RESPONSE OF JAPANESE QUAIL CHALLENGED WITH VARYING CONCENTRATIONS OF NEWCASTLE DISEASE VIRUS

Fayeye*[*1], *T. R. and Omole*[2], *E. B.

ABSTRACT

*[1]Department of Animal Production, University of Ilorin, PMB. 1515, Ilorin, Nigeria [2]College of Wild-Life Resources Management, New Bussa, Niger State, Nigeria. *e-mail: fayetiro@yahoo.com +234(0)80601395678*

Quail is believed to be resistant to most diseases of poultry and as such, its production does not often involve any vaccination regime. However, limited report has shown that Japanese quail could be an important carrier for the Newcastle disease virus. The present study assessed the growth performance, haematological, biochemical and histo-pathological indices of Japanese quail challenged with varying concentrations of Newcastle disease virus.

Two hundred (200), two week-old, mixed sex quails were randomly allotted to 5 treatments (T1 to T5) groups with different concentrations of Newcastle disease virus. Data was collected on haematological, biochemical and histo-pathological indices. The results showed that mortality increased progressively with increase in dose level of inoculums (0-60%). Generally, all the haematological indices (packed cell volume, white blood cell count and heamoglobin concentration) decreased ($p<0.05$) with the age of birds before sampling. However, the mean corpuscular haemoglobin concentration remained unchanged. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of all groups were higher than the referenced ranged for the two metabolites. The biochemical indices declined ($p<0.05$) with the concentration of the inoculum except in Albumin/Globulin ratio and true protein. Serum antibodies increased with the concentration of Newcastle disease virus. pathologic degenerative changes observed in the organs of birds challenged with New castle disease virus include mild to severe haemorrhagic ulceration of the intestinal mucosa and the myocardium; congestion and focal haemorrhage of the lung; necrosis of hepatocytes and mild to severe haemorrhage of the caeca tonsil. The control groups (unvaccinated, unchallenged) has high levels of AST and ALT that is indicative of subclinical degeneration of liver and or muscle functions.

The study revealed the need to incorporate regular vaccination regime against Newcastle disease, especially in commercial quail farms.

Keywords*: Biochemical indices, Histo-pathological indices, Newcastle disease, Quail*

INTRODUCTION

Inadequate supply of day old chicks, diseases (such as Newcastle), and high cost of drugs has led to the introduction of quail birds into Nigeria (Haruna *et al.*, 1997). Quails are highly prolific and hardy (Anon, 1991) and are well adapted to the tropical environment. They have less feed requirements (Ani *et al.,* 2009). They mature in about 6 weeks and are usually in full egg production by 50 days, with hens laying up to 200-300 eggs in their first year of lay (Smith, 2001).). The meat and eggs are low in cholesterol (Schwartz and Allen, 1981). The production of quail has become increasingly popular in Nigeria urban and peri-urban communities due to their nutritional and health benefits. Babangida and Ubosi (2005) opined that the Japanese quail has the potential as an excellent and cheap source of animal protein for Nigerians.

Newcastle disease (ND) is a globally endemic infectious disease of poultry which is caused by avian paramyxoviruses which have a single, negative sense, elliptical RNA strand with roughly 16,000 nucleotides (Ashraf and Shah, 2014). Alders *et al.*, (2001) stated that Newcastle disease is a major constraint against the development of both industrial and village poultry production in Africa and Asia. The use of vaccination is the only effective way of controlling Newcastle disease in most countries. Research has shown that quail are resistant to most of the diseases of poultry (Ani *et al*., 2009). The outbreak of ND in quail has not been documented in field condition in Nigeria. There is also no vaccination regime for farmed quail due to the belief that quail are not susceptible to Newcastle disease virus (Ani *et al.,* 2009). However, Silva Lima *et al.* (2004) opined that Japanese quail could be considered an important carrier for the Newcastle disease virus. There is therefore the need for further work to determine the response of unvaccinated Japanese quail experimentally challenged with of Newcastle disease virus. Disruption of functional integrity of an organ is usually evaluated by means of associated clinic-pathologic changes. However, there is the need to confirm diagnostic methods (such as clinical signs and pathological findings) with haemagglutination inhibition test for a reliable conclusion. The objectives of the study are to evaluate the haematological, biochemical and histo-pathological indices of quail subjected to sub-lethal and lethal doses of Newcastle disease virus.

MATERIALS AND METHODS

Experimental site

The study was conducted at the Teaching and Research farm of Federal College of Wildlife Management, New Bussa, Niger State, Nigeria. New Bussa is located in the Nigerian Guinea Savannah. It has geographical coordinates of 9° 53' 0" North, 4° 31' 0". New Bussa has an altitude of 170 meters (Lawal *et al.,* 2014).

Animal and management

The Two Hundred (200) day old quail chicks used for the experiment were purchased from B4U farm in Oyo State, Nigeria. Anti-stress was given to birds on arrival. Chicks were kept in deep litter housing. Water and feed were supplied *ad libitum*. Composition of starter diet (Table 1) fed to chicks was as recommended by Dafwang (2006). The treatment design used for the study was a Completely Randomized Design. At two weeks of age, the unvaccinated, un-sexed chicks were randomly divided into 5 experimental treatments (T1, T2, T3, T4 and T5), with 4 replicates per treatment and 10 birds per replicate. The birds in T1 were transferred to a rearing facility far from T2 to T5. Birds in T1 serve as control (i.e. un-inoculated and free from aerosol viral transmission).

Inoculation of birds and data collection

150 ml of sterile injection water was used to reconstitute 300 doses of komorov vaccine. Birds on T2, T3, T4 and T5 were reared in the same building and were given 0, 0.5, 1.0, and 1.5ml of the reconstituted vaccine, respectively. The sub-lethal and lethal doses of the strain of avian paramyxovirus-1 were administered intramuscularly via the breast muscle. Birds on treatment T2 differs from T1 because they were reared in the same building as T3 to T5 and were therefore susceptible to aerosol viral transmission. Mortality (%) was recorded throughout the experimental period.

One bird per replicate (4 birds per treatments) was randomly selected at days, 1, 8, 15 after inoculation with New castle disease virus. Selected birds were slaughtered; 0.5 ml of blood sample was aspirated from the jugular vein using the needle and syringe. The aspirated sample was transferred into sterilized heparinized bottle for haematological analysis (i.e. for the determination of Pack cell Volume, Haemoglobin concentration, White Blood Cell count, Red Blood Cell count, Mean Corpuscular Haemoglobin, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin Concentration). Another 0.5ml of blood was aspirated from each bird into sterilized non-heparinized sample bottle. The blood samples in the non-heparinized sample bottles were centrifuged for ten minutes to separate the serum. Newcastle disease antibody in the sera was detected using Neogen Elisa kit (Neogen Corporation) with X L800 reader (Adair *et al*., 1989).

Post mortem examination for lessons of Newcastle disease was carried out on all the slaughtered birds. The liver, lung, caeca tonsils, intestine, proventriculus and heart of slaughtered birds were removed for histopathological analysis at the Air Force Hospital and Federal College of Wildlife Management New-Bussa. Microscopic slides of these internal organs were also prepared. The photo-micrographs obtained were used to determine the histo-pathological effect of viral dose levels on birds' tissues.

Statistical Analysis

Data collected on haematological, biochemical and serum antibody indices of inoculated Japanese quail were subjected to a one way analysis of variance.

Table 1: Gross and proximate composition of experimental diet

Ingredients	Percentage composition
Maize offal	20.00
Blood meal	12.00
Lysine	0.10
Premix	0.25
Maize	35.00
Soya (full fat)	30.00
Bone meal	2.40
Methionine	0.25
Total	100.00
Metabolizable energy (Kcal per kg)	2,836.00
Protein%	28.10
Lysine%	0.10
Calcium%	0.53
Total phosphorus%	0.25

RESULTS

Table 2 showed total mortality (%) in quail chicks belonging to different treatment groups. The results showed that mortality increased progressively with increase in dose level of inoculum. The haematological indices of Japanese quail at 1, 8, 15 days post-inoculation is as shown in Table 3 below. There were no significant ($p>0.05$) differences in Packed Cell Volume (PCV), White Blood Cell count (WBC) and Mean Corpuscular Haemoglobin Concentration (MCHC) of sampled birds at one (1) day after inoculation with Newcastle disease virus. All the haematological indices decreased with the length of time before sampling, except the value for MCHC. Generally, haematological indices were not significantly different ($p>0.05$) among birds in different treatment groups one (1) day post-inoculation. However, birds in T3 (with 0.5 ml viral inoculum) had significantly lower ($p<0.05$) MCH and MCV than other treatment groups. There was a wide variation ($p<0.05$) in the values of the biochemical

indices at 8 and 15 days post-inoculation. However, the variation in the biochemical indices did not show consistency with the dose level of inoculum.

Table 2: Mortality (%) in quail chicks challenged with varying doses of Newcastle disease virus

	T1	T2	T3	T4	T5
Total number of birds	40	40	40	40	40
Total mortality		4	8	10	24
Percentage mortality	0%	10%	20%	25%	60%

T1 and T2 represent un-inoculated-unexposed and un-inoculated-exposed chicks, respectively. T3, T4 and T5 represent chicks exposed to 0.5ml, 1.0ml and 1.5ml doses of avian paramyxoviruses-1

Table 3: Haematological indices of Japanese quail inoculated with Newcastle disease virus

Parameters	T1	T2	T3	T4	T5	SEM
		1 day post-inoculation				
PCV (%)	30.25	28.00	29.80	30.90	32.20	1.25
Hb (g per 100ml)	10.02a	9.30b	10.0a	10.30a	10.73a	0.42
WBC ($x10^3\mu l$)	15.05	12.68	10.65	9.98	9.70	0.95
RBC ($x10^6 I\mu$)	9.20	8.98	10.50	9.00	10.20	0.46
MCH (pictograms)	10.98ab	10.41ab	9.50b	11.9a	10.50ab	0.30
MCV (fl)	3.32ab	3.14ab	2.85b	3.60a	3.15ab	0.09
MCHC (%)	33.08	32.80	33.00	33.28	33.23	0.11
		8 days post-inoculation				
PCV (%)	40.20a	30.65b	20.25c	21.48c	9.40d	2.46
Hb (g per 100ml)	13.42a	10.22b	6.75c	7.15c	3.13d	0.82
WBC ($x10^3\mu l$)	19.05a	9.95b	7.18c	6.25c	4.05d	1.21
RBC ($x10^6 I\mu$)	12.03a	8.50b	7.95c	6.60c	3.08d	0.68
MCH (pictograms)	11.15a	3.61b	2.57b	3.63b	3.09b	0.74
MCV (fl)	3.34c	12.02b	8.35b	12.10a	10.31ab	0.83
MCHC (%)	33.38a	33.36b	33.34a	33.54a	33.80a	0.08
		15 days post-inoculation				
PCV (%)	43.38a	32.05bc	33.03b	33.55b	26.08c	1.52
Hb (g per 100ml)	14.48a	10.75bc	11.03b	11.18b	8.70c	0.51
WBC ($x10^3\mu l$)	21.90a	2.73b	6.83b	1.66b	1.08b	2.02
RBC ($x10^6 I\mu$)	13.65a	7.50b	6.13bc	5.66cd	4.45d	0.78
MCH (pictograms)	10.59a	4.38b	5.37b	4.93b	5.88b	0.57
MCV (fl)	3.17c	14.58b	17.98ab	20.44a	19.58a	1.58
MCHC (%)	33.37a	33.28a	33.39a	33.31a	33.36b	0.02

[a, b, c, d] means in the same row with different superscript differ significantly ($P< 0.05$). PCV, Hb, WBC, RBC, MCH, MCV, MCHC represent Pack cell Volume, Haemoglobin concentration, White Blood Cell count, Red Blood Cell count, Mean Corpuscular Haemoglobin, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin Concentration, respectively. T1 and T2 represent un-inoculated-unexposed and un-inoculated-exposed chicks, respectively. T3, T4 and T5 represent chicks exposed to 0.5ml, 1.0ml and 1.5ml doses of Newcastle virus

The biochemical indices of quails exposed to varying concentrations of Newcastle disease virus are presented in Table 4. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST), Albumin (ALB) and globulin (GLO) values of un-inoculated birds were significantly ($p< 0.05$) higher than those of inoculated bird, except in ALB/GLO ratio and true protein. Generally, all the biochemical indices of unchallenged birds were higher than those challenged with varying doses of Newcastle disease virus. There was a wide variation ($p<0.05$) in the values of the biochemical indices, which generally did not show consistency with the dose level of inoculum.

Titre values obtained for serum antibodies ranged between 0.14 and 78.17 (Table 5). The titre value for serum antibodies in birds increased significantly ($p<0.05$) with increase in the concentration of Newcastle disease virus.

Pathologic signs were observed only in birds challenged with Newcastle disease virus (Table 6, Fig. 1). The degenerative changes observed in the various organs include mild to severe haemorrhagic ulceration of the intestinal mucosa and the myocardium; congestion and focal haemorrhage of the lung; diffuse, hydropic degeneration and necrosis of hepatocytes in the liver and mild to severe haemorrhage of caeca tonsil.

DISCUSSION

The mortality due to Newcastle disease in the present report was lower compared with morbidity and mortality outbreak of 100 percent reported by Czirják *et al.* (2007). One hundred (100%) percent mortality was also obtained by Shaban (2012) in his work on unvaccinated broiler chicks that were challenged at 25 days of age. The higher mortality in contact but uninfected birds (T2) compared with birds in T1 suggests a horizontal transmission

from infected birds. Van Boven *et al.* (2008) observed that Newcastle disease virus is able to spread extensively in birds with low antibody titres, thereby leading to the infection of almost all contact birds.

Table 4: Biochemical indices of Japanese quail inoculated with Newcastle disease virus

	T1	T2	T3	T4	T5	SEM
Parameters		1 day post-inoculation				
ALT (U per L)	28.75c	25.50c	72.25a	26.25c	5.00b	4.88
AST (U per L)	38.25a	48.10a	25.25b	22.35b	24.08b	2.77
Albumin(g dL^{-1})	3.40	3.27	3.04	2.68	2.82	0.12
Globulin (g dL^{-1})	2.17ab	2.74a	1.09b	1.05b	1.65ab	0.22
ALB/GLO ratio	1.65	1.81	1.96	2.50	2.15	0.24
True Protein(g dL^{-1})	5.58ab	6.02a	4.11bc	3.77c	4.45bc	0.28
		8 days post-inoculation				
ALT (U per L)	33.50b	64.05a	46.50ab	36.25b	42.00ab	3.91
AST (U per L)	31.90ab	38.79a	22.71bc	20.10c	25.77bc	1.95
Albumin(g dL^{-1})	3.96b	5.23a	2.48b	3.90b	4.77ab	0.24
Globulin (g dL^{-1})	2.78a	2.30ab	1.63b	1.99ab	2.27ab	0.14
ALB/GLO ratio	2.00	2.00	2.00	2.00	2.00	0.24
True Protein(g dL^{-1})	6.74a	6.49a	4.11b	5.89ab	6.71a	0.35
		15 days post-inoculation				
ALT (U per L)	42.25a	10.75b	11.03b	11.18b	8.70b	3.49
AST (U per L)	36.15ab	57.98a	37.19ab	10.37c	30.26bc	4.60
Albumin(g dL^{-1})	4.76a	2.77b	3.25b	2.77b	3.10b	0.19
Globulin (g dL^{-1})	3.59a	1.45bc	1.24bc	1.94b	0.99c	0.24
ALB/GLO ratio	1.54	2.10	3.87	1.58	4.72	0.51
True Protein(g dL^{-1})	7.85a	4.22b	4.50b	4.70b	4.30b	0.34

T1 and T2 represent un-inoculated-unexposed and un-inoculated-exposed chicks, respectively. T3, T4 and T5 represent chicks exposed to 0.5ml, 1.0ml and 1.5ml doses of Newcastle virus

Table 5: Serum Antibody in Japanese quail exposed to varying concentration of Newcastle disease virus

	T1	T2	T3	T4	T5	SEM
Level of anti-body produced	-	+	++	+++	++++	
Antibodies (quantified)	0.14c	0.77c	1.68c	26.25b	78.17a	7.19

T1 and T2 represent un-inoculated-unexposed and un-inoculated-exposed chicks, respectively. T3, T4 and T5 represent chicks exposed to 0.5ml, 1.0ml and 1.5ml doses of Newcastle virus

Table 6: Histopathological evaluation of internal organs of Japanese quail exposed to varying concentration of Newcastle disease virus

Parameters	T1	T2	T3	T4	T5
Lung	NVI	Mild congestion of the lung	Congestion of the lung	Severe congestion of the lung	Focal haemorrhage and congestion of the lung
Heart	NVI	NVI	NVI	NVI	Haemorrhage in the myocardium
Liver	NVI	Diffuse fatty infiltration	Diffuse, hydropic degeneration and necrosis of the hepatocytes	Diffuse fatty infiltration	Haemorrhage degeneration and necrosis of the hepatocytes
Caeca tonsils	NVI	NVI	Mild haemorrhage	Haemorrhagic caeca tonsil	Severe haemorrhage
Intestine	NVI	Mild ulceration in the wall of the intestine	Focal ulcer in the intestinal wall	Ulcers in the wall if the intestine	Severe ulceration in the wall of the intestine

T1 and T2 represent un-inoculated-unexposed and un-inoculated-exposed chicks, respectively. T3, T4 and T5 represent chicks exposed to 0.5ml, 1.0ml and 1.5ml doses of Newcastle virus. NVI= No visible Indicator

The PVC, WBC, Hb, RBC of unchallenged birds in this report were generally higher than 33-37%, 8-10($x10^3$µl), 11.1-12.3 (g per100ml), 5.7-6.3 ($x10^6$Iµ) reported (respectively) by Odunsi *et al.* (2013). Haematological indices are indicators of systemic relationship, physiological adaptations and general health condition of birds (Kamal *et al*., 2007). Most haematological indices were also higher than that reported for unchallenged Japanese quail by Omonona and Jarikre (2014). Variations in haematological and serum biochemical indices are not uncommon in literatures and may be influenced by such factors as age and sex of the quail chicks (Ayoola *et al*, 2015). The

large variation in AST obtained in this study is not uncommon in literatures. For instance, Sokół *et al.* (2015) obtained a range of 64.67- 140.50U per L in their work on Japanese quail infected with coccidia. Scholtz *et al*. (2009) reported a referenced range for AST as 243-562U/L. A narrower AST and ALT range were however reported for normal human liver function (IAC, 2016). The ALT values obtained in this study were higher than 3.00 - 6.25 reported by Sokół *et al*. (2015). However, the ALT values were generally close to those obtained by Rabie and Abo El- Maaty (2015) in their evaluation of the effects of by dietary protein level and enzyme supplementation on growth performance of Japanese quails. Scholtz *et al*. (2009) reported a referenced range for ALT as 4.5 - 8.5. Although there was no mortality among the control group, the high ALT obtained suggests subclinical liver and muscle damage. According to Sokół *et al.* (2015), changes in enzyme activity levels can serve as indicators of damage to liver and intestinal cells in birds under sub-clinical infection. The biochemical (e.g. ALT) and pathological indices observed in this study are indicators that Japanese quail is susceptible to sub-lethal and lethal doses of Newcastle disease virus. Normal ALT activities may be recorded in individuals with severe liver damage as a result of low level of enzyme activity in the liver cells of such species. The protein concentration in all the birds was within the range of 1-10 typically found in the serum or plasma.

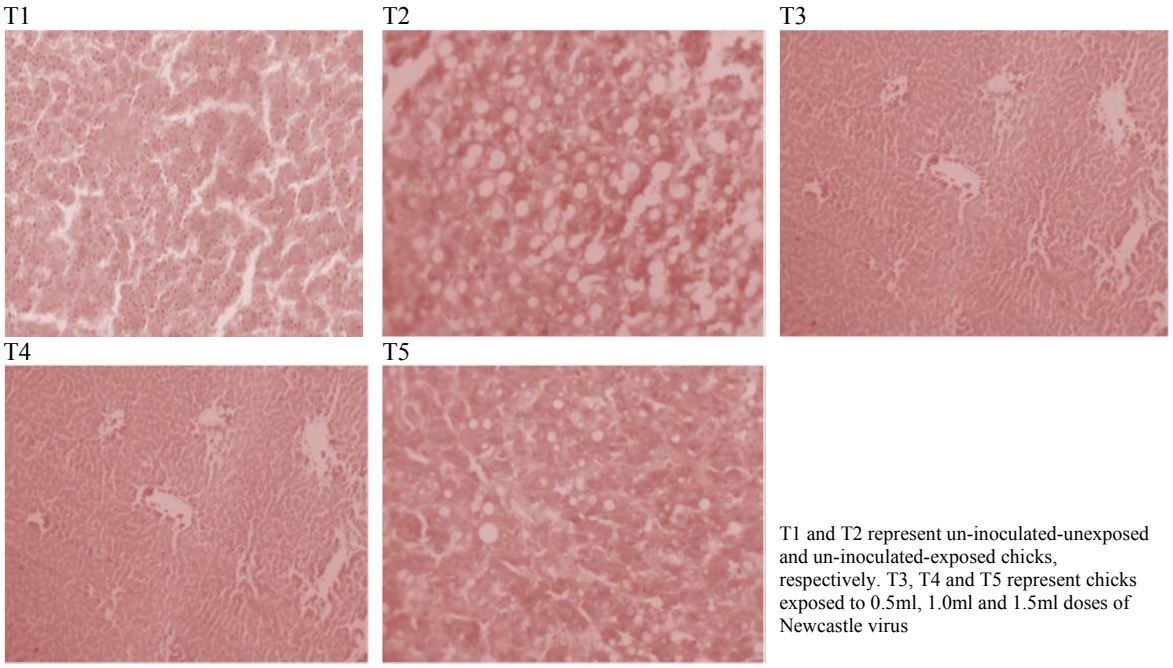

Fig. 1: Slides showing Histopathological effects of Newcastle disease virus on the liver of Japanese quails challenged with varying doses of Newcastle disease virus

The antibody titer of unchallenged (T1, T2) birds were lower than that reported for local Ethiopian chickens by Anebo *et al.* (2014). It was also lower than that reported for 60 days old unvaccinated white leghorn broilers by Akele *et al.* (2014). The production of antibodies by challenged birds is an immune response to neutralize the HN and F glycoprotein of Newcastle disease virus. Miller *et al.* (2013) opined that antibodies against the HN are responsible for blocking viral attachment, while antibodies against the F glycoprotein can inhibit viral fusion with the host cell membrane. Although vaccinated birds with low or undetectable antibody titres may be protected against disease and mortality, however, Van Boven *et al.* (2008) reported that infection and Newcastle disease transmission may still occur in such a herd. They then concluded that herd immunity can only be achieved if more than 85% of vaccinated birds have $\log_2$ haemagglutination inhibition titre of at least 3. Pathologic signs such as hemorrhagic or necrotic lesions in mucosa of intestine, caecal tonsils, proventriculus and gizzard have been reported in birds challenged with Newcastle disease virus (Ashraf and Shah (2014). Czirják *et al.* (2007) observed similar hemorrhagic condition as well as necrosis of the alimentary tract in challenged birds. They also reported microscopic lesions in the central nervous system.

CONCLUSION

The study revealed that quails birds experimentally challenged with New castle disease virus show high morality as well as biochemical and pathologic signs of Newcastle disease. Although no fatality was observed in unchallenged birds, the high level of ALT is indicative of subclinical degeneration of liver and or muscle

functions. The study recommends the need to introduce routine vaccination against Newcastle disease in commercial quail farms.

REFERENCES

Adair, B. M., McNulty, M. S., Todd, D., Connor, T. J. and Burns, K. 1989. Quantitative estimation of Newcastle disease virus antibody levels in chickens and Turkeys by Elisa, *Avian Pathology*, 18(1):175-192.

Alders, R. G., Costa, R., Dias, P., Fringe, R., Fumo, A., Lobo, Q., Mata, B. V., Silva, A. and Young, M. P. 2001. Investigations into the Control of Newcastle Disease in Village Chickens in Mozambique: Review of work done in Mozambique. Report on the ACIAR/INIVE Newcastle Disease Control Project Coordination Meeting, Tofo, Inhambane Province, Appendix 3.

Akele, Y. R., Tatfeng, Y. Ojiezeh, I. T., Chollom, C. S. Enitan, S. S. and Olayanju, O. A. 2014. Evaluation of the efficacy of Newcastle disease (lasota) live vaccines sold in Jos, Plateau state, Nigeria, *European Scientific Journal*, 10(27): 132-141.

Anebo, Z. G., Teklemichael, K., Bacha, B., Habte, T. and Hunde, A. 2014. Evaluation of the Newcastle disease antibody level after vaccination regimes in chickens in Debrezeit Agricultural Research Center, Ethiopia, *Journal of Veterinary Medicine and Animal Health*, 6(1):7-12

Ani, A. O., Okeke, G. C. and Emeh, M. B. 2009. Response of growing Japanese quail chicks to diets containing different energy and protein levels. Proceeding of 34th Annual conference. Nigeria society for Animal Production. 15th – 18th March, Uyo Pp 328 – 331.

Anon, L. 1991. Little known animals with promising economic future (micro-livestock). Board of science and technology for international development. National Academy press. Washington D.C, pp 147-155.

Ashraf, A. and Shah M. S. 2014. Newcastle Disease: Present status and future Challenges for developing countries. *African Journal of Microbiology Research*, 8(5): 411-416.

Ayoola, A. A., Egbeyale, L. T., Sogunle, O. M., Ekunseitan, D. A. and Adeyemi, A. A. 2015. Effects of age and sex on haematological and serum biochemistry in Japanese quails. *Bulletin of Animal Health and Production in Africa,* 63(1):43-51

Babangida, S. and Ubosi, C. O. 2005. Effects of varying dietary protein levels in the performance of laying Japanese quail (*Coturnix coturnix japonica*) in a semi-arid environment. *Nigeria Journal of Animal Production,* 33(1): 45-52.

Czirják, G. Á., Köbölkuti, L. B., Cadar, D., Ungvári, A., Niculae M. and Bolfã P. 2007. An outbreak of the Newcastle disease in Japanese quail (C*oturnix coturnix japonica*). *Bulletin USAMV-CN*, 64:1-2.

Dafwang, I. I. 2006. Nutrient Requirement and feeding regiment in quail production. A paper Presented at the National workshop on quail production for sustainable household protein in-take. National Agricultural Extension and Research Liason Services, Ahmadu Bello University, Zaria. September 11th-13th, 2006.pg 12-19.

Haruna, E. S., Musa, U., Lumbin, L. H., Tat, P. M., Shamaki, P. D., Okewole, J. P., Molokwu, J. U. 1997. Introduction to quail production in Nigeria. *Veterinary Journal*, 18:104-107.

IAC, 2016. What is the normal range for ALT and AST liver tests? IAC Publishing Labs Company.

Kamal, M., Shah, A., Khane, F., Rizvi, M., Siddique, A. and Sadeeq, U. R. 2007. Effect of cypermetrin on haematological parameters in rabbit. *Pakistan Veterinary Journal,* 27 (4): 171-175.

Lawal, A., Hakimi, D. and Laminu, I. 2014. Stochastic Modelling of Annual Rainfall at New Bussa. *OSR Journal of Mathematics*, 3 (2): 23-27.

Miller, P. J., Afonso, C. L., Attrache, J. E., Dorsey, K. M., Courtney, S. C., Guo, Z. and Kapczynski, D. R. 2013. Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses. *Developmental and Comparative Immunology*, 41(4): 505–513

Odunsi, A. A., Akinwumi, A. O. and Falana, O. I. 2013. Replacement value of hatchery waste meal for fish meal in the diet of laying Japanese Quail (*Coturnix coturnix japonica*). *International Food Research Journal,* 20(6): 3107-3110

Omonona, A. O. and Jarikre T. A. 2014. Effect of Garlic Supplemented Diets on the Haematologic and Lipid Profiles of Japanese Quail (*Cotunix coturnix japonica*). *Nigerian Veterinary Journal*, 35(2): 981·988.

Rabie, M. H., Abo and El-Maaty, H. M. A. 2015. Growth Performance of Japanese Quail as Affected by Dietary Protein Level and Enzyme Supplementation. *Asian Journal of Animal and Veterinary Advances,* 10: 74-85.

Scholtz, N, Halle, I, Flachowsky, G. and Sauerwein, H. 2009. Serum chemistry reference values in adult Japanese quail (*Coturnix coturnix japonica*) including sex-related differences. *Poultry Science,* 88: 1186-1190.

Schwartz, R.W. and Allen W. K. 1981. The effect of aging on the protein requirement of mature Female Japanese quail for egg production. *Japanese poultry science,* 60 (2): 342 – 348

Shaban, K. S. 2012. Exacerbating effect of Newcastle disease virus (NDV) infection on sub clinical caecal coccidiosis in broilers vaccinated against NDV. *Researcher*, 4(1): 55-59

Silva, L. F., Santin, E., Paulillo, A. C. and Doretto Jr. L. 2004. Japanese quail (*Coturnix coturnix japonica*) as Newcastle Disease Virus carrier. *International Journal of Poultry Science,* 3(7), 483-484.

Smith, A. J. 2001. Poultry. The Tropical Agriculturist. Revised edition. London: Macmillan Education Ltd

Sokół, R., Gesek, M., Raś-Noryńska, M., Michalczyk, M. and Kozi, S. 2015. Biochemical parameters in Japanese quails (*Coturnix coturnix japonica*) infected with coccidian and treated with Toltrazuril. *Polish Journal of Veterinary Sciences*, 18(1): 79–82

Van Boven, M., Bouma, A., Fabri, T. H. F., Katsma, E., Hartog, L. and Koch, G. 2008. Herd immunity to Newcastle disease virus in poultry by vaccination. *Avian Pathology*, 37(1):1–5.