on egg quality of laying pullets

The observed egg weight range is within the ranges of 53.23g to 62.54g reported by Orunmuyi *et al.* (2007) and 55.12g to 62.78g reported by Abutu *et al*. (2008). Thus, protein intake of the experimental birds was adequate for egg production. High protein and water intake are reported to be some of the factors that determine egg size (Reid, 1976; Quereshi, 1985). Observed shell weight values indicate that SOPM did not negatively affect shell weight. Result is comparable with 5.59 - 6.32g obtained in a study to determine the effect of calcium sources and limestone deposits on laying performance and egg shell quality by Tion and Njoku (2001). Egg shell thickness of the birds obtained in the study is better than 0.31mm observed by Chineke (2001) and 0.32mm reported by Abutu *et al.* (2008). Shell thickness was adequate and capable of reducing percentage crack during handling (Stadelman, 1977; Quereshi, 1985). The experimental birds received adequate minerals especially calcium and phosphorus needed for shell formation. Yolk height, yolk diameter and yolk index values show that incorporation of SOPM apparently did not negatively affect them. This indicates that the quality of the yolk of control birds was similar to those of experimental birds or vice versa. Observed yolk index range is higher than 0.42 to 0.45 observed by Ezieshi *et al.* (2001) and 0.44 to 0.47 reported by Garba *et al.* (2010). Haugh unit values obtained show that the internal quality of the eggs from the experimental dietary groups was similar with the control. The observed values suggest that all the eggs were fresh and of high quality. Yolk colour of the control was similar with the SOPM based groups. Observed values using Sennelier Egg Tempera chart indicate that SOPM did not adversely affect yolk colour of the eggs. Yolk colour in all the eggs examined during the study was either Lemon yellow-coded 501 or Naple yellow-coded 567 (Sennelier, 2009).

Effect of 24-hour fermented SOPM on haematology constituents of laying pullets

The obtained PCV values are within the reference range 24.9 - 45.2% for healthy birds reported by Mitruka and Rawnsley (1977). Hb values are normal and within the range of 7.40g/dl - 13.10g/dl for healthy birds (Mitruka and Rawnsley, 1977). The RBC values in the trial fall within the range 3.7 - $7.5x10^6$ µl reported by Hewitt *et al.* (1989). The WBC values are normal and within 5.0 - $15.00x10^3mm^3$ reported by McDonald (1996). This indicates that substitution of SOPM for maize did not have negative effect on haematopoiesis. Observed MCH values are on the high side of 19.29pg - 23.20pg for normal birds reported by Ayoola *et al*. (2010). MCV values are better than reference range of 60.0fl - 65.7fl (Ayoola *et al*., 2010). MCHC values are within the reference range of 31 reported by Ameen *et al.* (2007). A indices values obtained in the feeding trial appear to be normal. This indicates that experimental diets were balanced with sufficiently high quality protein, adequate vitamins and minerals, especially iron. Iron, in conjunction with high quality protein are critical for the formation and maintenance of RBC and prevention of anaemia. The experimental birds did not show any sign of anaemia.

Effect of 24-hour fermented SOPM on serum constituents of laying pullets

Total protein values compare with the reference range of 5.6g/dl to 5.9g/dl for birds reported by Ayoola *et al.* (2010), and are within the range of 3.25g/dl to 7.61g/dl observed by Rajurker *et al.* (2009). Observed values are therefore normal, and suggest that the experimental birds were not under stress likely due to dehydration, disease or malnutrition (McDonald, 1996). Globulin values are higher than the range of 2.13g/dl to 3.02g/dl reported by Adeyemo (2008). However, observed value for the control birds is similar to those of birds on the SOPM-based diets. The experimental birds were therefore not under nutritional stress due to substitution of maize with SOPM. Albumin range is within the range of 1.25g/dl to 2.20g/dl observed by Akinmutimi and Onen (2008). This appears normal and suggests that substitution of maize with SOPM did not adversely affect the nutritive quality of the experimental diets. Uric acid concentration is close to 3.68mg/dl to 3.77mg/dl reported by Sogunle *et al.* (2007). Since uric acid level is similar between the control group and the other diet groups, this may be indicative that the experimental birds in the study did not suffer kidney damage but rather utilized protein adequately, and were able to excrete nitrogen in form of uric acid (Eggum, 1970; Jorinkeets, 2009; Wildlifeedus, 2009). SGOT levels are lower than the range of 143µl/ml to 187µl/ml reported by Fasina *et al.* (2004), while SGPT values are within 32 to 62µl/ml reported by Fasina *et al.* (2004). It may be inferred therefore that the experimental birds did not suffer from kidney or liver damage because of the inclusion of SOPM in their diets. Serum urea concentrations which tended to increase significantly

($p<0.05$) with high inclusion of SOPM are within the range of 14 to 21.50mg/100ml reported by Fasina *et al.* (2004). This suggests that the protein quality and amino acid balance of the test diets were adequate (Eggum, 1970).

CONCLUSION

When sweet orange peel was fermented for 24 hours, it was capable of sustaining laying pullets and at the same time furnished adequate nutrients for stable body physiology and good quality egg production even at 30% level of substitution for maize in layer diet.

REFERENCES

Abutu, J. A., Ugwu, S. O. C. and Onyii (2008). A comparison of physical characteristics of eggs laid by exotic and local hens at various ages. *Proceedings of the 42nd Annual Conference of the Agricultural Society of Nigeria* (ASN), pp.635–639.

Adeyemo, G. O. (2008). Effects of Cottonseed cake based diets on haemotology and serum biochemistry of egg-type chickens. *International Journal of Poultry Science 7(1):23–27.*

Akinmutimi, A. H. and Onen, G. E. (2008). The response of Broiler finisher birds fed graded levels of Yam peel meal in place of maize–based diets. *International Journal of Poultry Science 7(5):474–479.*

Amhakhian S.O. (2010). Evaluation of phosphorus status in some soil of Kogi State. North central Nigeria. *Unpublished Ph.D Thesis Edo State University Ekpoma, Nigeria* 167p.

Ameen, S. A., Adedeji, O. S., Akingbade, A. A., Olayeni, T. B., Ojedapo, L. O. and Aderinola, O. A. (2007). The effects of different feeding regimes on haemological parameters and immune status of commercial broilers in derived savannah zone of Nigeria. *Proceedings of the 32nd Annual Conference of the Nigerian Society for Animal Production* (NSAP), pp.176–178.

AOAC (1995). Official Methods of Analysis (16th ed). *Association of Official Analytical Chemists, Washington DC.* pp.69-88.

Ayoola, M. O., Alabi, M. O., Sokunbi, O. A., Adewumi, A. A., Essien, A., Aderemi, F. A., Lawal, T. E. (2010). Physiological response of broiler starter chickens to oral supplementation with *Telfairia occidentalis* leaf extract. *Proceedings of the 35th Annual Conference of the Nigerian Society for Animal Production* (NSAP), pp.126-128.

Baker, F. J. and Silverton, R. E. (1985). *Introduction to Medical Laboratory Technology*. 6th edition. *Butterwoth*, *England*.pp.127-156.

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

Dacie, J. V. and Lewis, S. M. (1991). *Practical Haematology*. 7th Edition, *Livington, (ELBS) Church hill Publishers; England*.722p.

Eggum, B. O. (1970). Blood urea measured as a technique for assessing protein quality. *British Journal of Nutrition 24:983-988.*

Ezieshi, E. U., Omoregie. A. and Olomu, J. M. (2001). Performance and some physical and internal qualities of eggs of laying chickens fed palmkernel cake based diets. *Proceedings of the 26th Annual Conference of the Nigerian Society for Animal Production* (NSAP), pp.199-201.

Fasina, O. E., Ologhobo, A. D., Adeniran, G. A., Ayoade, G. O., Adeyemi, O. A., Olayode, G. and Olubanjo, O. O. (2004). Toxicological assessment of *Veronica amygdaliana* leaf meal in nutrition of starter broiler chicks. *Nigerian Journal of Animal Production 31(1):3-11.*

Fayeye, T. R., Adeshiyan, A. B. and Olugbami, A. A. (2005). Egg traits, hatchability and early growth performance of the Fulani-ecotype chicken. *Livestock research for Rural Development.www./lrrd.org/lrrd17/8/faye17094htm.*

Garba, S., Jibir, M. and Omojola, A. B. (2010). Egg quality of commercial laying hens fed diets with increasing substitution levels of metabolizable energy of pearl millet for corn. *Proceedings of the 35th Annual Conference of the Nigerian Society for Animal Production* (NSAP), pp.308-310.

Hewitt, C. D., Innes, D. J., Savory, J. and Wills M. R. (1989). Normal biochemical and haematological values for Newzealand white rabbits. *Clinical Chemistry 35(8):1777-1779.*

Jain, C. N. (1986). *Schalms Veterinary Haematology.*4th edition, *Lea and Febiger Publishers, Philadephia*. pp.564-575.

Jorikeets (2009). Gout.*www.jorikeets.com/gout.htm. Retrieved 29/11/2009.*

Kelly, W. R. (1979). *Veterinary Clinical Diagnosis.* 2nd edition, *Bailliere Tyndall Publishers*, *London*. 336p.

McDonald, S. (1996). Complete blood count. *Avian quarterly. http://www.parrottalk.com/cbc.html. Retrieved 29/11/2009.*

Minitab Statistical Software (1991). Version 14.2 : *Minitab Inc*. P.A.; USA.CD Rom.

Mitruka, B. M. and Rawnsley, H. (1977). *Clinical biochemistry and hematological reference values in normal experimental Animals*. 1st edition, *Masson Publishing Inc*. *New York, USA*. pp.54-55.

Ojedapo, L. O., Akinokun, O., Adedeji, T. A., Ameen, S. A., Olayeni, T. B., Amao, S. R., Ige, A. O. Rafiu, T. A. Ojediran, T. K. and Akinniran, T. K. (2009). Effect of strains and age on egg quality characteristics of two strains of layer chicken kept in cages in derived savannah zone of Nigeria. *Proceedings of the 35th Annual Conference of the Nigerian Society for Animal Production* (NSAP), pp.41- 43.

Oluremi, O. I. A., Okafor, F. N., Adenkola, A. Y. and Orayaga, K. T. (2010). Effect of fermentation of sweet orange (*Citrus sinensis*) fruit phytonutrients and the performance of *International Journal of Poultry Science* 9(6):546-549.

Oluyemi, J. A. and Roberts, F. A. (2000). *Poultry Production in warm wet climate.* 2nd edition *Spectrum Books, Ibadan*, Nigeria. 244p.

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.318-322.

Pauzenga, U. (1985). Feeding Parent stock. *Journal of Zootecnica International, December, 1985, pp.22-24.*

Quereshi, A. A. (1985). How feed effects egg quality. *Poultry International, February, pp.44-48.*

Rajurker, S., Rekhe, D. S., Maini, S. and Ravikanth, K. (2009). Acute toxicity studies of polyherbal formulation (Methiorep premix). *Veterinary World. 12(2)58-59.*

Reid, B. L. (1976). Estimated daily protein requirement of laying hens. *Poultry Science 55(5):1641–1645.*

Roland, D. A. (1980). Egg shell quality 1 and 2. Effect of dietary manipulation of proteins, amino acids, energy and calcium in aged hens on egg weight, shell weight, shell quality and egg production. *Poultry Science 59:2038-2054.*

Sennelier (2009). Sennelier Egg Tempera. *http://www.e.artstore.net/paints_tempera_sen.html. Retrieved 2/02/2009.*

Sogunle, O. M., Fanimo, A. O., Abiola, S. S. and Bamgbose A. M. (2007). Growth response, body temperature and blood constituents of pullet chicks fed cassava peel meal supplemented with cashew nut reject meal. *Nigerian Journal of Animal Production 34(1):32-44.*

Stadelman, W. J. (1977). Quality identification of shell eggs. In: *Egg Science and Technology.* (Editors: Stadelman, W.J. and Cotterill, O.J.). Avi. *Publishing Co. Westport, Conn.* pp.41-47,102.

Steel, R. G. D. and Torrie, J. H. (1980). *Principles and procedures of statistics*. *McGraw-Hill Book Co. Inc. New York*. 633p.

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

Wildlifeedus (2009). Uric acid. *www.jwildliffeedus.org/./583pdf. Retrieved 25/09/2009.*

Yusuf, A. M, Garba, M. H., Irokanulo, U. O., Olayinka, O. O., Fajobi, E. A. Obun, C. O. and Adeleke, A. M (2010). Effect of mango (*Mangifera indica*) seed kernel meal based diets on the haematology of grower rabbits. *Proceedings of the 35th Annual Conference of the Nigerian Society for Animal Production (*NSAP), pp.194-196.