INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

BIOCHEMICAL CHARACTERISATION OF MUSCOVY AND MALLARD DUCKS IN NIGERIA

Akinyemi, M. O., Osaiyuwu, O.H. & Ajayi, A.Y.
Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan, Ibadan, Nigeria
Corresponding author's email: larakinyemi@gmail.com

ABSTRACT
Protein polymorphism and alleleic variation were investigated in the blood and egg proteins of the Muscovy and Mallard duck breeds found in Southwestern Nigeria. Four proteins namely haemoglobin, transferrin, carbonic anhydrase, and albumin in the blood and two egg white proteins:ovalbumin and conalbumin were analysed .A total of 50 ducks comprising of 30 Mallard duck and 20 Muscovy ducks and 20 eggs from each breed were used for the analysis. Separation of blood and egg protein genotypes was achieved using cellulose acetate electrophoresis. Two co-dominant allele A and B controlling three genotypes AA, AB and BB were observed at haemoglobin, transferrin and carbonic anhydrase loci investigated for both breeds. A third allele C was observed at the albumin locus in the Mallard breed. . Allele A was the most predominant at the Hb, Tf and CA locus in the Muscovy breed with frequencies 0.775, 0.575, and 0.675 respectively and in the Mallard at the Tf, CA and Al locus with frequencies 0.667, 0.567 and 0.767 respectively. The frequencies of allele A and B at the conalbumin locus were 0.425, 0.575; 0.525, 0.475 in the Muscovy breed. Estimates of heterozygosity were 0.428 and 0.430 in the Muscovy and Mallard ducks respectively. Dendogram generated from the genetic distance values revealed that the two breeds are closely related. High genetic similarity was observed between the two breeds.

KEY WORDS: local duck, egg white, genetic variation, protein polymorphism, electrophoresis.

INTRODUCTION

The poultry industry is one of the most important sectors of the Nigerian economy contributing substantially to the sector nation's Gross Domestic Product (GDP) (Ambali *et al*., 2003). In 2005 the poultry population was estimated at about 190 million (Orajaka, 2005) comprising of 8.0 % exotic breeds and over 90 % indigenous species (Nwanta *et al*., 2006). The genetic resources of poultry are mainly represented by domestic local chickens (*Gallus gallus domesticus*), guinea fowl (Numida *meleagris*) and ducks (*Cairina* spp.) (Youssao *et al*., 2010) These local avian species are bred under traditional breeding system and constitute a fast means of bridging the protein deficiency gap prevalent in most developing countries (Jibir and Usman, 2003). They also serve as a means of providing additional income to the generally resource-poor small holder farmers (Gueye, 2004) Chickens dominate the poultry egg and meat sector, in several parts of the world. However duck represent the second largest population in Africa after chicken. The local ducks in Nigeria constitutes about ten percent of the local poultry population (Oluyemi and Ologbobo, 1997). There are two breeds of ducks found in Nigeria together with their crosses. These include the Muscovy or Barbary and the Mallard Ducks. (Suliman, 2010) They differ considerably in size, plumage colour and other characteristics (Adesope and Nodu, 2002) and are maintained traditionally with uncontrolled mating system causing genetic dilution of the local stocks (Muzani *et al*., 2005). They are hardy, less exigent for feed quality, less susceptible to diseases than chicken and quite promising among indigenous poultry species because of their rapid growth rate and the dressed weight of drakes (Duru *et al*., 2006). They also have the ability to withstand extremely hot ambient temperatures than chickens (Obinne, 1997). Despite these advantages little attention has been paid to the genetic characterization and possible improvement of these birds. Many gap exist in the understanding of identification and conservation of these duck breeds as well as the genes controlling advantageous traits. (Yakubu, 2009). In the absence of information about the genetic attributes of each breed available for a breeding programme, development of local breeds is often ignored in favour of introduction of germplasm from exotic breeds, about which more environmental adaptation information is generally unavailable. These pose a great risk for the loss of valuable genes. To surmount the situation the Food and Agriculture Organization of the United Nations (FAO) recommended establishing conservation programmes for the maintenance of animal genetic resources. These include among many other actions the identification and characterization of local breeds (Esquivelzeta *et al*., 2011). Phenotypic comparison based on morphological characters can provide to some extent a reasonable representation of genetic difference among populations (Yakubu *et al*., 2011). Ducks in Nigeria have been morphologically characterized (Yakubu *et al*., 2011) and in Africa (Teguia *et al*., 2008: Manuel, 2008) using both univariate and multivariate discriminant analysis. However, the accuracy of phenotypic characterization of domestic animals is often affected by the influence of the environment and the underlying genetic complexity. (Yakubu *et al*., 2011). This present study is designed to evaluate the occurrence of variations in blood and egg protein markers and investigate the

genetic relationship between the Mallard and Muscovy duck breeds in Nigeria. Therefore providing useful genetic information essential for developing effective management plans for the breeding and improvement of these genetic resources as well as conserving them.

MATERIALS & METHODS

Animals

Genetically unselected birds were sampled from several free range flocks in Ibadan, Oyo State, Nigeria. Ibadan is located in south western Nigeria and lies between latitude 72347N and 3550E. Blood samples were collected from 50 individuals comprising 30 Mallard and 20 Muscovy ducks. Only individuals who conform to the phenotypic description of each breed were sampled. Egg white was collected from 20 Mallard and 20 Muscovy ducks

LABORATORY ANALYSIS

Blood samples were collected from the wing vein into tubes containing heparin as the anticoagulant and kept refrigerated during transportation. Plasma and erythrocyte samples were separated from the heparinized whole blood by centrifugation. The electrophoresis of blood protein and enzyme system of Haemoglobin (Hb), Transferrin (Tf), Albumin (Al) and Carbonic anhydrase (Ca) and egg white protein of Ovalbumin (*Ov*), Conalbumin (*Cnb*) were performed on cellulose acetate membrane following the procedure described by RIKEN (2006) and Myint *et al*. (2010) with slight modifications. Details of the electrophoretic protocol are presented in Table 1. The analysis was done in the Animal Breeding and Genetics Laboratory of the Department of Animal Science, University of Ibadan, Nigeria.

TABLE 1: Electrophoretic protocols for proteins and enzyme analyzed in the Mallard and Muscovy duck breeds

LOCUS	Fraction	Buffer system	Staining Reagent
Haemoglobin	Red blood cell	Tris EDTA Borate pH8.4	Ponceau stain
Transferrin	plasma	Tris Glycine pH8.5	Ponceau stain
Carbonic anhydrase	Red blood cell	EDTA Trihydrate pH5.6	Ponceau stain
Albumin	plasma	Tris Citrate pH5.0	Amido blue solution
Ovoalbumin	Egg white		Amido blue solution
Conalbumin	Egg white		Ponceau stain

DATA ANALYSIS

Allele frequencies were calculated by direct gene counting method for all the loci studied. To assess the genetic variability within and between breeds, allele frequencies and test of Hardy- Weinberg equilibrium (HWE) were performed using Tools for Population Genetics Analysis (TFPGA), (Miller, 1997) Genetic variability within the population was quantified by measuring the average heterozygosity (Het) and genetic distance values as described by Nei (1972).The matrix of the distances was used to construct a dendogram of relationships according to the unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1973)

RESULTS

Blood protein polymorphism

Observed alleles at the investigated loci and their frequencies are presented in Table 2. All loci investigated in the blood proteins and enzymes were found to be polymorphic. Two alleles A and B controlling three genotypes (AA, AB and BB) were observed at Haemoglobin, Transferrin and Carbonic anhydrase loci.

TABLE 2: Gene frequencies of blood protein loci and average heterozygosity of Muscovy and Mallard Ducks

Locus	Allele	Gene frequencies in local ducks	
		Muscovy n=20	Mallard n=30
Haemoglobin(Hb)	A	0.775	0.300
	B	0.225	0.700
Transferrin	A	0.575	0.667
	B	0.425	0.333
Carbonic anhydrase	A	0.675	0.567
	B	0.325	0.433
Albumin	A	0.325	0.767
	B	0.675	0.217
	C	0.000	0.016
Average heterozygosity		0.428	0.430

At the albumin locus three alleles A, B and C were observed. Hb^A (0.775) and Tf^A (0.575)occurred at a high frequency in the Muscovy duck breeds. CA^A was the most frequent in the two breeds occurring at 0.675 and 0.567 in Muscovy and Mallard respectively. Al^A occurred at a higher frequency (0.767) in Mallard ducks while Al^B occurred at a higher frequency (0.675) in Muscovy ducks. However Al^C was only observed in the Mallard ducks with a frequency of 0.016 (Table 2). Mean heterozygosities were 0.428 and 0.430 for Muscovy and Mallard ducks respectively. Significant deviations from HWE was observed at the Hb locus in Muscovy ($p<0.001$) and Mallard ($p<0.05$). Allele frequencies were used to generate D_A genetic distance between the two breeds .(Table 3) A dendogram of relationships constructed by the UPGMA method revealed that the two breeds belong to the same cluster suggesting that the two breeds are closely related. (Fig 1).

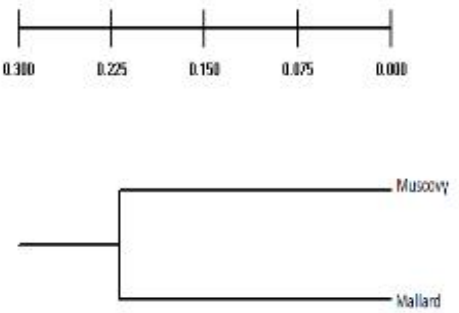

FIGURE 1: Dendogram showing genetic relationship between Mallard and Muscovy ducks

Egg protein polymorphism

In this study two alleles (A and B) controlling three genotypes AA, AB and BB were observed at Ovoalbumin and Conalbumin locus. Observed allelic distribution at the ovoalbumin locus of the two breed's revealed similar frequency with Ov^B and Ov^A 0.450 occurring at 0.550 in Muscovy and Mallard respectively.At the Conalbumin locus, CnbA was higher in Mallard while the B allele was higher in Muscovy. Average heterozygosities for the egg protein were 0.492 and 0.497 for Mallard and Muscovy ducks respectively. (Table 3)

TABLE 3: Gene frequencies of egg white proteins in Muscovy and Mallard duck breeds

Locus	Gene frequencies in local ducks		
	Allele	Muscovy n=20	Mallard n=20
Conalbumin	A	0.425	0.525
	B	0.575	0.475
Ovoalbumin	A	0.450	0.450
	B	0.550	0.550
Average heterozgosity		0.492	0.497

TABLE 4: Genetic distance values of blood protein between the duck breeds Nei's (1972/1978) identities/distances

Population	Mallard	Muscovy
- Blood protein loci:		
Mallard	0.0000	
Muscovy	0.2188	0.0000
-Egg protein loci		
Mallard	0.0000	
Muscovy	0.0099	

DISCUSSION

In this study two haemoglobin alleles; Hb^A and Hb^B controllling three genotypes (AA, AB and BB) were observed. The frequency of Hb^A was higher than Hb^B in Muscovy ducks while Hb^B was higher in Mallard duck breeds. Polymorphism at this locus was also observed by Ismoyowati (2008) in Tegal ducks and in Mallard ducks by Oates and Principato (1994) and in Nigerian local fowls (Ajayi et al., 2013). The inherent advantages that this locus confers on poultry breeds have been previously reported (Das and Deb, 2008). Ismoyowati (2008) reported that Tegal Duck with AA genotype on all loci had higher egg production than BB and CC homozygote. In this study we did not however observe the C allele in any of the breeds studied. Polymorphism at the Albumin locus has been observed in Magelang, Tegal and Mojosari ducks in Indonesia (Johari *et al*., 2012) and Azmi *et al*. (2006) in Talangbenih ducks. A high frequency of Al^A (0.767) recorded for Mallard duck in this study compares very closely with frequencies recorded for Magelang (0.719) and Tegal (0.800) by Johari et al 2012.This is also in line with the report of Azmi *et al*. (2006) in Talangbenih ducks, in which the gene frequency of Al^A was higher than the two other genes, namely Al^B and Al^c. The results obtained for Muscovy ducks in this study is in agreement with the results by Ismoyowati (2008), in which the Al^Bgene frequency of Tegal duck was higher than Al^A and Al^C.. However the result from this study suggests that Al^A confers a selective advantage on duck breeds with respect to their laying potential. The high frequency of gene Al^A in Mallard is comparable to the values reported for the Magelang duck which is reported to have a henday production(HDP) potential of 73.63+20.68 percent compared to Tegal and Mojosari with 42.42+17.72 and 69.25+22.16 respectively (Purwatini *et al.*, 2002). This likely lays credence to the better laying ability of the Mallard duck breed over the Muscovy. Duru *et al.*, 2006 reported that Mallard duck can lay up to 300 eggs a year while Muscovy has the potential to lay about 195 eggs in a year (Swan, 2004). From the genetic point of view

transferrin is a heterogenic protein and it shows large differentiation among individuals and breeds. The results of transferrin loci in the two duck breeds showed that there were two alleles, A and B which is in agreement with the findings of Johari *et al*. (2012) in three breeds of Indonesian local ducks (Magelang duck, Tegal duck and Mojosari duck) and Zhang *et al*. (2002) in some native Chinese chicken breeds. The frequency of the Tf^A gene for both Mallard and Muscovy duck breeds in this study was relatively higher than the frequency of Tf^B gene, this is similar to the values reported in Mojosari ducks (Johari *et al*., 2012) and Kerinci ducks (Nur et al., 2012). Tf^B however occurred at a higher frequency in Talangbenih ducks (Azmi *et al.*, 2006) and Magelang and Tegal ducks (Johari *et al*., 2012). However, Ismoyowati (2008) reported three alleles A, B,C at this locus in Tegal ducks, this observed disparity may have reflected breed differences. Observation at the Carbonic anhydrase locus showed that there were two different types of alleles; CA^A and CA^B controlling 3 genotypes. CA^A occurred at a higher frequency in both breeds with the CA^A of Muscovy occurring at a higher frequency of 0.675 than Mallard 0.567. Das and Deb (2008) however reported that six genotypes *viz*. AA, BB, CC, AB, AC BC was identified controlled by three co-dominant alleles (CA-1A, CA-1B and CA-1C) located at an autosomal locus CA-1. However the activity of CA has been positively correlated with egg shell thickness.(Das and Deb 2008).This may aid in selection for increased shell thickness in the breeds to guide against cracks and breakages in egg production. A high frequency of Ov^A recorded for Mallard ducks agrees with the result obtained by Johari *et al*. (2012) in three Indonesian local duck breeds and the reports of Myint *et al*. (2010) on Japanese native chicken breeds. However Johari *et al.* (2008) found that there was a relationship among body weight, egg weight and ovalbumin, in which the AA genotype had a greater body weight and heavier eggs production than other genotypes. This may lay further credence to the inherent ability of Mallard as a better layer suggesting the need for genetic improvement and selection for increase egg production in the breed. Previous research report revealed that at the Conalbumin locus Cnb^B is generally the most occurring in most poultry breeds (Myint et al., 2010, Johari et al., 2013, Inafuku et al., 1998) Observation at this locus showed that the frequency of Cnb^B was higher than Cnb^A in the Muscovy while Cnb^B was higher in Mallard. Estimates of heterozygosities which are considered to be good measures of genetic diversity, ranged between 0.428 and 0.430 at the blood protein loci, these values are comparable to 0.484, 0.447 and 0.479 reported by Johari *et al.*, 2012 in Magelang, Tegal and Mojosari ducks respectively. The average value of heterozygosity in this study differ significantly from the average heterozygosities of local ducks in Vietnam that was 0.098 to 0.179 (Okabayashi *et al*., 1999) and in Laos which was 0.118 to 0.132 (Okabayashi *et al*., 2000). The disparity obtained in these values could be because of the differences in the number of alleles in this study since the number of alleles per locus has a great effect on accuracy of estimation of genetic diversity. Thus a lower heterozygosity value for the Vietnamese and Laos breeds was due to monomorphism at some of the loci studied. All the loci investigated in the two populations sampled in the present study were however polymorphic. Heterozygosity values from the egg protein loci investigated were 0.497 and 0.492 in Mallard and Muscovy ducks respectively. These values are comparable to those reported by Johari *et al. (*2012) for Mojosari ducks but higher than the values reported for local ducks in Vietnam, (0.098 to 0.179) (Okabayashi *et al*., 1999) and for local ducks in Laos, 0.118 to 0.132 (Okabayashi *et al*., 2000). The low heterozygosities likely points to the decreasing genetic variability at the investigated egg protein loci in the reported duck populations. The D_A genetic distance estimate 0.218 from the blood protein loci indicated a very high degree of genetic similarity between the two breeds. However lower genetic distance values ranging between 0.0168-0.0322 were reported by Johari *et al*. (2012) on Indonesian local ducks. Estimate of 0.0099 obtained at the egg protein loci could be as a result of the number of samples used and the number of proteins sampled. The value obtained could also be because of a short time since the divergence of the two breeds to allow accumulation of more genetic differences.

CONCLUSION

The two breeds examined are genetically close with respect to the blood and egg proteins examined. This result is however subjected to certain limitations due to the sample size and the number of markers used. The similarity and distances obtained between the two breeds should be subjected to further analysis using more protein markers and complemented with molecular DNA markers.

REFERENCES

Adesope, O. M. and Nodu, M. B. (2002) A note on acceptance of duck as table-meat among inhabitants of selected communities in the Niger Delta zone, Nigeria. *Livestock Research for Rural Development*, **14**: http://www.lrrd.org/lrrd14/6/ades146.htm.

Ajayi, F. O., Agaviezor, B. O. & Wihioka, S. N. (2013) Haemoglobin genotypes in the Nigerian indigenous Chicken in the Niger delta region of Nigeria. *International Journal of.Advanced Biological Research* **3**(1), 13-16

Akinyemi, M. O. and Salako, A. E. (2010) Haemoglobin Polymorphism and Morphometrical Correlatesin the West African Dwarf Sheep of Nigeria. *International Journal of Morphology*, **28**(1), 205-208

Akinyemi, M.O. and Salako, A. E. (2012) Genetic Relationship among Nigerian Indigenous Sheep Population using Blood Protein Polymorphism; *Agricultural Science and Technology*, **4**(2), 107-112

Ambali, A.G., Abubakar, M.B. and James, T. E. (2003) An assessment of poultry health problems in Maiduguri, Borno state, Nigeria. *Tropical Veterinarian* **21** (3), 138-145.

Arora, R., Bhatia, S., Mishra, B.P. and Joshi, B.K**.** (2011) Population structure in Indian sheep ascertained using microsatellite current and future improvement options information. *Animal Genetics* **42** (3), 242–250.

Azmi, G. and Suharnas, E. (2006) Charateristic of morphologic and genetik on talangbenih duck in Bengkulu In: Proceeding of Seminar Teknologi Peternakandan Veteriner. Pusat Penelitiandan Pengembangan Peternakan. Badan Penelitiandan PengembanganPertanian Department Pertanian, September 5- 6, 2006. Bogor, 716-722.

Das, Ananta Kr. and Deb, RajibIndian (2008) Biochemical Polymorphism and its relation with some traits of importance in poultry. *Veterinary World*, **1** (7), 220-222**.**

Food and Agriculture Organization, (2000a) FAOSTAT Statistical database of Food and Agriculture Organisation of the United Nations, Rome., Italy.http://www.fao .org/dad-is/ Food and Agriculture Organization of the United Nations, Rome, Italy.

Guéye E. F. (2004) Gender aspect in family poultry management systems in developing countries. 2005. *World Poultry Science Journal* **61** (1), 39-46

Ismoyowati, I. (2008) Detection study of egg production of Tegal duck through protein polymorphisms). *Journal of Animal Production* **10** (2), 122-128.

Inafuku, K., Y. Maeda, S. Okamoto, S.M. Ardiningsasi and Hashiguchi, T. (1998) Polymorphisms of egg white proteins in native chickens in Indonesia. *Japanese Poultry Science* **35** (5), 278-284.

Jibir, M. and Usman, A.U. (2003) Duck production in the tropics, *AgVet International* **4**, 10-12.

Johari, S., Kurnianto, E. & Hasviara, E. (2008) Blood protein polymorphism of Kedu chicken. *Journal Pengembangan Peternakan Tropis* **33** (4), 313-318.

Johari S., Ekasari, S. and Kurnianto, E. (2012) Genetic variation in three breeds of Indonesian local ducks based on blood and egg white protein polymorphism. *Journal of the Indonesian Tropical Animal Agriculture* **38** (1), 20-26

Kinoshita, K.S.T., Okamoto, K., Shinogiri, T. Kawabe, R. Nishida, R. Kakizawa, Y. Yamamoto and Y. Maeda (2002) Gene constitution of egg white proteins of native chicken in Asian countries. *Asian-Australian Journal of Animal Science* **15**(2), 157-165.

Mahfudz, L.D., Wulandari, A.R. and Johari, S. (2011) Genetic variation through polymorphism of blood and egg white protein in three kinds of Kedu chickens at laying period. *Journal of Animal Production* **13**(2), 83-88.

Muzani, A., Brahmantiyo, B., Sumatri, C. and Tapyadi, A. (2005) Pendugaanjarak genetic padaitik Cihateup, Cirebon dan Mojosari. Media Peternakan **28**(3), 109-116.

Mwacharo J.M., C.J. Otieno, A.M. Okeyo, and R.A.Aman (2002). Characterization of indigenous fat tailed and fat-rumped hair sheep in Kenya: Diversity in blood proteins. *Tropical Animal Health and Production*, **34**, 515-524.

Myint S.L., T.Shimogiri, K. Kawabe, T. Hashiguchi and Y. Maeda (2010) Characteristics of seven Japanese native chicken breeds based on egg white protein polymorphism. Asian – Australasian Journal of Animal Sciences, **23** (9), 1137-1144

Nei, M. 1972. Genetic distance between populations .*The American Naturalist* **106**, 283-292.

Nur H., Y. Yusrizal and F. Manin (2012) Study of Blood Polymorphism of Kerinci Duck. *International Journal of Poultry Science* **11** (10), 641-643

Nwanta, J.A., J.U. Umoh, P.A. Abdu, I. Ajogi. and J.K. Ali Balogun (2006) Management of losses and Newcastle disease in rural poultry in Kaduna state, Nigeria. *Nigerian Journal of Animal Production* **3** (2), 274-285.

Obeidah, A.,P. Merat, and L. Durand (1977) Polymorphism of egg white proteins, egg weight and components weight in Fayoumi hen. *Genetics Selection Evolution* **9**(3), 301-306.

Okabayashi, H., Okamoto, A., Tanabe, Y., Yamamoto, Y. Namikawa, T. and Buahom, B. (2000) Genetic constitutions of the Laotian indigenous ducks with emphasis on their phylogeny. *Japanese Poultry Science* 37, 95-100.

Orajaka L.J.E. (2005) The role of local chicken in poultry production in Nigeria. *Nigerian Veterinary Journal* **26** (2), 68-72.

Ola, S.I. (2000) Growth and carcass characteristics of the Nigerian Muscovy duck, *in*: The proceedings of the XXI World's Poultry Congress, August 20-24 2000, Montreal, Canada.

Oluyemi, J.A. & Ologbobo, A. D. (1997) The significance and management of the local duck in Nigeria. Proceedings of the 2nd Annual Conference of Anim. Sci. Association of Nigeria, Sept. 16-17 Lagos, Nigeria, pp: 96-103.

Rege, J.E.O. and Okeyo, A.M. (2006) Improving our knowledge of Animal Genetic. Training Resource Version 2, 2006.Ojango J.M., Malmfors, B. and Okeyo A.M. (eds) International Livestock Research Institute Nairobi, Kenya and Swedish University of Agricultural Science, Uppsala, Sweden.

Riken, (2006) Available at http://www.riken.go.jp/engn/r-world/research/lab/index.html.

Yakubu, A. (2009) an assessment of sexual dimorphism in AfricanMuscovy ducks using morphological measurement and discriminate analysis. Proceeding of 4th water fowl conference 2009 Kerala India pp69-75.

Yakubu, A. and Ugbo, S. B. (2011) An assessment of biodiversity in morphological traits of Muscovy ducks in Nigeria using discriminant analysis. *International Conference on Biology, Environment and Chemistry* ***1,*** *389-391*

Youssao, I.A.K., Tobada, P. C., Koutinhouin, B.G., Dahouda, M.N.D., Idrissou G.A, Bonou, U.P., Tougan,

S., Ahounou, V. Yapi-Gnaore, B. Kayang, X. Rognon and Tixier-Boichard, M. (2010) Phenotypic characterisation and molecular polymorphism of indigenous poultry populations of the species Gallus gallus of savannah and forest ecotypes of Benin. *African Journal of Biotechnology* 9 (3): 369-381.

Zhang, X., Leung, F. C., Chan, D. K. O., Yang, G. and Wu, C. (2002) Genetic diversity of Chinese native chicken breeds based on protein polymorphism, randomly amplified polymorphic DNA, and microsatellite polymorphism. *Poultry Science* **81** (10), 1463-1472