Allelic polymorphism of insulin-like growth factor 1 gene and its relationship with growth traits in repeated backcrossed laying chickens

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Abstract

The relationship of insulin-like growth factor-1 (*IGF-1*) polymorphism with growth traits was investigated in repeated backcrossed laying chickens reared in derived savanna zone of Nigeria which was conducted at Poultry Unit of Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Department of Animal Breeding and Genetics, Biotechnology Laboratory , Federal University of Agriculture, Abeokuta, Ogun State. Nigeria. One-hundred and thirty-three birds were used for the study and comprises of 4 different genotypes. Genomic DNA was extracted from obtained bloods and the polymorphism of *IGF-I* gene was detected by PCR-RFLP-PstI. Both allelic and genotypic frequencies were calculated and Statistical analysis of data was done using LSD (least significant difference) method as applied in the General Linear Model (GLM) procedure (SAS 9.1). Genotyping of birds revealed two alleles A (0.54), C (0.46) and three genotypes AA (41), AC (60), CC (32). AC genotype has the highest frequency of 53% while for AA and CC the frequencies are 30% and 17% among selected repeated backcrossed chickens. Analyzing different genotypes reveals non- significant effects on growth traits of the chickens involved. Based on the results of this study, the application of *IGF-1* locus in Marker Assisted Selection programs can be advised.

Key words: Insulin-like Growth Factors (*IGF1*), repeated backcrossed chickens, growth traits, gene polymorphism, Hardy-Weinberg equilibrium

Introduction

The candidate gene approach has been used as a powerful method for evaluating the effect of contributing genes which are involved in the expression of quantitative variations between individuals in the population (Kwon & Goate 2000; Nagaraja *et al.*, 2000). Candidate genes that are extensively studied in chickens are those of the growth hormone axis, because of the wide range of biochemical and physiological processes that they regulate. Abbasi, and Kazemi, (2013) described Insulin-Like Growth Factors (*IGF*) to consist of a family of polypeptide hormones structurally linked with insulin with multiple metabolic and anabolic functions. The *IGFs* have been shown to regulate body and muscle growth in chickens (Duclos, 1998). Several studies have shown that circulating *IGF-I* affects growth rate in poultry (Goddard *et al*., 1988; Scanes *et al*.,

1989; Ballard *et al*., 1990). Insulin like growth factor (*IGF1*) is associated with several phenotypic features in chicken including production and reproduction traits. *IGFI* gene could be linked to the quantitative trait loci (QTL) that control egg production, egg quality, body weight and reproductive traits in chicken (Kim *et al.*, 2004; Li *et al.*, 2010; Tang *et al.*, 2010). The aim of this study was to evaluate the *IGFI* gene polymorphism and its relationship with growth traits (body weight, body length, keel length, thigh length and wing length) in repeated backcrossed laying chickens.

Materials and Methods

The experiment was carried out at the Poutry Unit of Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Department of Animal Breeding and Genetics, Biotechnology Laboratory and Central Biotechnology Laboratory, Federal University of Agriculture, Abeokuta, Ogun State. Nigeria. Blood samples were obtained randomly from 133 chickens from 4 different genotypes through the wings vein using 2ml syringe and transferred to EDTA bottles to serve as anti-coagulating agent, after which the samples were stored in the laboratory at -20^{o}C. On weekly basis, the growth traits variables such as body weight (BDW), body length (BL), cheast girth (CH), keel length (KL), shank length (SHL) and thigh length (TL) were recorded from day old to 20 weeks of age by the procedure described by Amao and Ojedapo (2016). Genomic DNA was extracted from the blood, using the Qiagen DNA extraction kits following manufacturer protocol. Working dilutions of extracted DNA were prepared for each individual at a concentration of 50 ng/µg. Primers forward (5'-GACTATACAGAAAGAACCAC-3') and reverse (5'-TATCACTCAAGTGGCTCAAGT-3') were used for the polymerase chain reaction (PCR) amplification (Nagaraja *et al.*, 2000). Each cycle was consisted of initial denaturation at 94°C for 5 minutes, 94°C for 4 minutes, 45 sec at 60°C, 60 sec at 72^{o}C and final extension at 72°C for 10 minutes. The PCR products were run in an agarose gel electrophoresis to verify the result. The PCR product or amplicon were digested with a restriction enzyme, *Pst1* and digested products were electrophoresed for 1 h at 80 V on a 2.5% agarose gel. Individual PCR-RFLP fragment sizes for each gene were determined by visualizing the banding pattern under ultraviolet light. Genotyping was obtained manually following the scoring procedure described by Wheto *et al.* (2016). The data obtained were subjected to one way analysis of variance while significance between genotypes was assessed by Duncan multiple range test of SAS (2009).

Results and Discussion

Table 1 showed the allele and genotype frequencies of the *IGF1* gene polymorphism. The result indicated that two types of allele frequencies were identified as A of 0.54 of the population and C of 0.46 of the population. The frequency of A allele was higher than that of C while the genotypic frequencies of 30 %, 53 % and 17 % were observed for genotype AA, AC and CC. The population of the birds used, the chi-squared for the genotypic frequency was estimated and it was observed that there was no significant effects which indicated that the frequency of the genotype does not varied from expectation of Hardy-Weinberg equilibrium and this was in accordance with the previous findings of Wheto *et al.*(2016) and Abbasi and Kazemi (2013) reported that chi-squared test on the genotype frequencies shows no deviation from Hardy-Weinberg equilibrium in the Mazandaran native fowls population. The three genotypes found namely AA, AC and CC which show fragments at different base pair (bp) using *Pst1* for PCR-RFLP with allelic frequency A and C in the present result indicated that allele A was higher than that of C and genotypic values of AC was highest followed by AA and least value recorded for CC. The observed frequency was in accordance with the findings of Wheto *et al.* (2016); Babayi *et al.* (2014). They observed that

frequency of genotype (AA) homozygote individual was lower than the frequency of homozygote individual with AC. Ghelghachi *et al.* (2013) reported higher allele A than that of C in Arian broilers while Mehdi *et al.* (2014) found that the allele A was higher than B in Azarbaijan native chicken. The genotypic frequencies observed presently was also similar to the findings of Ali *et al.* (2016) for Desi chicken, Mariandayan *et al.* (2013) for Indonesian native chicken and Ghelghachi *et al.* (2013) for Arian broilers. These authors reported that heterozygote individual (AB) was superior in value than either AA or BB homozygote individual frequencies. However, the observation of Jafari *et al.* (2015) contradicted the present findings who noted that genotypic frequency of AA homozygote individual had more value than that of AB heterozygote individual and BB homozygote individual respectively for Isfahan native fowls with highest genotypic frequency for homozygote individual (BB) than homozygote individual AA and heterozygote individual (AB) for Mazandaran native fowls.

The least square means and the standard errors of genotype and *IGF-1* gene polymorphism interaction on the growth traits of repeated backcrossed chickens' population is presented in Table 2. Interestingly, there were no significant (P>0.05) effects between the genotype and *IGF-1* gene polymorphism. Although, the polymorphism of *IGF-1* gene polymorphism shows that it is a potential candidate gene associated with growth and body composition (Whetto *et al*., 2016; Bhattachanya *et al.*, 2015), egg production (Aminafshar and Fathi, 2012) and egg quality traits (Li *et al.*, 2010) but the non-significant effects of *IGF-1* gene polymorphism on the growth traits observed in the present study was in accordance with findings of Wheto *et al*. (2016), Ali *et al.* (2016) and Ghelighachi *et al.* (2013). These authors on their different studies with *IGF-1* gene polymorphism on chickens reported that *IGF-1* gene polymorphism had no significant effects on either growth or carcass traits of chickens. Babayi *et al.* (2014) found no significant differences on *IGF-1* gene polymorphism with some growth traits in West-Azerbaijan native chicken in Iran. Reports of Mazurowski *et al.* (2015) on *GH* polymorphism of body weight and selected body dimension in Peckin and Mallard ducks found non-significant effects of *GH* gene polymorphism on the body weight and other selected body conformation measured.

Conclusion

The *IGF1* gene polymorphism did not indicates any significant relationship with the growth traits of the chickens but it's a potential candidate gene that expressed relationship with growth traits in chickens.

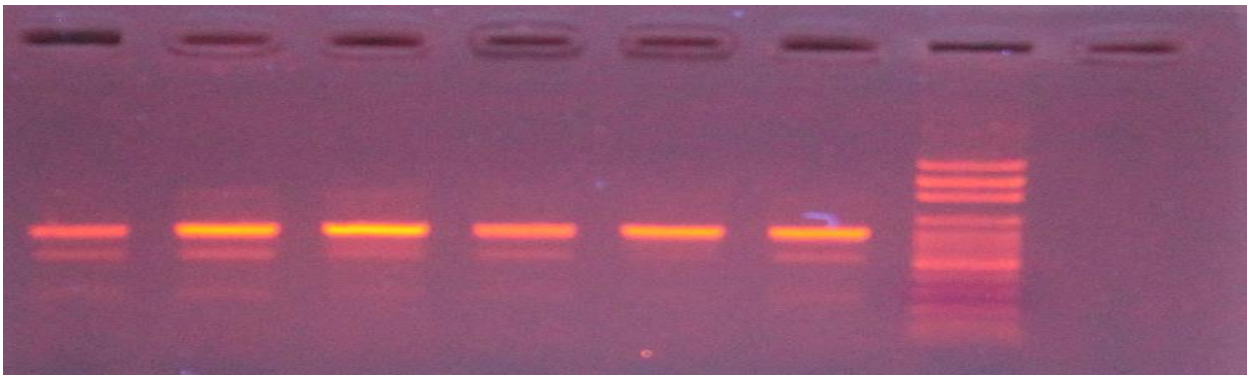

Plate 1: Insulin –like growth factor (*IGF-1*) gene optimization on agarose gel

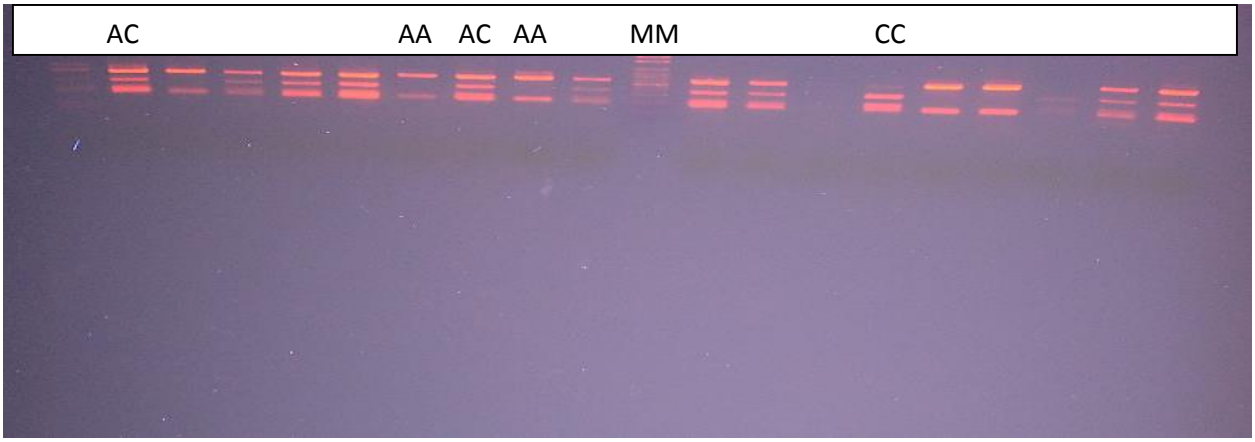

Plate 2: PCR- RFLP pattern for Insulin – like growth factor (*IGF-1)* gene polymorphism

Table 1: The allele and genotype frequencies of the *IGF1* gene polymorphism

	Allele Frequencies		Genotype Frequencies		
Locus	**A**	**C**	**AA**	**AC**	**CC**
IGF-1 gene	0.54	0.46	0.30	0.53	0.17

IGF-1 = Insulin – like growth factor gene

Table 2: Least square means and standard errors of genotype x *IGF-1* gene polymorphism interaction on growth traits of chickens' population

Genotype	*IGF-1*	BDW		BL		CH		KL		SHL		TL	
RIFE	AA	1309.00	±	21.55	±	22.53	±	15.90	±	9.88	±	14.35	±
		20.99		1.11		2.34		1.91		0.45		0.78	
	AC	1337.00	±	21.84	±	22.64	±	17.30	±	9.83	±	14.45	±
		56.54		1.01		1.89		1.34		0.89		0.67	
	CC	1360.00	±	23.37	±	22.63	±	18.12	±	9.86	±	14.55	±
		45.22		1.13		1.56		1.67		0.99		0.35	
RIFF	AA	950.25	±	20.80	±	22.90	±	15.10	±	9.00	±	15.95	±
		12.89		0.56		2.13		1.67		0.83		0.51	
	AC	941.25	±	20.78	±	22.74	±	15.07	±	8.91	±	15.87	±
		11.89		0.67		1.90		1.34		0.45		0.89	
	CC	940.00	±	20.77	±	22.78	±	15.08	±	8.92	±	15.90	±
		11.11		1.04		1.89		1.67		0.56		0.59	
RINF	AA	1251.25	±	23.81	±	25.81	±	17.37	±	10.46	±	18.55	±
		11.99		1.23		2.10		1.78		0.67		0.56	
	AC	1285.11	±	23.79	±	25.78	±	17.47	±	10.45	±	18.68	±
		21.90		1.45		1.89		1.22		0.89		0.34	
	CC	1253.00	±	23.90	±	25.90	±	17.20	±	10.42	±	18.45	±
		23.12		1.68		2.05		1.45		0.99		0.67	

RINN	AA	1860.00	±	28.55	±	26.60	±	18.00	±	11.00	±	18.00	±
		13.88		1.56		2.13		1.90		0.78		1.04	
	AC	1675.00	±	28.37	±	26.40	±	17.95	±	11.03	±	18.54	±
		23.33		1.44		2.56		1.67		0.67		0.90	
	CC	1695.00	±	28.11	±	26.18	±	17.96	±	11.02	±	18.50	±
		23.45		1.67		1.67		1.89		0.56		0.89	

BDW = body weight, BDL = body length, CH = chest girth, KL = keel length, SHL = shank length, TL = thigh length, *IGF-1* = Insulin – like growth factor gene, AA = homozygote dominant gene, AC = heterozygote gene, CC = homozygote recessive gene, RIFE = Reciprocal Improved Fulani Ecotype, RIFF = Reciprocal Improved frizzle feather, RINF = Reciprocal Improved Normal feather, RINN = Reciprocal Improved Naked neck.

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