

Insulin-like growth factor-1 (Igfi) gene polymorphism and its effect on egg quality traits of three chicken genotypes reared in hot humid tropics

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Abstract

A study was conducted on the effect of gene polymorphism of Insulin like Growth Factor-1 (IGFI) on egg quality traits in three chicken genotypes. A total of 250 chicken comprising 150 FUNAAB-Alpha (50 Normal feather, 50 Naked neck and 50 Frizzle feather), 50 Kuroiler and 50 Sasso were used for this experiment. At point of lay, 30 hens per genotype were selected and transferred into a battery cage of one unit per bird. Data was collected on the egg quality traits, 30 eggs for each genotype was collected. All collected data was subject to analysis of variance using a completely randomized design, of which genotype was the interest factor. At 16 weeks, 1ml of blood was collected from each hen and extraction of genomic DNA from the blood was done. PCR was conducted using the pair of primer and condition as described by Nagaraga, et al. (2000). The PCR amplicons were digested using PstI restriction enzymes following the manufacturer's procedure. The resulting fragments were analyzed using GenAnalyzer (GenAlEx 6.502) was used for the genetic diversity of the IGFI locus. This data was subject to the PROC GLM of SAS 9.2. Results showed the chicken genotypes significantly ($P < 0.05$) affect all the egg-quality traits except the shell weight, yolk ratio and albumen ratio. The IGFI gene polymorphism had no significant effect ($P > 0.05$) on egg quality traits for except the egg length and egg width.

Keywords: Growth hormone, SNPs, FUNAAB Alpha, Sasso, Kuroiler, Indigenous.



Polymorphisme du gène comme croissance analogue à l'insuline- 1 (Igfi) et son effet sur les caractéristiques de qualité des œufs de trois génotypes de poulet élevés dans des régions tropicales chaudes et humides

Résumé

Une étude a été menée sur l'effet du polymorphisme génique du facteur de croissance analogue à l'insuline (IGFI) sur les caractéristiques de qualité des œufs chez trois génotypes de poulet. Un total de 250 poulets comprenant 150 FUNAAB-Alpha (50 plumes normales, 50 cou nu et 50 plumes Frizzle), 50 Kuroiler et 50 Sasso ont été utilisés pour cette expérience. Au moment de la ponte, 30 poules par génotype ont été sélectionnées et transférées dans une cage en batterie d'une unité par oiseau. Des données ont été collectées sur les traits de qualité des œufs, 30 œufs pour chaque génotype ont été collectés. Toutes les données recueillies ont fait l'objet d'une analyse de variance selon un design complètement randomisé, dont le génotype était le facteur d'intérêt. À 16 semaines, 1 ml de sang a été prélevé sur chaque poule et l'extraction de l'ADN génomique du sang a été effectuée. La PCR a été réalisée en utilisant la paire amorce et condition décrite par Nagaraga et al. (2000). Les amplicons PCR ont été digérés à l'aide d'enzymes de restriction PstI selon la procédure du fabricant. Les fragments résultants ont été analysés à l'aide de GenAnalyzer (GenAlEx 6.502) a été utilisé pour la diversité génétique du locus IGFI. Ces données ont été soumises au PROC GLM de SAS 9.2. Les résultats ont montré que les génotypes de poulet affectent de manière significative ($P < 0,05$) tous les caractères de

qualité des œufs, à l'exception du poids de la coquille, du rapport de jaune et du rapport d'albumen. Le polymorphisme du gène IGFI n'a eu aucun effet significatif ($P>0,05$) sur les caractères de qualité des œufs, sauf la longueur et la largeur des œufs.

Mots-clés : Hormone de croissance, SNP, FUNAAB Alpha, Sasso, Kuroiler, Indigène.

Introduction

The improved Nigerian local chickens are unique breeds which serve as the nation's heritage. They have an appreciable body weight and are dual purpose in production (Addisu, 2013). In this study, the dual-purpose breeds of Sasso, Kuroiler, and FUNAAB Alpha were used. Three genotypes of the dual-purpose FUNAAB Alpha chicken exist: normal feather, naked neck, and frizzle feather. (Durosaro *et al.*, 2021).

According to many researchers, the external egg quality is demonstrated by the egg's weight, shape, percentage of eggshell, and thickness, all of which depend on the species, breed, variety, nutrition, management, and environment (Atsbaha *et al.*, 2022). Similarly, internal egg quality is represented by albumen quality and yolk quality, which are in charge of the nutritional content of breed-specific eggs and decide whether or not consumers would accept them. (Zita *et al.*, 2009; Sinha *et al.*, 2017; Atsbaha *et al.*, 2022). The qualities of an egg are what determine whether a consumer will accept it. Therefore, in today's production-oriented market, ongoing genetic evaluation of several egg quality features has become crucial to maintaining supremacy in an egg's overall quality (Sreenivaset *et al.*, 2013; Pradepta *et al.*, 2015).

Economic traits in animals show continuous variation, although they exhibit a complex genetic nature. Molecular marker assisted selection has proven to be efficient in helping to improve both productive and reproductive abilities. Nonetheless, an individual gene approach is a great method to understanding the genetic basis directive which aids the expression of differences that are quantitative amongst individuals as new latest technologies in molecular genetics provides opportunities to evaluate the variability of genes at the level of DNA (Kaya and Yildiz, 2008; Anh *et al.*, 2015). The growth hormone in chickens (cGH) and

IGF-1 genes are ingenious genes that improve chicken performance whilst enhancing certain quality traits (Anh *et al.*, 2015). They are mitogenic polypeptides with similarities to insulin, playing a major role for cellular growth, assisting in mediating growth hormone actions, affecting biological processes such as growth and reproductive differentiation in poultry. Authors also have suggested that the IGF-I show association amongst the body weight, carcass and reproductive traits of the chicken (Tang *et al.*, 2010). Wu *et al.* (2015) reports that the IGF-1 was strongly expressed in the liver according to the patterns of expression and SNP analyses, and its mutation was linked to characteristics related with egg-laying. Moreover, it is important to identify and understand the part of this candidate gene in order to speed up the rate of selection in reproductive performance traits in Nigerian local poultry, therefore the motive of this project was to probe the associations between IGF-I genes and egg quality traits of the laying performance in the selected chicken breed.

Materials and methods

Experimental site

The research was carried out at the PEARL Poultry Breeding unit of the University farm, Federal University of Agriculture, Alabata, Abeokuta (FUNAAB). The molecular analysis was carried out at the FUNAAB, College of Animal Science and Livestock Production (COLANIM); Breeding and Genetics Biotechnology laboratory. Alabata is located in Odeda Local Government Area of Ogun State, Nigeria (latitude 7°100 N and longitude 3°20 E). This region in the south-western section of Nigeria has a tropical climate with an annual rainfall of about 1037 mm. The typical ambient temperature ranges from 28 degrees Celsius in December to 36 degrees Celsius in February, with a yearly average relative humidity of roughly 82%. The vegetation is a transitional zone between tropical rainforest and derived

savannah (Iloriet *al.*, 2017; Durosaroet *al.*, 2021).

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Experimental animals

Two hundred and fifty (250) chickens comprising of 150 FUNAAB - Alpha (50 normal, 50 naked-neck and 50 frizzled-feathered), 50 Kuroiler and 50 Sasso dual purpose breed were used for this experiment. The chickens were generated via artificial insemination (AI) from the flocks in the PEARL poultry breeding unit, Directorate of the University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun state, Nigeria.

Rearing and Management

The chicks generated from AI were brooded and raised under deep litter system, they were subsequently moved to cages. They were pedigreed along genotype for proper identification. They were fed with commercial feed (Chick mash, grower mash and layer mash). Medication and vaccination were administered as at required stages. At sexual maturity, cocks were separated from the hen and the hens were transferred into a battery cage (one bird/unit) in order to monitor their reproductive performances.

Egg collection

Thirty (30) eggs per genotype was collected from the birds at 32 weeks of age, which was a peak period of laying. The eggs were used for analysis of the external and internal traits. The chickens were similar in age as they were hatched on the same day.

Egg quality evaluation: external qualities of the egg

The following were measured:

Weight of the egg - Mettler weighing balance.

Length and width of egg - digital vernier caliper.

Egg width - the distances between the widest cross-sectional regions of the ends.

Egg length - as distance between the broadest and narrowest ends of the egg.

Egg Shape Index (ESI) = Egg Width / Length

The shell weight was obtained as a percentage of the weight of the air-dried egg shells to that of the whole egg. Whilst shell thickness was

obtained from the air-dried shell using a micrometer screw gauge (Kgwatalalae *al.*, 2016).

Internal qualities of the Egg

A spherometer was used to measure the height of the albumen. A Mettler-weighing balance was used to measure the separated yolk. Yolk weight was obtained as the percentage weight of the yolk and whole egg. The difference obtained from the weight of the egg, sum of the yolk weight and dry egg shell was taken as albumen weight and this was expressed as a percentage of the total weight of egg. Haugh Unit (HU) = $100 \log (AH+7.5-1.7We^{0.37})$. (Haugh, 1937; Atsbahaet *al.*, 2022).

AH = Albumen height (mm),

We = weight of egg

Blood Collection, DNA extraction and PCR-RFLP and Analysis

One (1) ml of blood sample was collected per chicken using a needle and syringe from the wing vein. The collected blood was transmitted into Ethylene Diamine Tetra acetic Acid (EDTA) bottle as an anticoagulant agent. The blood samples were transported to the laboratory for the extraction of genomic DNA extraction using Isolate II Blood DNA kits following the manufacturer procedures.

DNA purity and concentration were done with a Nanodrop spectrophotometer and 1% gel electrophoresis. The primer sequences used for Polymerase Chain Reaction (PCR) amplification was as described by Nagaragaet *al.* (2000). Polymerase Chain Reaction was done with a terminal reactive volume of 20µl. Each of one of these had 4µl of 5X Firepol PCR premix, 2µl of genomic DNA, 1µl each of forward and reverse primer and 12µl of Nuclease Free water. These reactive mixtures were subject to prior denaturation at 94°C for 5 minutes, 35 cycles of 94°C for 45 seconds, 60°C for 45 seconds and an extension at 72°C for 1 minute then followed by a final extension at 72°C for 10 minutes.

About 10µl of the PCR product was subjected to 1.5 percent agarose-gel which contained gel red as a stain and ran at a 100 Volts steadied for 30 min utilizing 1X TAE buffer. A 100 base pair gene ladder was used to examine the size of the molecules of the bands that migrated. The product thus amplified were viewed under gel

documentation system and their photograph taken.

Digestion of the amplified fragment was done using *PstI* restriction enzyme to detect polymorphism. The restriction enzyme digestion was performed using 10µl of the PCR product mix with 1µl of *PstI* and incubated at 37°C for 20 min. Enzyme deactivation was at 80°C for 5 minutes. The digested products were subjected to gel electrophoresis of 2% which ran at 100 volts for 45 minutes, the fragments resulting from these were viewed with a gel documentation system. The gel picture was taken and analyzed using GelAnalyzer software (Peakall and Smouse, 2012).

Statistical analysis

The PROC GLM of SAS 9.2 software was used for the variance analysis of all collected data. The effect of *IGFI* gene polymorphism and egg quality traits was analyzed using this procedure. The ANOVA model is

$$Y_{ijk} = \mu + G_i + B_j + (GB)_{ij} + \Sigma_{ijk}$$

Where:

Y_{ijk} = Traits of interest (the egg quality traits)

μ = Overall mean

G_i = Fixed effects of *i*th genotype (*i*=1, 2, 3, 4, 5)

B_j = Fixed effects of the *IGFI* marker genotype (*j*=1, 2, 3)

$(GB)_{ij}$ = The interaction effects of genotype and *IGF* marker genotype

Σ_{ijk} = Randomised error.

GenAlEx 6.502 (Peakall and Smouse, 2012) was used to analyze the genetic diversity parameters. The allelic frequency distribution was compared using χ^2 test. Correlation coefficients among egg quality traits were determined using correlation procedure of SAS.

Results

The genetic impact on the exterior egg-quality characteristics of chickens is shown in Table 1. With the exception of shell weight, all external factors examined in the study were significantly influenced by genotype. Additionally, Kuroiler exceeded the rest of the breeds in terms of egg weight, length, and width (58.08 ± 0.79, 55.42 ± 0.31 and 43.29 ± 0.23). One of the economic elements that affects how well an egg retains is the thickness of the shell; compared to other eggs, Sasso eggs have the softest shells (0.37 ± 0.01). This is closely related to how easily the eggs hatch and lose moisture. For the naked neck FUNAAB Alpha, the value of the shell thickness was highest (0.43 ± 0.01).

Table 1: Genotype effect on the external egg-quality traits of five chicken genotype (Mean ± SEM)

Variables	Frizzle	Kuroiler	Normal	Naked neck	Sasso
Egg weight	54.36 ± 1.22 ^b	58.08 ± 0.79 ^a	52.55 ± 0.83 ^b	52.33 ± 0.81 ^b	54.92 ± 1.16 ^b
Egg Length	54.44 ± 0.45 ^{ab}	55.42 ± 0.31 ^a	54.21 ± 0.41 ^{ab}	53.55 ± 0.28 ^{ab}	46.33 ± 1.04 ^c
Egg width	42.84 ± 0.34 ^{ab}	43.29 ± 0.23 ^a	41.60 ± 0.23 ^b	41.70 ± 0.24 ^b	34.25 ± 0.93 ^c
Shape Index	78.78 ± 0.63 ^a	78.16 ± 0.46 ^{ab}	76.83 ± 0.52 ^b	77.88 ± 0.38 ^{ab}	73.80 ± 0.68 ^c
Shell weight	5.27 ± 0.10	5.45 ± 0.07	5.27 ± 0.10	5.17 ± 0.10	5.30 ± 0.15
Shell thick.	0.41 ± 0.01 ^a	0.40 ± 0.01 ^{ab}	0.42 ± 0.02 ^a	0.43 ± 0.01 ^a	0.37 ± 0.01 ^b
Shell ratio	9.80 ± 0.25 ^{ab}	9.30 ± 0.27 ^b	10.14 ± 0.22 ^a	9.94 ± 0.22 ^{ab}	9.44 ± 0.21 ^{ab}

^{a, b, c} the superscript shows a significant ($p > 0.05$) difference between the means within the rows.

Table 2 illustrates how the genotype affects the internal characteristics of the chicken. The Sasso chicken genotype received the highest ratings for the bulk of the internal characteristics assessed in this study. It also had the highest Haugh unit. The Haugh unit, which also predicts

how much protein will be present in relation to albumen height, is used to assess egg quality. On the other hand, genotype had no appreciable ($p < 0.05$) impact on each chicken's yolk and albumen ratio.

Table 2: Genotype effect on the internal egg-quality traits of three chicken genotype (Mean ± SEM)

Variables	Frizzle	Kuroiler	Normal	Naked neck	Sasso
Alb. Weight	35.06 ± 1.27 ^{ab}	37.35 ± 0.77 ^a	33.25 ± 0.64 ^b	32.70 ± 0.69 ^b	35.43 ± 0.87 ^{ab}
Alb. Height	6.64 ± 0.21 ^b	5.38 ± 0.22 ^c	6.64 ± 0.21 ^b	6.88 ± 0.27 ^{ab}	7.51 ± 0.25 ^a

Yolk Colour	8.31± 0.20 ^a	6.94 ± 0.21 ^b	8.30 ±0.19 ^a	6.91 ± 0.18 ^b	8.40 ± 0.18 ^a
Yolk weight	14.04 ± 0.31 ^b	15.82 ± 0.28 ^a	14.04 ± 0.31 ^b	14.46 ± 0.23 ^b	14.19 ±0.27 ^b
Haugh unit	82.39 ± 1.43 ^b	71.54 ± 1.75 ^c	83.05 ± 1.30 ^b	84.23 ± 1.66 ^{ab}	87.57 ± 1.32 ^a
Yolk ratio	26.20 ± 0.84	26.33 ± 1.10	26.77 ± 0.56	27.75 ± 0.49	25.94 ± 0.36
Alb. Ratio	63.96 ± 1.09	62.27 ± 0.97	63.19 ± 0.63	62.35 ± 0.52	64.41 ± 0.36

^{a, b, c} the superscript shows a significant (p>0.05) difference between the means within the rows.

The genotype and allele frequency of the IGFI polymorphisms found in the chicken genotypes are shown in Table 3. In this investigation, the A allele was more frequent. When genotypic

frequency was considered, it was found that the AC genotype was more common in this study population

Table 3: Frequency of IGFI gene genotypes and alleles in the three chicken genotypes

	Genotypic Frequency			Allele Frequency		χ^2	Probability
	AA	AC	CC	A	C		
Frizzle feather	0.233	0.499	0.267	0.483	0.517	60.00	0.000
Kuroiler	0.284	0.498	0.218	0.533	0.467	60.00	0.000
Normal feather	0.360	0.480	0.160	0.600	0.400	60.00	0.000
Naked neck	0.381	0.473	0.147	0.617	0.383	60.00	0.000
Sasso	0.321	0.491	0.187	0.567	0.433	60.00	0.000

Table 4 displays the impact of the IGFI gene polymorphism on the egg characteristics of the chicken population. With the exception of egg length and width, the IGFI gene polymorphism

had no effect (P>0.05) on the egg quality parameters. The AC genotype's eggs had the longest lengths and the widest widths.

Table 4: The Effect of IGFI gene polymorphism on egg traits of three chicken genotype (Mean ± SEM)

Variables	AA	AC	CC
Egg weight	53.33 ± 0.90	55.14 ± 0.59	53.55 ± 1.32
Egg Length	52.01 ± 0.71 ^{ab}	53.41 ± 0.48 ^a	51.59 ± 0.97 ^b
Egg width	40.09 ± 0.70 ^{ab}	41.23 ± 0.41 ^a	39.81 ± 1.06 ^b
Shape Index	76.94 ± 0.50	77.18 ± 0.34	77.00 ± 1.06
Shell weight	5.19 ± 0.10	5.36 ± 0.05	5.16 ± 0.17
Shell thick.	0.40 ± 0.01	0.41 ± 0.01	0.41 ± 0.02
Alb. Weight	33.89 ± 0.69	35.31 ± 0.55	33.96 ± 1.19
Alb. Height	6.73 ± 0.20	6.56 ± 0.17	6.61 ± 0.26
Yolk weight	14.25 ± 0.23	14.63 ± 0.18	14.43 ± 0.37
Haugh unit	83.16 ± 1.32	80.97 ± 1.14	82.52 ± 1.60
Shell ratio	9.75 ± 0.15	9.79 ± 0.11	9.68 ± 0.32
Yolk ratio	26.80 ± 0.34	26.38 ± 0.45	27.14 ± 0.82
Alb. Ratio	63.44 ± 0.39	63.83 ± 0.50	63.18 ± 0.98

^{a, b} the superscript shows a significant (p>0.05) difference between the means within the rows.

The phenotypic relationships between the characteristics that determine egg quality in the five chicken genotypes employed in this investigation are shown in Table 5. Egg weight was positively correlated with egg length (0.45-0.80), width (0.48-0.94), and shell weight (0.61-0.77), with the exception of the frizzle feathered FUNAAB Alpha chicken which had a non-significant value (-0.02) between egg weight and shell weight. The same genotype however,

exhibited a significant (p<0.001) inverse correlation coefficient between egg weight and shell ratio (-0.76), egg weight and yolk ratio (-0.68). All genotypes showed a negative connection (p<0.001) with yolk – albumen ratio (-0.6 -0.86) and most had positive relationships (p<0.05) with albumen weight: egg weight (0.69-0.97), egg length (0.4-0.76), egg width (0.43–0.92) and shell weight (0.43–0.72). Egg width did not significantly (p>0.05) affect the

yolk weight for the FUNAAB Alpha chicken strains with frizzle (-0.11), naked neck (0.33) and normal feather (0.34).

Except for the connection ($p < 0.001$) between shell thickness and shell ratio in the naked neck FUNAAB Alpha chicken (0.55), Sasso (0.8), and between shell thickness and yolk ratio ($p < 0.05$) for Sasso chicken (-0.38), there was no significant link between most of the reported features. For all genotypes, albumen height and Haugh unit had a strong and positive connection ($p < 0.001$), with the normal and Sasso showing the highest close-range value (0.98). Only the

Sasso chicken had no discernible impact ($p > 0.05$) on the yolk weight and albumen ratio (0.25), as well as the yolk weight and ratio (-0.15). For the shell-to-yolk ratio, only the frizzle chicken exhibited a significant ($p < 0.001$) value (0.76). While the shell to albumen ratio was positively ($p < 0.05$) correlated (0.86) and negatively correlated (-0.54) for the frizzle and naked feather FUNAAB chickens, respectively. With the kuroiler genotype having the highest value (-0.99), all genotypes in this investigation demonstrated a high ($p < 0.001$) inverse relationship between yolk and albumen ratio

Table 5a: The phenotypic correlations amongst egg quality traits in the three chicken genotypes

G	TR	EW	EL	ED	SW	ST	SI
FZ		1	0.64***	0.84***	-0.02	0.07	0.19
NK		1	0.79***	0.92***	0.65***	-0.06	-0.16
N	EW	1	0.80***	0.94***	0.61***	-0.1	0.24
SA		1	0.45**	0.48**	0.77***	0.25	0.26
K		1	0.71***	0.92***	0.77***	0.08	0.16
FZ			1	0.46**	0.17	0.42*	-0.51**
NK			1	0.51**	0.52**	-0.04	-0.72***
N	EL		1	0.61***	0.53**	-0.12	-0.36*
SA			1	0.94***	0.29	0.05	0.28
K			1	0.42**	0.53**	0	0.56**
FZ				1	-0.16	0.12	0.53**
NK				1	0.55**	-0.08	0.22
N	ED			1	0.49**	-0.11	0.51**
SA				1	0.35	0.12	0.59***
K				1	0.67***	0.14	0.51**
FZ					1	0.08	-0.33
NK					1	0.18	-0.15
N	SW				1	0.38*	0
SA					1	0.70***	0.28
K					1	0.19	0.09
FZ						1	-0.26
NK						1	-0.02
N	ST					1	0
SA						1	0.23
K						1	0.13
FZ							1
NK							1
N	SI						1
SA							1
K							1

* represents sig. diff ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

G- GENOTYPE, TR-TRAITS, EW- EGG WEIGHT, EL- EGG LENGTH, ED- EGG WIDTH, S - SHELL WEIGHT, ST- SHELL THICKNESS, SI- SHAPE INDEX,

Table 5b: The phenotypic correlations amongst egg quality traits in the three chicken genotypes

G	TR	AH	AW	YW	YC	HU	SR	YR	AR
FZ		0.06	0.97***	0.02	0.02	-0.23	-0.76***	-0.68***	0.74***
NK		0.31	0.90***	0.48**	0.27	0.13	-0.21	-0.24	0.26
N	EW	0.2	0.95***	0.41*	-0.23	0.02	-0.26	-0.52**	0.57***
SA		0.56***	0.97***	0.74***	0.31	0.39*	0	0.46**	0.46**
K		0.24	0.69***	0.69***	0.18	0.04	-0.39*	0.03	0.06
FZ		-0.19	0.58**	0.14	0.12	-0.37*	-0.36	-0.34	0.36***
NK		0.35	0.61***	0.60***	0.37*	0.22	-0.15	0.05	-0.01
N	EL	0.40*	0.76***	0.3	-0.14	0.28	-0.15	-0.45**	0.47**
SA		0.07	0.40*	0.48**	0.26	-0.05	-0.1	0	0.05
K		-0.08	0.48**	0.63**	-0.08	-0.24	-0.29	0	0.03
FZ		-0.08	0.84***	-0.11	0.09	-0.33	-0.72***	-0.66***	0.71***
NK		0.23	0.88***	0.33	0.19	0.05	-0.25	-0.38	0.37*
N	ED	0.12	0.92***	0.34	-0.3	-0.06	-0.34	-0.54**	0.61***
SA		0.14	0.43*	0.49**	0.27	0.3	-0.03	-0.04	0.06
K		0.34	0.66***	0.59**	0.33	0.18	-0.43*	-0.06	0.09
FZ		-0.03	-0.22	0.46**	0.2	0.01	0.66***	0.36	-0.46**
NK		-0.03	0.43**	0.46**	0.2	-0.14	0.60***	0.03	-0.17
N	SW	-0.04	0.53**	0.12	-0.15	-0.15	0.61***	-0.42*	0.22
SA		0.42*	0.72***	0.45**	0.06	0.27	0.64***	-0.52**	0.2
K		0.12	0.51**	0.58**	0.16	-0.02	0.29	0	0.01
FZ		-0.33	0.05	0.06	-0.03	0.03	0.05	0.04	-0.04
NK		0.09	-0.08	-0.04	-0.09	0.12	0.29	0.01	-0.08
N	ST	-0.3	-0.14	-0.08	-0.01	-0.29	0.55***	0.02	-0.18
SA		0.05	0.23	-0.01	-0.26	0	0.80***	-0.38*	-0.02
K		0.31	-0.04	0.12	0.02	0.32	0.16	0.11	-0.12
FZ		0.1	0.27	-0.24	-0.04	0.03	-0.35*	-0.33	0.35
NK		-0.22	0.02	-0.42	-0.26	-0.22	-0.03	-0.35*	0.33
N	SI	-0.29	0.26	0.07	-0.2	-0.37*	-0.24	-0.14	0.21
SA		0.21	0.24	0.21	0.14	0.17	0.14	-0.1	0.03
K		0.39*	0.14	-0.06	0.38*	0.39*	-0.12	-0.04	0.06

* represents sig. diff ($p < 0.05$), **($p < 0.01$), ***($p < 0.001$).

G- genotype, IGF-1 polymorphism, TR-traits, EW- egg weight, EL- egg length, ED- egg width shell weight, ST- shell thickness, SI- shape index, AH- albumen height, AW- albumen weight, YW- yolk weight, HU- haugh unit, SR- shell ratio, YR- yolk ratio, AR- albumen ratio, FZ - frizzle, NK- naked neck, N-normal, SA-sasso, K-kuroiler

Table 5c: The phenotypic correlations amongst egg quality traits in the three chicken genotypes

G	TR	AH	AW	YW	YC	HU	SR	YR	AR
FZ		1	0.04	0.08	0.16	0.95***	-0.06	0.02	0
NK		1	0.34	0.07	0.15	0.97***	-0.36*	-0.15	0.22
N	AH	1	0.18	0.17	-0.16	0.98***	-0.21	-0.02	0.08
SA		1	0.60***	0.24	0.08	0.98***	-0.03	-0.51**	0.52**
K		1	0.22	0.06	0.47**	0.97***	-0.18	-0.08	0.1
FZ			1	-0.26	-0.04	-0.24	-0.85***	-0.86***	0.90***
NK			1	0.06	0.23	0.17	-0.40*	-0.63**	0.66***
N	AW		1	0.13	-0.29	0.02	-0.31	-0.74***	0.79***
SA			1	0.61***	0.34	0.44**	-0.06	-0.61***	0.63***
K			1	0.41*	0.05	0.08	-0.31	-0.74***	0.76***
FZ				1	0.16	0.1	0.28	0.70***	-0.62**
NK				1	0.16	0.02	0.12	0.73***	-0.68***
N	YW			1	0.12	0.08	-0.24	0.56***	-0.46**
SA				1	0.21	0.09	-0.18	0.25	-0.15
K				1	0.18	-0.04	-0.21	0.61**	0.52**
FZ					1	0.12	0.12	0.1	0.11
NK					1	0.07	-0.02	-0.03	0.03
N	YC				1	-0.14	0.04	0.33	-0.33
SA					1	0.04	-0.3	-0.19	0.34
K					1	0.45**	-0.08	0.11	-0.1
FZ						1	0.18	0.24	-0.23
NK						1	-0.31	-0.08	0.15
N	HU					1	-0.17	0.06	-0.01
SA						1	-0.06	-0.48**	0.50**
K						1	-0.09	0.08	0.08
FZ							1	0.76***	0.86***
NK							1	0.03	-0.54**
N	SR						1	0.03	-0.32
SA							1	-0.25	-0.25
K							1	-0.02	-0.07
FZ								1	-0.98***
NK								1	-0.97***
N	YR							1	-0.95***
SA								1	-0.87***
K								1	-0.99***

* represents sig. diff ($p < 0.05$), **($p < 0.01$), ***($p < 0.001$).

G- genotype, IGF – IGF-1 polymorphism, TR–traits, AH– albumen height, AW– albumen weight, YW– yolk weight, HU– haugh unit, SR– shell ratio, YR– yolk ratio, AR– albumen ratio, FZ – frizzle, NK-naked neck, N-normal, SA-sasso, K-kuroiler

Table 6 display the phenotypic relationships between the egg weight and length for the IGF-1 polymorphism discovered in this study. The AA naked neck FUNAAB Alpha chicken had more influenced ($p < 0.05$) traits than other genotypes,

this was on EL (0.72), EW (0.95), SW (0.61), AW (0.93), YW (0.64). Meanwhile, the frizzle feather FUNAAB Alpha chicken had only one significant ($p < 0.05$) coefficient (0.97), which was between EW and AW. The AC

polymorphism had the highest influence on the traits observed in this study. With the Normal feather FUNAAB Alpha chicken having more correlated ($p < 0.05$) features, this between EW and the following: EL (0.82), ED (0.94), SW (0.64), AW (0.96), YC (-0.58), YR (-0.69), AR (0.72). Moreover, the Sasso chicken had the highest significant ($p < 0.001$) correlation between EW and AW (0.97). The naked neck FUNAAB Alpha chicken had no significant value ($p < 0.05$) in all the measured traits for the CC polymorphism. Whilst the Sasso chicken had the highest egg and albumen weight relationship (0.99), and a similar inverse relationship between egg weight and yolk ratio (-0.99).

The Sasso chicken had a high correlation coefficient ($p < 0.001$) for EL and EW in the AA IGF-1 polymorphism. Frizzled feathered FUNAAB Alpha chicken had no significant value for all the observed qualities in this study for the AC and CC IGF-1 polymorphism, it however, had one significant ($p < 0.05$) value between EL and YW (0.89). The kuroiler chicken had no significant ($p > 0.05$) trait for the AC IGF-1 polymorphism. Whilst the normal FUNAAB Alpha chicken had a higher number of influenced traits. Although, the Sasso chicken had a significant ($p < 0.001$) value for EL and ED (0.94). Only the naked neck FUNAAB Alpha and Sasso chicken had significant ($p < 0.05$) values in the CC IGF-1 polymorphism. EL and (0.99) SW, (0.95) SI, (0.97) YW, (0.98) YR and (-0.97) respectively for the former chicken, with (0.92) between EL and YW for the latter. Overall, the AC IGF-1 polymorphism had the highest influence on the measured traits.

Discussion

The quality of an egg affects how it is acceptable to the final consumer. It also predicts its price for both hatching and table eggs (Stadelman, 1977; Rajaravindra et al., 2015). This is influenced by several factors one of which is the genotype of the bird. These egg quality traits are determined by a large number of genes and can be improved by selective breeding (Tumova et al., 2009; Obikeet et al., 2014). The following factors are considered during the selecting process: egg weight, length, width, and thickness of its shell (Parmaret et al., 2006). The majority of

the parameters assessed in this study were significantly ($p < 0.05$) influenced by genotype, with the exception of shell weight, yolk ratio, and albumen ratio. Contrary to reports by Kgwatala et al. (2016) and Alkanet et al. (2010), which found a substantial difference in egg shell weight between several strains of Tswana chicken and lines of Japanese quails, respectively, this is the case with the egg weight of the eggs.

The size of the chicken is what gives the Kuroiler breed its supremacy in terms of egg weight, length, and width. The size of a chicken's egg and its weight are positively associated, which indicates that the larger the egg, the heavier the chicken. Due to their impact on embryonic development and chick hatchability, these have a stronger impact on egg quality and reproductive fitness in chickens (Islam et al., 2001). The data from this study are comparable to those from Serkalem et al. (2019) agro-ecological study of production attributes in domestic and foreign hens. Although the majority of the traits in this analysis showed slightly higher numbers.

ESI, the shape index of the egg is defined as the average of the egg width and length, it remains an indicator in terms of uniformity in the egg-size. A higher shape index augur uniformity of the eggs. This plays a part during the incubation period especially with the movement of the embryo for the utilization of nutrients during the direction of turning (Hristakieva et al., 2017). The Frizzled feather (FUNAAB Alpha) had the highest egg shape index, this establishes a report of better egg uniformity in the bird relating to healthy production and hatchability. Similar results were reported by Rajaravindra et al. (2015) in PB-2 chickens at different ages but higher than Hristakieva et al. (2017) for 34 - 46 weeks old turkey layers.

Egg albumen constitutes about 58.5% of the absolute egg weight, hence exhibiting a majority effect on its inner quality. In this study, the albumen height and weight displayed a varied significant ($P < 0.05$) effect across the chicken genotype, Sekeroglu and Altuntas (2009) reported that the overall weight of an egg appreciates alongside its albumen height.

Three Chicken Genotypes.

Table 6: The phenotypic correlations between the IGF-1 polymorphism and the egg quality traits in the

G	IGF	TR	EW	EL	ED	SW	ST	SI	AH	AW	YW	YC	HU	SR	YR	AR
F	AA		1	0.58	0.66	-0.39	-0.52	0.22	-0.46	0.97*	0.15	0.14	-0.56	-0.8	-0.79	0.8
NK			1	0.92***	0.93***	0.77**	-0.03	-0.42	0.12	0.97***	0.65*	0.25	-0.12	-0.23	-0.52	0.53
N			1	0.72**	0.95***	0.61*	0	0.19	-0.08	0.93***	0.64*	0.36	-0.24	0.01	-0.16	0.16
SA			1	0.27	0.24	0.80***	0.1	0.13	0.55	0.99***	0.77**	0.74*	0.34	-0.6	-0.51	0.58
K			1	0.81	0.82	0.79	-0.66	-0.13	-0.08	0.99**	0.96**	0.72	-0.32	-0.66	-0.53	0.65
F	AC	EW	1	0.67**	0.87***	-0.1	0.06	0.13	0.006	0.56***	0	-0.03	-0.23	-0.78***	-0.68***	0.74***
NK			1	0.66***	0.95***	0.54*	-0.05	0.21	0.25	0.92***	0.25	0.33	0.08	-0.31	-0.49	0.51*
N			1	0.82***	0.94***	0.64**	-0.05	0.2	0.46	0.96***	0.22	-0.58*	0.32	-0.33	-0.69**	0.72**
SA			1	0.53*	0.59**	0.81***	-0.4	0.31	0.49*	0.97***	0.85***	0.37	0.36	-0.33	-0.09	0.21
K			1	0.58	0.66	-0.39	-0.52	0.22	-0.46	0.97	0.15	0.14	-0.56	0.8	-0.79	0.8
F	CC		1	0.77	0.95**	-0.29	0.34	0.75	0.57	0.98**	-0.25	0.14	0.12	-0.84	0.9*	0.89*
NK			1	0.84	0.57	0.82	0.36	-0.62	0.79	0.01	0.91	0.61	0.73	0.47	0.83	-0.79
N			1	0.69	0.99**	0.39	0.11	0.8	-0.37	0.98*	0.03	-0.71	-0.51	-0.77	-0.77	0.89
SA			1	0.54	0.68	0.88*	0.82	0.57	0.91*	0.99***	0.89*	-0.64	0.8	0.58	-0.99***	0.82
K			1	0.85	-0.16	0.4	-0.1*	-0.51	-0.54	0.98	-0.99	0.99	-0.57	0.13	-0.1*	0.93
F	AA		1	0.75	0.23	0.16	-0.17	-0.72	0.42	0.8	0.88	-0.78	0.19	-0.3	0.9	
NK			1	0.74*	0.58	0.1	-0.73**	0.38	0.86***	0.74**	0.36	0.16	-0.4	-0.34	0.42	
N			1	0.5	0.23	-0.34	-0.54	0.41	0.87***	0.15	0.32	0.31	-0.27	-0.52	0.65*	
SA			1	0.98***	0.16	-0.31	0.75**	0.33	0.2	0.45	0.07	0.23	-0.11	0.17	-0.14	
K			1	0.37	0.65	-0.5	0.67	0.61	0.76	0.89*	0.6	0.27	-0.5	0.06	0.27	
F	AC	EL	1	0.45*	0.18	0.41	-0.56**	-0.13	0.58**	0.19	0.11	0.32	0.39	-0.3	0.34	
NK			1	0.42	0.21	-0.24	-0.59*	0.07	0.61**	0.19	0.42	-0.06	-0.37	-0.29	0.35	
N			1	0.64**	0.86***	0.18	0.36	0.56*	0.73**	0.31	-0.47	0.48	0.14	-0.49	0.42	
SA			1	0.94***	0.49*	0.1	0.13	-0.29	0.47	0.55*	0.41	-0.2	-0.08	0.1	-0.06	
K			1	0.75	0.23	0.18	-0.18	-0.72	0.42	0.8	0.88	-0.78	-0.19	-0.04	0.09	
F	CC		1	0.59	-0.33	0.35	0.2	0.19	0.82	-0.63	-0.01	-0.19	-0.69	-0.87	0.83	
NK			1	0.03	0.99**	0.62	0.95*	0.86	-0.5	0.97*	0.67	0.84	0.86	0.98*	-0.97*	
N			1	0.58	0	0.38	0.12	-0.76	0.54	0.66	-0.01	-0.8	-0.76	-0.13	0.31	
SA			1	0.92*	0.12	0.16	0.27	0.43	0.62	0.39	0	0.35	-0.29	0.47	0.81	
K			1	-0.67	0.83	-0.87	-0.89	-0.91	0.73	-0.9	0.9	-0.91	0.64	-0.88	0.6	

* represents sig. diff ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$). G- genotype, IGF – IGF-1 polymorphism, TR–traits, EW– egg weight, EL– egg length, ED– egg width, S – shell weight, ST– shell thickness, SI– shape index, AH– albumen height, AW– albumen weight, YW– yolk weight, HU– haugh unit, SR– shell ratio, YR– yolk ratio, AR– albumen ratio, FZ – frizzle, NK-naked neck, N-normal, SA-sasso, K-kuroiler

A high albumen height was observed in this study, which was in accordance with Olawumi and Ogunlade (2008) findings although, it is lower than Yakubuet *al.* (2008) reported. The Sasso breed had better Haugh unit due to the higher albumen height observed in this study, thus indicating better internal egg quality than the others. There was a high and positive ($p < 0.001$) correlation between the Haugh unit and albumen height, this is in line with the result of Rafea (2019) although there was an inverse correlation between haugh unit and egg weight in this study, no significant ($p > 0.05$) correlation was recorded. The albumen height and Haugh unit measures the viscosity of an egg's albumen. The values obtained in this study are higher than the standard (HU= 70) as reported by North (1978); Olawumni *et al.* (2020).

Selection based on genetic factors had been considered as a practical approach for improving animal's production in breeding program. There exists a direct relationship between IGF-1 gene polymorphism with the reproductive indices such that the reproductive trait of chicken increases with IGF-1 gene polymorphism. The effect of IGF-1 polymorphism on egg length and weight in this report is in accordance with previous records of Wu *et al.* (2014, 2016); Gabillard *et al.* (2003); Revolet *al.* (2005), who revealed that the IGF-1 axis applies a major play over growth and reproduction values in animals, indicating the presence of both axes during early development. This study suggests that IGF-1 exhibits similar modal activities in the growth hormone/insulin like growth factor axes, thus regulating reproductive traits in chicken. Similar findings were recorded by Shimizu *et al.* (2008), Wu *et al.* (2016) for different mammals and chicken populations. The absence effects of *IGFI* gene polymorphisms on some reproductive traits noticed in the study can be imputed to dissimilarities in gene structure and unequal linkage in the chicken's publication.

The result from the study observed that the IGF-1 polymorphism had no significant effect on its egg-quality traits except the egg length and width. This is not in accordance with the result of Lei *et al.* (2005) and Tang *et al.* (2010), in their findings they discovered that the SNP within the promoter region, is significantly associated with the following: body weight, egg production, shell weight and quality. This study

reports that the *PstI* digested PCR products of the IGF-1 gene reveals three (3) polymorphic fragments and this was in consistent with the findings of Esmailnejad and Nikbakht (2017), although it was not in line with the findings of Nagaraja *et al.* (2000). The genotypic and allelic frequency observed in this study showed the population to be in Hardy-Weinberg equilibrium. The different population genetic backgrounds and the breeding objectives might be the main cause of the differences observed among the chicken population.

Conclusion

In conclusion, all egg-quality parameters, with the exception of shell weight, yolk, and albumen ratio, were significantly influenced by chicken genotype. While the Sasso chicken is preferable in terms of protein quality due to its high albumen height, the Kuroiler chicken performed better in all observed attributes for this study compared to other birds. Except for egg length and width, the *IGFI* gene polymorphism did not significantly affect the egg features in this investigation. Egg length and width had high values in the AC chickens, coupled with many linked traits. However, the FUNAAB Alpha chicken with normal feathers outperformed the competition in terms of higher correlation values for the qualities that were examined. The IGF-1 gene may be suggested as a genetic marker for selection to increase the length and width of the eggs of the breeds employed in this study based on these findings.

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