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Biochemical Polymorphism in Newzealand White X Chinchilla Rabbit Crosses

Osaiyuwu*, O. H., Akinyemi, M. O., Akinlabi, J. O. and Fatai, R. B
Animal Breeding and Genetics Unit, Department of Animal Science, University of Ibadan. Ibadan. Nigeria.

Corresponding author: cosamede@yahoo.com, Phone: +234 806 466 6076

Target Audience: Molecular geneticist, Rabbit breeders, Researchers

Abstract

The study evaluated diversity within New Zealand white and Chinchilla rabbit crosses using four structural protein loci: Hemoglobin (Hb), Albumin (Alb), Transferin (Tf) and Carbonic anhydrase (CA). Blood (4mls) was sampled from a total of 49 rabbits through ocular venipuncture. The samples collected were analysed using cellulose acetate electrophoresis to estimate the genetic variability within the populations. The degree of heterozygosity, deviation from Hardy-Weinberg's Equilibrium (HWE), F_{IS}, and F_{ST} values were estimated. All four proteins loci studied were found to be polymorphic. CA and Alb produced two codominant alleles which controlled three different genotypes. While the two observed codominant alleles in Hb and Tf controlled two genotypes. The haemoglobin locus had the highest heterozygosity value (0.87) while the lowest value (0.53) was recorded at the Albumin locus. Significant deviation from Hardy-Weinberg equilibrium ($P < 0.05$) was observed at Hb and Tf loci while CA and Alb loci were found to conform with Hardy-Weinberg equilibrium ($p > 0.05$). Negative F_{IS} values were obtained for all the studied loci indicating the deficiency of homozygotes in the population and that mates were less related in comparison to the average relationship of the population. The results obtained could serve as a reference point in the genetic improvement of the rabbit genetic resource.

Key words: Heterozygosity, Rabbit crosses, Polymorphism, Hardy-Weinberg, Protein electrophoresis

Description of Problem

Demand for meat has being on the increase in developing countries due to population growth and increases in economic activities (1, 2). Thus, to ensure sustainable and affordable animal protein supply, the productivity of the fast-growing and efficient converter of available fodder to food livestock such as rabbit needs to be improved. Rabbits exhibit several traits of economic importance including: short gestation period, high production potential, rapid growth rate, ability to utilize forage and by-products as major diet components, early maturity, and efficient feed utilization (3).

The first step to genetic improvement is assessment of genetic diversity. The knowledge of the genetic diversity is important as it forms the basis for designing breeding programs and making rational decisions on sustainable utilization of animal genetic resources

(4). The genetic diversity found in domestic breeds allows breeders to select and develop new breeds in response to changes in demand, environment or climate (5). The FAO global strategy for the management of farm animal genetic resources places a strong emphasis on the use of molecular methods to assist the conservation of endangered breeds and to determine the genetic status of breeds. Although, DNA-based technologies are now the methods of choice for genetic characterization of livestock (6), several alternative assays, such as protein polymorphisms remain tremendously useful, especially in developing countries, because of their utility, ease, cost and simplicity of data interpretation (7).

Protein electrophoresis has been widely used in studying genetic diversity in several farm animals and poultry (5, 8, 9, 10, 11 and 12). However, there are very few published reports on the genetic diversity in rabbits particularly at the biochemical level. Therefore, this study aimed at assessing the genetic diversity within Newzealand white and Chinchilla rabbit crosses using biochemical markers. This may provide useful genetic information essential for developing effective management plans for the conservation and improvement of these genetic resources.

Materials and Methods

The study was conducted at the Animal Breeding and Genetics Laboratory, Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan. Blood (4ml) was randomly sampled by ocular venipuncture from 49 Newzealand white and Chinchilla rabbit crosses of both sexes bred in the Teaching and research farm of the University of Ibadan. Blood samples were carried in tubes containing lithium heparin as anticoagulant and transported in ice pack to the laboratory for analysis. The red blood cell was prepared from the erythrocyte fraction of heparinized blood by centrifugation at 3000rpm for 10m minutes at 4°C. The RBC was washed in normal saline three times and centrifuged at 3000rpm for 5 minutes. The red blood cells were lysed with a four folds' volume of distilled water and stored in the freezer until electrophoretic analysis. The plasma fraction was separated from the erythrocyte fraction of the blood by centrifugation at 3000rpm for 10 min at 4°C, the supernatant was used. Each fraction of the sample was then subjected to cellulose acetate electrophoresis. The electrophoretic conditions, staining and distaining protocol were as previously described by (9 and 13). Allelic variant for each locus were marked in order of increasing mobility. The alleles with slowest and fastest mobility were represented as A and B respectively in hemoglobin (Hb), Transferin (Tf) and Albumin (Alb) while F and S were used to designate the fastest and the slowest allele at carbonic anhydrase (CA) locus.

Statistical Analysis

Allele and genotypic frequencies for each locus in each sample were computed by direct gene counting method and tested for fit to Hardy-Weinberg ratios using goodness of fit test. The observed and expected heterozygosity were calculated according to (14) while the genetic differentiation and fixation indices (F_{IS})

were analysed according to (15) using (16) F-statistic. All computations were performed using Popgene program (17).

Results and Discussion

All four studied loci were polymorphic generating eight allelic variants. The most frequent allele was Alb^A while the least frequent was Tf^B with the frequencies of 0.63 and 0.36, respectively (Table 1).

The heterozygous genotypes (AB and FS) had the highest genotypic frequency at all the studied loci (Table 2). The Hb and Tf loci were observed to have deviated significantly ($P<0.05$) from Hardy-Weinberg equilibrium while CA and Alb loci conform with Hardy-Weinberg equilibrium ($P>0.05$) (Table 3). Negative F_{IS} values were obtained for all the studied loci (Table 4).

Two reported hemoglobin genotypes AB and BB in this study were in agreement with the findings of (18) in New Zealand white rabbits. Additional genotype AA was also reported by (19**).** However, Hb locus was monomorphic in the studies of (20) and (21) in rabbit. In studies of Hb in sheep, Hb^A has been implicated to have high affinity for oxygen and it is

Table 1 Allele Frequency at four protein loci in Newzealand white x Chinchilla rabbit crosses

Locus	Allele	Frequency
Hb	A	0.4348
	B	0.5652
CA	F	0.4778
	S	0.5222
Alb	A	0.6327
	B	0.3673
Tf	A	0.6383
	B	0.3617

Hb = Hemoglobin, CA = Carbonic anhydrase, Alb = Albumin, Tf = Transferin, A, B, F and S = alleles.

Table 2 . Genotype Frequency at four protein loci in Newzealand white x Chinchilla rabbit crosses

Locus	**Genotype**	**Observed Frequency**	**Expected Frequency**
Hb	AA	0.000	0.1863
	AB	0.8696	0.4970
	BB	0.1304	0.3167
CA	FF	0.1778	0.2256
	FS	0.6000	0.5047
	SS	0.2222	0.2700
Alb	AA	0.3673	0.3978
	AB	0.5306	0.4696
	BB	0.1020	0.1324
Tf	AA	0.2766	0.1283
	AB	0.7234	0.4049
	BB	0.0000	0.4668

Hb = Hemoglobin, CA = Carbonic anhydrase, A lb = Albumin, Tf = Transferin, AA, AB, BB, FF, FS and SS = genotypes.

Table 3. Heterozygosity estimates for Rabbit Crosses

Allele	Sample size	Ho	He	HWE	Probability
Hb	49	0.8696	0.4915	26.47*	0.000
CA	49	0.6000	0.4990	1.6444	0.997
Tf	49	0.7234	0.4617	14.58*	0.0001
Alb	49	0.5306	0.4648	0.85	0.35
Mean		0.6809	0.4793		
Standard Deviation		0.1489	0.0188		

Hb = Hemoglobin, CA = Carbonic anhydrase, Alb = Albumin, Tf = Transferin, Ho = observed heterozygosity, He = expected heterozygosity, HWE = Hardy-Weinberg equilibrium. * Significant at $p<0.05$

Table 4. F_{IS} values for four protein loci in Newzealand white x Chinchilla rabbit crosses

Locus	F_{IS}
Hb	-0.7692
Alb	-0.1416
CA	-0.2024
Tf	-0.5667
Mean	-0.4207

Hb = Hemoglobin, CA = Carbonic anhydrase, Alb = Albumin, Tf = Transferin,

important for survival in areas of altitudes above 3000m (22). A possible correlation between Hb polymorphism and genetic resistance to helminth infection in sheep and goat has also been reported (23). Two co-dominant allele A and B which controlled three different genotypes AA, BB and AB were observed at Albumin (Alb) locus. The frequency of allele B (0.63) was higher than the frequency of allele A (0.37). Genotype AB had the highest genotypic frequency (0.53). These findings disagree with the report of (18) in rabbit where Genotype BB had the highest genotypic frequency value at albumin locus.

At the Carbonic anhydrates (CA) locus, FS genotype had the highest frequency 0.600 while FF had the lowest allelic frequency value (0.18). This trend is similar to the values obtained by (20) in Newzealand rabbit population. In a related study, (21) reported highest genotypic frequency value for FF genotype. The reported two alleles (A and B) and two genotypes (AA and AB) at transferrin locus in this study correspond with the result of (20). Allele A had the highest frequency value (0.64) while the highest genotypic value was recorded in genotype AB (0.72). Consequently, allele B and genotype AA had the lowest allelic and genotypic frequency value. (21), (24) however reported Transferin (Tf) locus to be monomorphic in all their studied population.

Estimates of heterozygosity which is a measure of genetic variation for each individual locus in the population revealed that, albumin has the lowest value of (0.53) and haemoglobin locus value (0.87) appeared highest. This result accounted for just 50% heterozygosity which is a little bit

deviated from the result of (20) who reported high level of heterozygosity in more than 80% of the studied rabbit population, the deviation could be as a result of the breed differences, or the effect of population size. Chi-square analysis for the differences between observed and expected genotype frequencies showed that the deviation was significant ($P<0.05$). Suggesting that the flock studied is in Hardy-Weinberg equilibrium for the hemoglobin and transferrin (Tf) Locus. Carbonic anhydrates (CA) and Albumin (Alb) locus were however, not at Hardy-Weinberg equilibrium ($P>0.05$). This is in agreement with the report of (21) in Newzealand and Californian rabbits.

The F_{IS} values for each locus were observed to be negative for all the loci studied, indicating that there is excess of heterozygotes the population studied. F_{IS} can be interpreted as a measure of inbreeding or the measure of allelic fixation of individuals relative to the subpopulations. Thus, the negative values of F_{IS} in the current study indicates the deficiency of homozygotes in the population and that mates are less closely related the average relationship of the population. This was to be expected since the sampled population was a population of crossbred rabbits. The population is a non-random mating population and genetic exchange between populations is evidenced.

Conclusion and Applications

The following conclusion could be made from the result of the current study:

1. Allele frequencies were higher for allele A in Tf and Alb locus but lower in Hb, while allele S had a higher frequency in the CA locus.
2. The population is a non-random mating population and genetic exchange between populations is evidenced.
3. Selection of rabbits on the bases of Hb polymorphism may provide genetic progress towards better genetic resistance to helminth as has been reported in sheep and goat.

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