EFFECT OF CARBONIC ANHYDRASE POLYMORPHISMS ON PERFORMANCE TRAITS IN INDIGENOUS CHICKEN GENOTYPES IN NIGERIA

H. MUHAMMAD[1], B. I. NWAGU[2], M. ORUNMUYI[3], A. A. MUSA[4], A. K. OLUTUNMOGUN[2] AND S. A. JOHN[5]

[1]Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna State [2]National Animal Production Research Institute, Shika, Zaria, Kaduna State.

[3]Department of Animal Production and Health, Federal University, Oye-Ekiti, Ekiti State.

[4]Department of Animal Production, Kogi State University, Anyigba, Kogi State.

[5]Department of Animal Science, College of Agricultural Science, Landmark University, Omu-Aran, Kwara State.

Correspondence: hassanmuhammad11116@gmail.com, 08069554750

ABSTRACT

The paper examined the possibility of performance differentiation and genetic characterization of indigenous chickens in Nigeria using carbonic anhydrase polymorphisms. F_1 offspring hatched from eggs produced by the pedigree mating of Normal feathered, Frizzle feathered and Naked Neck of Nigerian local cocks to their Normal feathered, Frizzle feathered and Naked Neck hens respectively were used. The birds were weighed on a weekly basis to collect data on body weight, breast girth and tibia length. At 20weeks, 5ml of blood was collected from the wing vein of each chicken for carbonic anhydrase electrophoresis, after which direct gene counting method was used to score the bands which separated based on the electrophoretic mobility into fast moving band (homozygous, AA gene), slow moving band (homozygous, BB gene) and mixture i.e. midway between fast and slow (heterozygotes, AB gene).Data obtained were subjected to general linear model procedure of statistical analysis system (SAS, 2002) and significant means were separated using tukey honestly significant difference of the same software. Gene and genotypic frequencies were calculated and tested for Mendelian inheritance ratio using chi-square. Carbonic anhydrase polymorphism was distinct into three forms viz; CA^{AA}, CA^{AB} and CA^{BB}. The birds were more of heterozygotes (CA^{AB}) than either of the homozygotes (CA^{AA} or CA^{BB}) for the three loci considered. This means more of the heterozygotes adapted and survived better than the homozygotes. There were low chi-square values for Frizzle (1.00), Normal (0.44), Naked Neck (3.39) and the entire population (2.94) which was in consistence with Hardy Weinberg's equilibrium. The differencesobserved were not consistent among the various biochemical genotypic categorization of carbonic anhydrase in terms of body weight (g), breast girth (cm) and tibia length (cm).In conclusion, carbonic anhydrase polymorphism does not suitably serve for body weight (g), breast girth (cm) and tibia length (cm) selection in indigenous chickens as other protein allozyme may serve.

Keywords: Carbonic anhydrase polymorphism, local chickens, weight, gene and genotypic.

INTRODUCTION

Proteinpolymorphisms (biochemical) remain useful, especially in developing countries, because of their utility, ease, cost and amount of genetic information accessed or simplicity of data interpretation (Rege and Okeyo, 2006). Carbonic anhydrase was not directly reported to affect weight of chicken, but, it was recognized to affect the transportation and utilization of haemoglobin in the body of chickens (Das and Deb, 2008), while haemoglobin is known to affect growth rate in chickens. The effect of the carbonic anhydrase on heamoglobin could therefore indirectly translate into effect on the growth rate of chickens.The presumed effect of the carbonic anhydrase on performance traits in indigenous chicken genotypes was therefore tested using biochemical polymorphism in order to know the basic genetic activities which contribute to the differences observed in terms of weight in the performance of Nigerian local chicken genotypes especially as research of this kind is limited in the country.

MATERIALS AND METHODS

Location of the Experiment

The research was conducted at the Poultry Unit, Department of Animal Science Teaching and Research Farm, Ahmadu Bello University, Samaru – Zaria, Kaduna State, which lies between latitude 11° 09¹ 06¹¹ N and 7° 38¹ 55¹¹E, at an altitude of 706m above sea level. (Ovimaps, 2012).

Experimental Animal, Breeding and Management

Growing birds which served as the parent stock were sourced from Kogi State (Anyigba and Okene), Kaduna State (Shika and SabonGari markets) and KatsinaState (Funtua). The birds were wing tagged, housed separately according to genotype on deep litter floor and treated against worms and lice and vaccinated against Newcastle diseases. Each genotype consisted a total of 30 hens and 6 cocks which were mated in the ratio of 1 cock to 5 hens. Feeds and water were supplied to the birds *ad libtum*. Pure mating system was used in which males of Frizzle were mated to their female Frizzles, males of Normal feathered to females of Normal feathered and males of Naked Neck mated to females of Naked Neck. Eggs were collected separately according to the mating group, pedigreed and hatched using automated electric incubator at the optimum temperature 37^{0}C, relative humidity of 60 – 65% and were turned hourlyto produce F_1 offspring. Data on body weight, breast girth and tibia length were taken weekly.

At 20weeks of age, 5ml syringe was used to collect blood from the wing vein of each chicken into heparinized tubes each labeled according to the tag number of each birdfor electrophoretic analysis. The electrophoresis was carried out at a voltage of 150V for about 50minutes at a temperature of 4^{0}C. The direct gene counting method as explained by Christensen (2003) was then used to score the bands based on the separation of the electrophoretic mobility as; fast moving band (homozygous, AA gene), slow moving band (homozygous, BB gene) and midway between fast and slow (heterozygotes, AB gene)

Statistical Analysis

Data obtained were subjected to general linear model procedure of statistical analysis system (SAS, 2002) and significant means were separated using tukey honestly significant difference. Gene and genotypic frequencies were calculated using the Hardy Weinberg's equation by the expansion of the binomial equation below.

$$(p+q)^2 = p^2 + 2pq + q^2$$

Where p stands for the fast alleles and q for the slow types and pq is the midway (heterozygotes). Mendelian inheritance ratio for band (genotype) inheritance was tested using chi-square (χ^2) formula below.

$$\text{Chi-square } (\chi^2) = \frac{(\text{Observed genotype frequency} - \text{Expected Mendelian genotype frequency})^2}{\text{Expected Mendelian genotype frequency}}$$

The statistical model used for the association of blood polymorphic types with each traits was;

$$y_{ij} = \mu + b_i + e_{ij}$$

Where: y_{ij} = Result of the observation, μ = The overall mean, b_i= Effect of particular biochemical genotype (either of transferrin, haemoglobin and or carbonic anhydrase) and e_{ij} = Residual error.

RESULTS AND DISCUSSION

Table 1 shows the genotype, genotypic and allelic distribution of all the chickens in respect to carbonic anhydrase. There were three polymorphic forms of carbonic anhydrase as CA^{AA}, CA^{AB} and CA^{BB}, which were being controlled by two co-dominant alleles, this was also reported by Ige *et al.* (2013a). Das and Deb, (2008) however, reported six genotypes, controlled by three codominant alleles. There were more of heterozygotes than the homozygotes at the carbonic anhydrase locus for Frizzle, Normal and Naked Neck. Heterozygosityis a measure of genetic diversity occurring at both the intrapopulation and interpopulation levels (Yakubu and Aya, 2012). This also suggests that many of the homozygotes do not adapt well to survive as the heterozygotes did adapted and survived.Chi-square value of 5.99 at 2 degrees of freedom was the expected limit for which the calculated (observed) chi-square can be compared. The calculated chi-square value for the Frizzle population was 1.00, while that of Normal was 0.44, Naked Neck was 3.39 and the entire population was 2.94. The low calculated chi-square values indicates consistence with Hardy Weinberg's equilibrium.

Table 2 shows effect of carbonic anhydrase polymorphism on the Body Weight (g), Breast Girth (cm) and Tibia Length (cm) of the Indigenous Chickens.The carbonic anhydrase CA^{AB} and CA^{BB} maintained similar (P>0.05)

values in body weight from day old to 20 weeks of the experiment. The CA^{AA} however, showed no definite pattern of growth.

Significant differences ($P<0.05$) in tibia length were also observed between CA^{AB} and CA^{BB} genotypes except at 8 weeks when there was no significant difference ($P>0.05$) between them. The CA^{AA} genotype also did not show a consistent pattern of tibia length growth difference between CA^{AB} and CA^{BB} genotypes.The difference in the body weight, breast girth and tibia length of the birds in association to the type of carbonic anhydrase genotype showed that carbonic anhydrase has a trophic effect but the inconsistence in weight differentiation indicates that it cannot be relied upon for selection.Karhuma (2002) reported that carbonic anhydrase participate in the regulation of ion, water and acid-base balance, promote cell proliferation and act as trophic/growth factor.

CONCLUSION

Carbonic anhydrase polymorphism was distinct into three forms viz; CA^{AA}, CA^{AB} and CA^{BB}. The birds were more of heterozygotes (CA^{AB}) than either of the homozygotes (CA^{AA} or CA^{BB}) for the three loci considered. This means more of the heterozygotes adapted and survived better than the homozygotes. The total population was however in Hardy Weinberg's equilibrium in respect to carbonic anhydrase locus.The relationship between body weight(g), breast girth(cm), tibia length(cm) and the polymorphic forms of the carbonic anhydrase howeverdid not present consistent pattern growth whereby a particular polymorphic form exhibits significant difference between the separate indigenous chicken groups nor within the total population. This shows that carbonic anhydrase polymorphic form does not suitably serve for body weight (g), breast girth (cm) and tibia length (cm) selection in indigenous chickens.

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Table 1: Carbonic Anhydrase Genotype, Genotypic and Allelic Frequencies of the Frizzle, Normal and Naked Neck Indigenous Chicken of Nigeria

Group	Sex	No	Observed number of genotype			Allelic Frq(Gene)= 1		Observed Genotypic Frq = 1			Chi-square (χ^2)
			CA^{AA}	CA^{AB}	CA^{BB}	P	Q	p^2	2pq	q^2	
F	Male	20	7	12	1	0.65	0.35	0.35	0.60	0.05	2.03
	Female	17	10	6	1	0.76	0.24	0.59	0.35	0.06	0.01
	Total	37	17	18	2	0.70	0.30	0.46	0.49	0.05	1.00
N	Male	43	19	20	4	0.67	0.33	0.44	0.47	0.09	0.15
	Female	36	10	20	6	0.56	0.44	0.28	0.56	0.17	0.56
	Total	79	29	40	10	0.62	0.38	0.37	0.50	0.13	0.44
NA	Male	24	10	13	1	0.69	0.31	0.42	0.54	0.04	1.63
	Female	15	7	8	0	0.73	0.27	0.47	0.53	0.00	1.98
	Total	39	17	21	1	0.71	0.29	0.44	0.54	0.02	3.39
Total Male		87	36	45	6	0.67	0.33	0.41	0.52	0.07	2.64
Total Female		68	27	34	7	0.65	0.35	0.40	0.50	0.10	0.61
Population Total		155	63	79	13	0.66	0.34	0.41	0.51	0.08	2.94

Fre= frequency, F- Frizzle feather, N-Normal Feather, NA – Naked Neck chickens

Table 2: Effect of Carbonic Anhydrase Genotype on the Body Weight (g), Breast Girth (cm) and Tibia Length (cm) of the whole Population of the Indigenous Chickens.

Parameters	CARBONIC ANHYDRASE GENOTYPES			CV (%)	SEM	LOS
	CA^{AA}	CA^{AB}	CA^{BB}			
Day-old	24.63[b]	25.58[a]	25.85[a]	12.40	0.25	*
WT_4	95.41[b]	101.46[a]	100.15[a]	21.78	1.73	*
WT_8	306.79[b]	326.78[a]	319.62[a]	20.80	5.31	*
WT_{12}	561.00[b]	598.78[a]	582.38[a]	22.24	10.40	*
WT_{16}	691.11[b]	745.92[a]	734.46[a]	21.84	12.68	*
WT_{20}	1024.33[b]	1099.91[a]	1075.31[a]	20.59	17.65	*
BG_4	9.29[b]	9.44[a]	9.18[b]	9.22	0.07	*
BG_8	12.55[a]	12.70[a]	12.32[b]	8.66	0.09	*
BG_{12}	17.29[b]	17.65[a]	17.06[b]	8.93	0.13	*
BG_{16}	22.05[a]	22.00[a]	21.57[b]	12.14	0.21	*
BG_{20}	24.90[b]	25.09[b]	25.87[a]	11.11	0.22	*
TL_4	3.04[b]	3.11[a]	3.03[b]	11.13	0.03	*
TL_8	4.57	4.63	4.56	10.51	0.04	NS
TL_{12}	7.02[a]	7.02[a]	6.77[b]	11.33	0.06	*
TL_{16}	8.23[b]	8.76[a]	8.20[b]	1.65	0.13	*
TL_{20}	9.33[a]	9.35[a]	9.01[b]	10.79	0.08	*

[a,b,c]= Means with the same superscript within the same row for a particular parameter are not significantly different ($P<0.05$) different. CV = Coefficient of variation, SEM = Standard Error of Means, LOS = Level of Significance, NS = Not significant, WT_{4-20} = body weight for week 4, 8, 12, 16 and 20 weeks respectively, BG_{4-20} = breast girth for week 4, 8, 12, 16 and 20 weeks respectively, TL_{4-20} = tibia length for week 4, 8, 12, 16 and 20 weeks respectively