

GENETIC VARIATION OF THE JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*) BASED ON BIOCHEMICAL POLYMORPHISM

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Abstract. The study aimed at characterizing the Japanese quail using biochemical markers. Blood protein polymorphism of one hundred and sixty-six (166) Japanese quails of both sexes comprising of 83 each of mottled brown and white quails were analysed using cellulose acetate paper electrophoresis. Six loci which includes hemoglobin (Hb), transferrin (Tf), albumin (Alb), carbonic anhydrase (CA), alkaline phosphatase (Alp) and esterase-1 (Es-1) were tested. All the loci tested were polymorphic with each locus having two co-dominant alleles controlling three genotypes. Allele B was predominant at Hb, Tf and Es-1 locus with frequencies 0.90, 0.55, and 0.77, respectively while Allele A was predominant at Alb and Alp locus with frequencies 0.83 and 0.58 respectively. The Allele A had generally lower frequencies than B at the CA loci having values of 0.43 - Brown, 0.38 - White and 0.40 – overall. The mean observed heterozygosity (H_o) was 0.48 with brown and white quails having H_o values of 0.47 and 0.49 respectively, and the expected heterozygosity was observed to be higher in white quails (0.39) than in the mottled brown (0.31). The genetic distance (0.0534) between white and brown quails in this study showed little genetic differentiation between the brown and the white quails. Dendogram generated from the genetic distance values indicated that the two strains had common origin.

Key words: dendogram, Japanese quail, polymorphism, characterization, electrophoresis

Introduction

Livestock populations have evolved unique adaptation to their agricultural production systems and agro-ecological environments. The knowledge of their genetic diversity is important as it forms the basis for designing breeding programs and making rational decisions on sustainable utilization of animal genetic resources

(*Mwacharo et al., 2005*). Genetic characterisation through the use of molecular markers is providing new avenues for decision making choices for the conservation and rational management of Animal Genetic Resources (AnGR) (*Ajmone-Marsan et al., 2010; Groeneveld et al., 2010; FAO, 2011*). The order *Galliformes* includes many wild bird species and the entire row of domestic species and breeds, the majority of which is well known by their morpho-physiological and productive qualities. This group includes the Japanese quail (*Coturnix coturnix japonica*) which has a very wide natural distribution. Since the 1th century, the Japanese quail has been known as a meat and egg-type bird, but it has never been as popular as chicken because of its small body size (*Cheng and Kimura, 1990*). However, it has occupied an equivalent position with other popular experimental animals. The wide distribution of the Japanese quail as an experimental animal began two decades ago, during which time no particular attention was paid to the birds themselves other than being used in comparison in scientific research (*Cheng and Kimura, 1990*). A major factor contributing to the variations in the results of experiments on animals is their genetic backgrounds (*Van Zutphen et al., 1993*). This is a major reason for the study of the genetic variability of quail. Another reason why the Japanese quail is so interesting in respect to the protein polymorphism is its wide natural distribution in comparison with other birds of the order *Galliformes* (*Cheng et al., 1992*). The Japanese quail is highly adaptable to an extensive range of ecological conditions due to an unusually high frequency of polymorphic loci and average individual heterozygosity (*Baker, 1967*). In spite of the wide natural distribution of this galliform bird and its adaptability to a wide range of ecological conditions (*Cheng et al, 1992*), it is worth mentioning that the population of the Japanese quail have been observed dwindling in the last 3 decades (*Kimura, 1991*). At present, there are two strains of Japanese quail in Nigeria classified according to plumage colour. The most common strain is the mottled brown quail while the least common is the white quail and popularly known among farmers as 'albino' for its characteristic white plumage.

Genetic diversity studies are undertaken to classify individuals or populations; and has been accessed in farm animals through morphological, molecular or biochemical methods (*Mohammadi and Prasanna, 2003; Goncalves et al., 2009*). Several experiments have described various molecular genetic markers used for evaluation of genetic variability in different poultry species and breeds. The utilization of DNA as genetic marker to evaluate genetic variability of poultry breeds and lines was reported by *Semionova et al. (1996)*. The use of biochemical markers is also significant (*Cywa-Benko et al., 1994; Inafukuk et al., 1998*). The characteristic features of biochemical markers are high stability and conservativity. The allelic variants of protein visualized after electrophoresis are the products of certain genes. Studies of such polymorphic proteins may provide additional information on the genetic differences among separate individuals, populations, breeds or species and on the influence of natural or artificial selection on genetic

processes - gene drift, gene flow and others, which occur in populations and breeds (Kuznetsov, 1995). The present study was aimed at describing the genetic variability of the Japanese quail using biochemical markers.

Materials and Methods

Blood samples were randomly collected from a total of one hundred and sixty-six (166) birds of both sexes and 12-24 months of age comprising eighty-three (83) each of mottled brown quails and white quails sourced from reputable farms in Ibadan. Blood samples were collected by jugular venipuncture into tubes containing heparin as anticoagulant and kept refrigerated during transportation. Plasma and erythrocyte samples were separated from the heparinized whole blood by centrifugation. The electrophoresis of blood proteins and enzyme systems of Hemoglobin (Hb), Transferrin (Tf), Albumin (Alb), Carbonic anhydrase (CA), Alkaline phosphatase (Alp), Esterase-1 (Es-1) were performed on cellulose acetate membrane following the procedure described by RIKEN (2006) with slight modifications.

Allele frequencies for each locus in each sample were computed by direct gene counting method and tested to fit Hardy-Weinberg ratios using Chi square (χ^2) goodness of fit test. The observed (H_o) and expected heterozygosity (H_e) were calculated according to Nei (1972) with the correction for small samples (Levene, 1949). The genetic identity (I) and genetic distance (D) were calculated according to Nei's (1978). The matrix of the distances was used to construct a dendrogram of relationships according to Unweighted pair-group with arithmetic mean (UMPGA) (Sneath and Sokal, 1973). F-statistics (fixation indices F_{is} , F_{st} , F_{it}) was calculated. All computations were performed using Popgene (Yeh *et al.*, 1997) and Tools for Population Genetic Analyses (TFPGA; Miller, 1997).

Results

Allele frequencies of the analyzed loci were as presented in Table 1. All loci investigated polymorphic with two co-dominant alleles A and B. Hb^B (0.89, 0.92), CA^B (0.57, 0.62) and $Es-1^B$ (0.75, 0.78) had the most frequent occurrence in the brown and white quails respectively. Alb^A (0.88) was more frequent in white quails, while Alb^B (0.79) was more in brown. Tf^A (0.52) and Alp^A (0.79) were more in brown quails whereas Tf^B (0.62) and Alp^B (0.62) were most frequent in white quails. The result observed from this present study shows that the frequency of Alp^A was higher in brown quail than white quail while Alp^B was observed to be higher in white quail than brown quail.

Heterozygosity estimates were as presented in Table 2. The observed heterozygosity (H_o) was 0.47 and 0.49 for brown and white quail birds, respectively with an overall average of 0.48. The value of expected heterozygosity (H_e) was observed to be 0.33 and 0.30 for brown and white quails, respectively. The mean value of H_e for all population was 0.32.

Population differentiation examined by fixation indices F_{is} , F_{st} , F_{it} for each of the 6 loci studied across population were as shown in Table 3. F_{it} was estimated at 0.16 with Hb (-0.106) being the only locus with negative value. The heterozygote deficit within population evaluated by F_{is} was negative (-0.03 and -0.17) for Alb and Tf, respectively. The global breed differentiation (F_{st}) evaluated as 0.0439 with a range of 0.0017 (Hb) and 0.1727 (Alp). The gene flow values for each of the six loci studies ranged from 1.197 (Alp) to 149.75 (Hb). The mean gene flow for all loci was recorded as 5.45.

Table 1. Allele frequency of polymorphic loci

Locus	Allele	Observed number of alleles	POPULATIONS		
			Brown	White	Overall
Hb	A	32	0.11	0.08	0.10
	B	300	0.89	0.92	0.90
CA	A	134	0.43	0.38	0.40
	B	198	0.57	0.62	0.600
Alb	A	55	0.21	0.88	0.83
	B	277	0.78	0.12	0.17
Tf	A	149	0.51	0.38	0.45
	B	183	0.48	0.62	0.55
Es-1	A	78	0.25	0.22	0.24
	B	254	0.75	0.78	0.77
Alp	A	194	0.79	0.38	0.58
	B	138	0.21	0.62	0.42

Hb- Haemoglobin, CA Carbonic Anhydrase, Alb- Albumin, Tf- Transferrin, Es-1 - Esterase 1, Alp- Alkaline Phosphatase

Table 2. Heterozygosity (Ho, He) and Deviation from Hardy-Weinberg Equilibrium (DHWE) per strain across allozyme loci

Strain/Population	No	Heterozygosity*		DHWE
		<i>Ho</i>	<i>He</i>	
Brown	83	0.4687(0.1335)	0.3313(0.1335)	0
White	83	0.4928(0.1286)	0.3072(0.1286)	0
Overall	166	0.4807	0.3193	
St. Dev.		0.1233	0.1233	

N= number of samples; St. Dev = Standard deviation; *Standard deviation in parenthesis

The genetic distance is a measure of genetic difference between population and genetic variability within a population. The distance between the two population was 0.0517 and genetic identity of 0.95 which show a close similarity between the two quail population. According to Wright's values of genetic distance, a dendrogram (Fig. 1) was drawn using the unweighted pair-group clustering analysis (UPGMA). The dendrogram indicated the genetic processes.

Table 3. F-Statistics and Gene Flow for all Loci Studied

LOCUS	Sample Size	Fis	Fit	Fst	Nm*
Hb	332	0.1085	-0.1067	0.0017	149.750
CA	332	0.0969	0.0991	0.0024	103.390
Tf	332	-0.1723	0.1550	0.0148	16.6778
Alb	332	-0.0306	0.0106	0.0194	12.6361
Es-1	332	0.3285	0.3297	0.0018	137.333
Alp	332	0.4304	0.5288	0.1727	1.1974
Mean	332	0.1182	0.1569	0.0439	5.4479

*Nm= gene flow estimated from $F_{ST} = 0.25(1-F_{ST})/F_{ST}$; Hb- Haemoglobin, CA- Carbonic Anhydrase, Alb- Albumin, Tf- Transferrin, Es-1 - Esterase 1, Alp- Alkaline Phosphatase

Discussion

In this study two hemoglobin alleles; Hb^A and Hb^B were observed. *Dimiri (1981)* in a study of the effect of haemoglobin genotypes on growth and some physiological parameters in Japanese quails reported, three genotypes of hemoglobin (AA, AB and BB) which were controlled by two autosomal alleles A and B. *Mazumder et al. (1989)* reported frequencies of 0.96 (Hb^A) and 0.04 (Hb^B) for white leghorn chickens, and 1.00 (Hb^A) for broiler chickens which contradicted the results of this study as frequency of Hb^A were 0.108 and 0.08 in brown and white Japanese quail, respectively, while frequency of Hb^B were 0.89 and 0.92 respectively. Frequency of Hb^B was predominant in both populations. *Yakubu and Aya (2012)* reported Hb^A frequencies that were higher for all genetic groups of Nigerian indigenous chickens

in their study. In a study of blood protein polymorphism and genetic diversity in locally adapted Muscovy ducks in Nigeria, *Oguntunji and Ayorinde (2015)* reported the predominance of Hb^A over Hb^B in all the ecotypes studied. *Mazumder et al. (1989)* reported the presence of gene fixation as only genotype Hb^{AA} was identified in their study including broiler chickens. The preponderance of the B allele observed is similar to those obtained by *Okamoto et al. (2003)* in Asian native fowl. *Okamoto et al. (2003)* reported in general that Asian fowl was being fixed in Hb^B while Hb^A was detected at extremely low frequencies in some chickens. *Washburn et al. (1971)* related hemoglobin types with Marek disease and concluded that chickens with homozygous mutant hemoglobin genotypes were approximately 20% less susceptible to Marek disease. In the same way, *Dimri (1981)* reported that hemoglobin polymorphism affects growth rate and hatchability, with the highest in Hb^{AA} (62.20%) followed by Hb^{AB} (48.20%) and Hb^{BB} (31.50%). *Akinyemi et al. (2014)* reported that frequency of Hb^A was higher than Hb^B in Muscovy ducks while Hb^B was higher in Mallard ducks. 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*).

Table 4. Nei's Original Measures of Genetic Identity and Genetic distance Nei (1972)

Population	Brown	White
Brown	****	0.9496
White	0.0517	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Ismoyowati (2008) reported that Tegal Duck with Hb^{AA} genotype on all loci had higher egg production than Hb^{BB} and Hb^{CC} homozygote. *Ordas (2004)* reported that Hb^A has a higher affinity for molecular oxygen than Hb^{BB} because of differences in oxygen dissociation rates. Frequency of CA^B was higher in both mottled brown and white Japanese quail populations. This observation suggests a close relationship between the two populations. *Ige et al. (2013)* reported the predominance of CA^f in both Yoruba and Fulani ecotype population. *Oguntunji and Ayorinde (2015)* reported the predominance of CA^F over CA^S in various Muscovy ecotypes in Nigeria. *Akinyemi et al. (2014)* reported predominance of CA^A in both Muscovy and Mallard ducks in Nigeria. 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. The allele frequency of Alb^A was higher in brown quail, while for white quail it was Alb^B. Similar result has been reported for Japanese quails and their hybrids (*Vaida et al., 2000*). They reported higher frequency of Alb^A for brown (0.820), white (0.729) and hybrid (0.500) Japanese quail respectively. They also reported lower frequency of Alb^B (0.461). *Butkauskas*

et al. (2000) reported similar result with Alb^A of 0.5356 in Japanese quail. *Akinyemi et al.* (2014) reported higher frequencies for Alb^B in Muscovy ducks and higher values of Alb^A for Mallard ducks. The authors also reported the presence of Alb^C in Mallard ducks. All samples tested were polymorphic at the Tf locus with two Alleles (A and B). *Butkauskas et al.* (2000) reported the presence of Tf^C in quails, while Tf^D was reported to be present in turkey and Tf^E in chicken. *Okamoto et al.* (1999) reported gene fixation as only genotype Tf^B and Tf^C was identified in their study. *Akinyemi et al.* (2014) reported the presence of only Tf^A and Tf^B with Tf^A being predominant in both Muscovy and Mallard ducks. *Oguntunji and Ayorinde* (2015) reported the presence of $Tf^{A, B, C}$ and D with Tf^B (0.475) and Tf^C (0.419) being the most predominant in the populations of Muscovy ducks locally adapted in Nigeria. *Okamoto et al.* (1999) reported high frequency of Alp^B for the three breeds of chicken studied. Two co-dominant alleles F and S were reported for indigenous turkeys in Nigeria (*Fatai et al.*, 2017). The authors reported Alp^F to be more predominant than Alp^S in the studied population. The presence of two alleles at the Alp locus has also been reported by other authors. *Singh and Nordskog* (1981) reported the prevalence of the slow allele Alp^S in some lines of Leghorn chickens. *Das and Deb* (2008) reported that for sexual maturity, birds with the fast type allele Alp^F mature about 13 days before those with Alp^S . *Singh et al.* (1983) reported higher levels of Alp in pullets selected for higher production, suggesting that the enzyme likely plays a significant role in sexual maturity.

The observed heterozygosity is the proportion of heterozygotes observed at a locus while expected heterozygosity or gene diversity is the proportion of expected heterozygotes under random mating. The values were high for both mottled brown quail and white quail with an overall H_o of 0.48 with a value of 0.47 ± 0.13 and 0.49 ± 0.13 for brown quail and white quail respectively while the overall H_e was lower with values of 0.33 ± 0.13 and 0.31 ± 0.13 in brown and white quails. The result of gene diversity from this study was higher than those reported by *Maedal* (1999) and *Vaida et al.* (2000). This could be a contributing factor to better adaptability of the Japanese quail to the prevailing tropical conditions. However, *Meedal et al.* (1980; 1999) reported that there was no clear difference in heterozygosity in quail lines selected for large and small body weight and also between the selected and random bred lines. Heterozygosity of blood proteins and enzymes of brown and white quails showed no significant differences ($P > 0.05$). *Maeda et al.* (1999) reported that increasing the number of loci studied helps to detect small differences of heterozygosity, and it can be achieved now more easily through molecular markers

Chi-square test of Hardy-Weinberg proportions showed no significant differences in the observed and the expected frequencies of brown and white Japanese quails. This revealed that the gene and genotype frequencies of the two populations were in Hardy-Weinberg proportions as they were not affected by nonrandom mating, genetic drift, mutation, genetic migration and selection.

F-statistic values of F_{st} and F_{it} are measures of deviation from Hardy-Weinberg proportions and total populations, respectively. Positive values indicate a deficiency in heterozygotes and negative values an excess of heterozygotes. F_{is} can be interpreted as a measure of inbreeding (the measure of allelic fixation of individuals relative to the sub-populations). Thus, the negative value (-0.1067) of F_{it} observed at three loci out of the 6 loci that were studied for the quail populations indicates a deficiency of homozygotes in the populations and that mates were less related in comparison with the average relationship of the population. The negative values of F_{is} observed at two out of the six loci also indicate a deficiency of homozygotes in the populations. The observed excess of heterozygotes could be due to the non-random mating and genetic exchange between populations.

The estimated F_{ST} value, which corresponds to the proportion of genetic variability accounted for by the differences among breeds, was 0.0439. Thus, a larger part of the total genetic diversity can be explained by the variation within breeds (0.9561) and to a smaller extent by the variation among breeds (0.0439). This result indicated that the genetic diversity quantified by allozyme markers shows little genetic differentiation among the quail population studied. The degree of differentiation observed between the two quail population studied could be due to source, geographic proximity, similarities in environment and breeding practices. The standard genetic distance (*Nei, 1972*) obtained in this study between white and brown quails were 0.0534 indicating little genetic differentiation between the brown and the white quail. Similar trend was observed with *Nei (1976)* for the two strains under investigation (0.0517). *Vaida et al. (2000)* reported D value 0.0226 between white and motley quails using data from 10 loci. The estimated *Nei's* genetic identity (0.9480) obtained in this study was similar to 0.991 reported by *Vaida et al. (2000)* between white and brown quails.

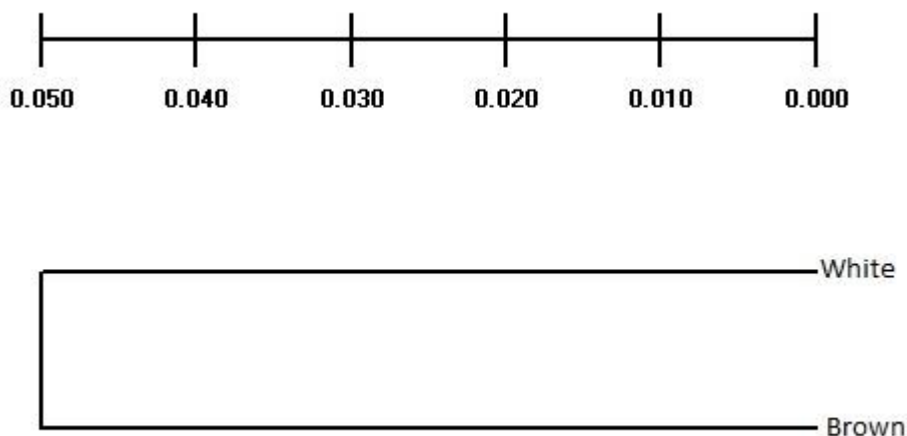


Figure 1. Dendrogram of genetic distance between two strains of Japanese quail based on six allozyme loci

Conclusion

The present study demonstrated the usefulness of protein markers to characterize Japanese quail. Genetic similarity as measured by dendrogram supported high gene flow between the two strains of Japanese quails; thus, indicating that the two populations were genetically related. Further studies should focus on other protein markers and DNA related studies on polymorphism. This may be useful as an initial guide in defining objectives for designing future investigations of genetic integrity and developing strategies for sustainable use of the Japanese quail genetic resource of in Nigeria.

Genetska varijacija japanske prepelice (*Coturnix coturnix Japonica*) bazirana na biohemijskom polimorfizmu

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Rezime

Cilj istraživanja je karakterizacija japanske prepelica koristeći biohemijske markere. Polimorfizam krvnih proteina od sto šezdeset šest (166) japanskih šarenih smeđih i belih prepelica, 83 svakog pola, analizirani su pomoću elektroforeze

celuloznog acetat papira. Ispitivano je šest lokusa koji uključuju hemoglobin (Hb), transferin (Tf), albumin (Alb), karbonatnu anhidrazu (CA), alkalne fosfataze (Alp) i esteraze-1 (Es-1). Svi testirani lokusi su polimorfni sa svakim lokusom koji ima dva ko-dominantna alela koja kontrolišu tri genotipa. Alel B je dominantan za Hb, Tf i Es-1 lokus sa frekvencijama 0,90, 0,55 i 0,77, dok alel A preovlađuje na Alb i Alpu lokusima sa frekvencijama 0,83 i 0,58 respektivno. Alel A imao je generalno niže frekvencije od B u CA lokusima sa vrednostima od 0,43 - Braon, 0,38 - Bela i 0,40 - ukupno. Prosečna opažena heterozigotnost (H_o) bila je 0,48 sa smeđim i belim prepelicama sa vrednostima H_o 0,47 i 0,49 respektivno, a očekivana heterozigotnost je bila viša kod belih prepelica (0,39) nego u šareno smeđim (0,31). Genetička distanca (0,0534) između bele i smeđe prepelice u ovoj studiji pokazala je malo genetske diferencijacije između. Dendogram koji je generisan iz vrednosti genetičke distance pokazuje da su dva tipa imala zajedničko poreklo.

Ključne reči: dendogram, japanska prepelica, polimorfizam, karakterizacija, elektroforeza

References

- AJMONE-MARSAN P. (2010): The Globaldiv Consortium A global view of livestock biodiversity and conservation–GLOBALDIV. *Animal Genetics*, 41, Suppl. 1, 1–5.
- AKINYEMI M. O., OSAIYUWU O.H., AJAYI A.Y. (2014): Biochemical Characterisation of Muscovy and Mallard Ducks in Nigeria. *International Journal of Science and Nature*, 5 (3), 557-562.
- BAKER C.M.A., MANWELL C. (1967): Molecular genetics of avian proteins: 8. Egg white proteins of the migratory quail, *Coturnix coturnix* new concepts of hybrid vigour. *Comparative Biochemistry Physiology* 23, 21-42.
- BUTKAUSKAS D., JUODKA R., SRUOGA A., TUBELYT-KIRDIEN V., MOZALIEN E., PAULAUSKAS I. A. (2000): Genetic Study Of Variability And Similarity In Three Different Poultry Species. Institute of Ecology of Vilnius University, Akademijos 2, LT-08412, Vilnius, Lithuania.
- CHENG K.M., KIMURA M., FUJII S. (1992): A comparison of genetic variability in strains of Japanese quail selected for heavy body weight. *Journal of Heredity* 83, 31-35.
- CYWA-BENKO K., BRODACKI A., SZWACZKOWSKI T. (1994): Comparative study of blood serum protein polymorphism in three breeds of hens. *Roczniki Naukowe Zootechniki*, 21, (1-2), 41-49.
- DAS A.K., DEB R. (2008). Biochemical polymorphism and its relation with some traits of importance in poultry. *Veterinary World* 1, 220-222.

- DIMRI C.S., SINGH H., JOSHI H.B., BIST G.S. (1981): The effect of haemoglobin genotypes on growth and some physiological parameters in Japanese quails (*Coturnix coturnix japonical*). Indian Journal of Animal Science, 51(9), 911-914.
- FATAI R.B., AKINYEMI M. O., OSAIYUWU O. H. (2017): Genetic Variation in Indigenous Turkey populations in South West Nigeria. Journal of Advances in Agriculture, 7, 2, 1021 -1029.
- FAO. (2011): Draft guidelines on molecular genetic characterization of animal genetic resources. Commission on Genetic Resources for Food and Agriculture, 13th Regular Session, 18-22 July, 2011, Rome. Available at <http://www.fao.org/docrep/meeting/022/am651e.pdf>.
- GONCALVES L.S.A., SUDRE C.P., BENTO C.S., MOULIN M.M. (2008): Divergencia genetica em tomate estimada por marcadores RAPD em comparacao com descritores multicategoricos. Horticultura Brasileira 26, 364-370.
- GROENEVELD L.F., LENSTRA J.A., EDING H., TORO M.A., SCHERF B., PILLING D., NEGRINI R., JIANLIN H., FINLAY E.K., GROENEVELD E., WEIGEND, S. (2010): The Globaldiv Consortium Genetic diversity in livestock breeds. Animal Genetics, 41, suppl. 1, 6-31.
- INFUKU K., MAEDA Y., OKAMOTO S., ARDININGSASI S. M., HASHIGUCHI T. (1988): Polymorphism of egg white proteins in native chickens in Indonesia. Japan Poultry Science, 35 (5), 278-284.
- ISMOYOWATI, I. (2008): Detection study of egg production of Tegal duck through protein polymorphisms). Journal of Animal Production 10 (2), 122-128.
- KIMURA, M., FUJII S. (1989): Genetic variability within and between wild and domestic Japanese quail populations. Japanese Poultry Science 26, 245-256.
- KUZNETSOV S. B. (1995): Polymorphism of blood plasma proteins in the geese of Anser and Branta genera. Biochemical Genetics, 33 (3/4), 123-135.
- MAEDA Y., HASHIGUCHI T., TAKETOMI M. (1971): Endocrine control of serum alkaline phosphatase isozyme in the Japanese quail, *Coturnix coturnix japonica*. Japan Poultry Science, 8, 224-230.
- MAZUMDER N.K., MAZUMDER A. (1989): Indian Journal. of Animal Science, 59 (11), 1425-1428.
- MWACHARO J.M., OTIENO C.J., OKEYO M.A. (2005): Suitability of blood protein polymorphisms in assessing genetic diversity in indigenous sheep in Kenya. In: Harinder, P.S. Makkar and Gerrit J. Viljoen (eds). Applications of gene-based technologies for improving animal production and health in developing countries. Springer, Netherlands. Pp. 585- 591.
- MOHAMMADI S.A., PRASANNA B.M. (2008): Analysis of genetic diversity in crop plans salient statistical tools and considerations. Crop Science 43:1235-1248.
- NEI M. (1972): Genetic distance between populations. American Naturalist 106, 283-292.

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- OGUNTUNJI, A.O., AYORINDE, K.L. (2015): Blood protein polymorphism and genetic diversity in Nigerian Muscovy duck (*Cairina moschata*). *Animal Genetic Resources*, 56: 9-18.
- RIKEN (2006): Genetic Quality Monitoring by Biochemical Isozymes. RIKEN Bio Resource Center.
- VAIDA T., DALIUS B., ALGIMANTAS P., ANIOLAS S. (2000): Variability of Blood Serum Proteins In The Japanese Quail (*Coturnix Coturnix*) Breeds and Hybrids. *Acta Zoologica Lituanica*, 10, 4.
- SINGH H., NORDSKOG A.W. (1981): Biochemical Polymorphic systems in inbred lines of chickens: A survey. *Journal of Biochemical Genetics* 19, 1031-1035.
- SINGH R.P., J. KUMAR P.K. DWARKANATH and D.S. BALAINE. (1983): Association of plasma 5-nucleotidase and alkaline phosphatase with production traits in chickens: Genetic and phenotypic variability. *British Poultry Science*, 24, 483-488.
- VAN ZUPTEN L.F.M., BAUMANS V., BEYNEN A.C. (1993): Principles of laboratory animal science. Elsevier Sci. Publ., Amsterdam.
- YAKUBU A., AYA V. E. (2012): Analysis of Genetic variation in normal feathered, naked neck and Fulani- ecotype Nigerian Indigenous Chickens Based on Haemoglobin Polymorphism. *Biotechnology in Animal Husbandry* 28 (2), 377-384.

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