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Effects of Season and Clutch, and Polymorphism of FGFBP 1 Gene on External Egg Quality Traits of Normal Feathered Chickens of Nigeria

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ABSTRACT

A study was conducted to determine the effects of season and clutch, and Polymorphism of FGFBP 1 Gene on egg quality traits of normal feathered local chickens of Nigeria. One hundred and thirty eight (138) eggs were collected from the hens of different ages in different production seasons and clutches and studied for: Egg number and quality traits such as egg weight (g), egg length (cm), egg width (cm), egg shape index (%), egg shell weight (g) and egg shell thickness (mm) for candidate gene analysis, blood samples were collected from 100 birds and taken to the laboratory where Genomic DNA was extracted from the blood using Promega, UK DNA extraction following manufacture's protocol. Sequencing was also done. Data were analyzed using analysis of variance (ANOVA). Variations due to clutch and season effects were significant (P<0.05) at all ages. Clutch affected egg quality traits; all the traits measured improved significantly (P<0.05) with production phase. Significant (P<0.05) seasonal variation was also observed for egg quality traits. External egg quality traits were better in wet season than the dry cold season, while dry hot season had lowest values. This study therefore concludes that, improvement of egg production and egg quality characteristics of indigenous chickens through selection can therefore be achieved by considering these environmental factors. Three genotypes identified with respect to all the external egg quality traits on FGFBP 1 gene polymorphism showed that individuals with heterozygous GA genotype generally, had the highest values followed by GG, while AA genotype had the lowest values. This shows that GA individuals are ideal for selection for external egg quality traits.

Keywords: Season, Clutch, FGFBP 1 Gene, Normal feathered, Egg Production, External Egg Quality Traits,

Introduction

Nigeria is endowed with vast number of heterogeneous chicken population estimated to be around 175 million of which indigenous chicken accounts for more than 60% (Fayeye, 2011). Indigenous chickens are widely distributed in rural areas of tropical and sub-tropical countries and majority are found with the rural dwellers, and are capable to thrive on little inputs in terms of feed, medication and shelter (Ajayi, 2010). They are categorized in to varieties such as the normal feathered, frizzled feather and necked neck, and studies have shown that normal feathered are the most prominent (Ajayi, 2010). Egg quality, according to,[2] is the most important price-contributory factor in both table and hatching eggs, as it portrays the characteristic of an egg that affects its acceptability to the consumers. Economic success of a laying flock depends solely on the number of quality eggs produced. External egg quality traits include size and shell qualities, while the internal egg quality traits include yolk and albumen qualities. In the egg producing industries. (Altan *et al.*, 1998) pointed out that egg shell weight, albumen and the yolk that form the egg as well as their ratio affect the amount and price of the product. Egg weight is an important egg trait, which influences egg quality as well as grading (Faruuq *et al.*, 2001). The candidate gene approach is an interesting way to study QTL affecting production traits in chickens. These genes and their associated carrier proteins and receptors that are in turn regulated by alleles are highly polymorphic as could be determined by markers such as Single Nucleotide Polymorphism (SNP). The use of single nucleotide polymorphism marker to study the association of FGFBP 1 gene with phenotypic traits could serve as a selection and improvement tool in chicken production (Clevenger *et al.*, 2001). This study therefore, aims at investigating some factors affecting egg production and egg quality traits and the association of FGFBP 1gene with these traits in normal feathered chickens of Nigeria.

Materials and methods

Experimental Site:

The study was carried out at the Poultry Production Unit (PPU) in Potiskum local Government Area, Yobe State. Potiskum is located on an altitude of 475m above sea level and is situated between latitude 11. 42 and 32.36^{0}N and longitudes 11.40 and 11.89^{0}E. The area falls within the Northern Sudan Savannah having an average rainfall of 713 mm/annum.

Experimental animals and data collection:

A total of 184 eggs were collected from hens of different ages. The collection was done for 3 clutches in different seasons (dry cold, dry hot and wet seasons). The birds were managed intensively under deep litter system and fed ration containing 18 %CP and 2650 Kcal ME/Kg and water *ad libitum.* Constant and regular sanitation of the pen including the feeders were carried out.

Blood sample collection

At 24 weeks of age, two and a half millilitres (2.5mls) of blood was collected from 100 birds (62 males and 38 females) through their wing veins. A portion of the vein was blocked to prevent flow back of blood until the required quantity of blood was drawn into labeled sample bottles containing drops of anticoagulant (EDTA). Blood contamination was prevented by using separate syringes and needles for individual birds. Samples were taken to the laboratory in ice boxes for analyses.

DNA extraction

Genomic DNA was extracted from the blood using Promega, UK DNA extraction following manufacture's protocol.

Nucleic acid quality and quantity

Concentration of nucleic acid was determined spectrophotometrically using nanodrop sphectrophotometer at absorbance of A260/A230 ratio as described by Sambrook *et al.* (2012). 1.5 µ l of the sample was placed on the sphectrophotometer for the measurement.

Polymerase Chain Reaction (PCR)

Primers were designed to amplify exon 2 of chicken sequences for *FGFBP1* gene deposited in Gene Bank using Primer3 computer program (http://frodo.wi.mit.edu/). The quality of the primers was analyzed by the Net Primer program. Gene fragments were amplified by PCR in a final volume of 25 µL consisting of 20 ng genomic DNA, 2.5 µL 10X buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.5), 0.3 mM MgCl2, 0.4 mM dNTP, 2 pmoL of each primer, and 1 U Platinum *Taq* DNA polymerase (Life Technologies). Fragment amplification was performed usinf real-time PCR technique as follows: initial denaturation at 95°C for 1 min, 30 cycles of 95°C for 1 min, the specific annealing temperature for each primer pair for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. The size of the amplified fragments were confirmed using a molecular weight marker ΦX174 and estimated on a 1% agarose gel.

DNA Sequencing

Polymerase Chain Reaction products were purified and sequencing at exon 2 of *FGFBP* I gene was performed according to the Big Dye Terminator Cycle Sequencing Ready Reaction protocol (Gordon *et al.,* 1998). Sequencing reactions were purified and applied in the automated sequencer, ABI PRISM 3100 Genetic Analyzer using Sanger method (Sanger, 1977). Single nucleotide polymorphisms (SNP) markers were used and nucleotide sequences were edited, assembled, and analyzed using the Phred, Phrap, and Consed programs, respectively.

Data Analysis

Data collected were on external egg quality traits included egg weight (EW), egg length (EL), egg width (EG), shell weight (SW) and shell thickness (STH). The egg weight (EW) was measured with an electronic balance, Scout™ pro-scale with 0.001g to 1000g sensitivity. Egg length and width were measured with a pair of Vernier caliper (mm). For shell weight and thickness, after the removal of the internal components (yolk and albumen), the egg shell was washed and sundried for 24 hours. Then, the shell was weighed using and electronic balance, scout™ pro-scale with 0.001g to 1000 g sensitivity.

Data measured on internal egg traits included albumen length (AL), albumen height (AH), albumen width (AWD), yolk height (YH), and yolk width (YD).Yolk and albumen indices were calculated as the ratio of their lengths to the widths, while haugh unit was estimated us the formular : 100 log $(H+7.57-1.7\ W^{37})$(Haugh, 1937).

Data analysis

Data collected were subjected to analysis of variance using the following model:

$Y_{ijk} = \mu + C_i + H_k + e_{ijk}$

Where;

Y_{ijkl} = L^{th} individual observation,

µ= overall mean in the population,

C_j = effect of J^{th} season (1, 2, 3),

H_k is the effect of kth clutch (1^{st}, 2^{nd} 3^{rd})

e_{ijkl} = residual random error

Results and Discussion

External Egg Quality Traits of Nigerian Indigenous Normal Feathered Chickens

Tables 1 shows the external egg quality traits of indigenous normal feathered chickens according to the age groups (22-24, 25-27, 28-30, 31-33 and 34-36 weeks of age). Results of the analysis showed egg weight and other external egg quality traits increased with age. Mean values obtained for egg number ranged from 7.15 - 9.52 per clutch. The annual egg production is a function of the number of clutches and size of the clutches. Dafwang (1990) reported 2-3 clutches per year and each clutch consists of an average of 8.75 eggs. Atteh (1990) reported 1.84 and 11 eggs as the average number of clutches and eggs per clutch, respectively for chickens sampled in the Western Middle Belt of Nigeria. In the Northern part of Nigeria, Hassan *et al*. (2000) and Otchere *et al.* (1990) reported clutch size of 10.4 eggs and 12.0 eggs, respectively which are slightly higher than the values obtained for the present study. Eshiett *et al.* (1989) reported 9.0 eggs per as clutch for the Owerri flock in South Eastern Nigeria. Sonaiya and Olori (1990), however, reported 4 - 14 eggs as clutch size for chickens sampled in South Western Nigeria.

Table 1: Least squares means of External Egg Quality Traits of Nigerian Indigenous Normal Feathered Chickens at Different Ages (weeks) as affected by Clutch Number and Season

Variables	Age (weeks)									
	22-24	N	25-27	N	28-30	N	31-33	N	34 -39	N
OM±SE	**29.33±0.34**	**(82)**	**32.27 ±0.50**	**(79)**	**33.98±1.06**	**(70)**	**37.88±0.77**	**(70)**	**39.63±0.91**	**(58)**
Clutch	**		***		***		**		***	
1^{st}	28.71±0.23^{b}	(34)	29.68±0.45^{c}	(32)	31.10±0.90^{c}	(28)	36.28±0.86^{b}	(28)	37.65±1.02^{c}	(22)
2^{nd}	29.53±0.31^{a}	(28)	32.70± 0.55^{b}	(27)	34.65±1.00^{b}	(25)	37.56±0.77^{b}	(25)	39.25±0.78^{b}	(20)
3^{rd}	29.74±0.42^{a}	(20)	34.40±0.45^{a}	(20)	36.20±1.02^{a}	(17)	39.78±0.79^{a}	(17)	42.00±0.90^{a}	(16)
Season	**		***		***		**		***	
Wet	30.61±0.39^{a}	(33)	32.86±0.56^{a}	(30)	35.95±0.25^{a}	(26)	40.52±0.71^{a}	(26)	42.08±0.90^{a}	(24)
Dry Cold	29.01±0.28^{a}	(25)	32.30±0.58^{a}	(25)	33.49±1.08^{b}	(26)	36.44±0.77^{b}	(26)	38.03±0.91^{b}	(18)
OM ± SE	4.52±0.06	(82)	4.63±0.06	(79)	4.74±0.07	(75)	5.03±0.08	(70)	5.30±0.07	(58)
Clutch	***		***		***		***		***	
1^{st}	4.20±0.06^{c}	(34)	4.40±0.09^{c}	(32)	4.51±0.06^{c}	(30)	4.80±0.09^{c}	(28)	5.06±0.08^{c}	(22)
2^{nd}	4.59±0.08^{b}	(28)	4.60±0.05^{b}	(27)	4.75±0.06^{b}	(26)	5.03±0.04^{b}	(25)	5.27±0.06^{b}	(20)
3^{rd}	4.76±0.08^{a}	(20)	4.81±0.08^{a}	(20)	4.97±0.08^{a}	(19)	5.23±0.06^{a}	(17)	5.44±0.06^{a}	(16)
Season	**		**		**		**		**	
Wet	4.82±0.05^{a}	(33)	4.86±0.05^{a}	(30)	4.91±0.05^{a}	(32)	5.17±0.08^{a}	(26)	5.44±0.06^{a}	(24)
Dry Cold	4.56±0.06^{b}	(25)	4.65±0.05^{b}	(25)	4.80±0.04^{b}	(22)	5.05±0.05^{b}	(26)	5.07±0.08^{b}	(18)
OM± SE	3.64±0.03	(82)	3.68±0.04	(79)	3.79±0.04	(75)	3.98±0.05	(70)	4.01±0.06	(58)
Clutch	Ns		**		**		**		***	
1^{st}	3.55±0.02	(34)	3.49±0.04^{b}	(32)	3.60±0.05^{b}	(30)	3.75±0.05^{b}	(28)	3.81±0.03^{c}	(22)
2^{nd}	3.68±0.03	(28)	3.60±0.05^{b}	(27)	3.70±0.04^{b}	(26)	3.80±0.04^{b}	(25)	4.00±0.07^{b}	(20)
3^{rd}	3.74±0.03	(20)	3.81±0.04^{a}	(20)	4.13±0.03^{a}	(19)	4.40±0.04^{a}	(17)	4.22±0.04^{a}	(16)
Season	**		**		**		***		***	
Wet	3.65±0.01^{a}	(33)	3.70±0.03^{a}	(30)	3.76±0.05^{a}	(32)	3.97±0.04^{a}	(26)	3.98±0.04^{a}	(24)
Dry cold	3.61±0.04^{a}	(25)	3.68±0.04^{a}	(25)	3.70±0.04^{a}	(22)	3.81±0.05^{b}	(26)	3.85±0.07^{a}	(18)
OM ± SE	78.35±0.78	(82)	80.15±1.40	(79)	83.36±1.33	(75)	77.18±0.98	(70)	73.52±0.94	(58)

Clutch	**		**		**		*		*	
1st	80.52±0.68 a	(34)	85.71±1.68 a	(32)	93.76±1.52 a	(30)	77.73±0.97 a	(28)	74.17±0.92 a	(22)
2nd	76.44±0.71 b	(28)	79.34±1.17 b	(27)	78.23±0.98 b	(26)	77.96±1.00 a	(25)	74.63±0.97 a	(20)
3rd	78.65±0.88 b	(20)	75.34±0.31 b	(20)	78.09±6.05 b	(19)	75.82±0.98 b	(17)	71.72±0.74 b	(16)
Season	**		Ns		**		**		Ns	
Wet	77.35±0.76 b	(33)	75.23±0.90	(30)	79.17±1.07 a	(32)	78.82±0.89 a	(26)	73.64±0.97	(24)
Dry Cold	77.25±0.78 b	(25)	75.52±1.09	(25)	78.21±1.57 a	(22)	75.60±0.91 b	(26)	73.88±0.94	(18)
OM ± SE	3.22±0.04	(82)	3.40±0.04	(79)	3.54±0.07	(75)	3.51±0.08	(70)	3.75±0.16	(58)
Clutch	Ns		***		***		***		***	
1st	3.20±0.03	(34)	3.22±0.04 b	(32)	3.36±0.05 b	(30)	3.41±0.07 b	(28)	3.48±0.15 b	(22)
2nd	3.22±0.02	(28)	3.36±0.05 b	(27)	3.54±0.05 b	(26)	3.48±0.08 b	(25)	3.72±0.16 ab	(20)
3rd	3.25±0.03	(20)	3.76±0.02 a	(20)	3.68±0.08 a	(19)	3.62±0.08 a	(17)	3.91±0.15 a	(16)
Season	**		**		**		**		**	
Wet	3.18±0.04 a	(33)	3.32±0.04 a	(30)	3.66±0.06 a	(32)	4.01±0.07 a	(26)	4.20±0.17 a	(24)
Dry Cold	3.05±0.03 b	(25)	3.25±0.04 a	(25)	3.44±0.07 b	(22)	3.47±0.08 b	(26)	3.92±0.15 b	(18)
OM± SE	0.24±0.02	(82)	0.26±0.03	(79)	0.27±0.01	(75)	0.31±0.02	(70)	0.33±0.02	(58)
Clutch	Ns		***		***		Ns		Ns	
1st	0.23±0.02	(34)	0.23±0.02 b	(32)	0.24±0.01 b	(30)	0.28±0.02	(28)	0.32±0.03	(22)
2nd	0.25±0.09	(28)	0.27±0.02 a	(27)	0.26±0.01 a	(26)	0.31±0.02	(25)	0.31±0.02	(20)
3rd	0.25±0.02	(20)	0.28±0.03 a	(20)	0.28±0.01 a	(19)	0.33±0.01	(17)	0.32±0.03	(16)
Season	Ns		Ns		***		***		***	
Wet	0.26±0.01	(33)	0.28±0.02	(30)	0.28±0.01 a	(32)	0.32±0.02 a	(26)	0.38±0.02 a	(24)
Dry Cold	0.25±0.01	(25)	0.27±0.02	(25)	0.28±0.01 a	(22)	0.30±0.01 a	(26)	0.31±0.02 b	(18)
Dry Hot	0.23±0.02	(24)	0.24±0.02	(24)	0.25±0.01 b	(21)	0.27±0.02 b	(18)	0.29±0.02 b	(16)

a, b, c= means within a subset having different superscripts are significantly different (***p<0.001,** p<0.01,*p<0.05); OM= overall mean, SE=standard error, N: number of observations.

T

Effect of Clutch Number on External Egg Quality Traits of Nigerian Indigenous Normal Feathered Chickens

There were significant differences (P < 0.05) among the clutches for egg number, egg weight, egg length, egg width, shell weight and shell thickness, but no significant differences (P > 0.05) existed for egg shell thickness among clutches (production cycle) across ages. This shows that external egg quality traits improved with increasing laying cycle.

Findings on the external egg quality traits with respect to production cycles (PC) affirmed the report of Silversides *et al.* (2006) and Tomova and Gous (2012) that the age of the hen and laying phase affected egg quality. Egg weight was least during the first production cycle (PC1) but showed improvement by PC3. Haunshi *et al.* (2011) also observed that egg weight increased as the production phase of the hen increased. Generally, this indicates that eggs from Nigerian normal feathered chickens can be classified as small within the PC1 and subsequently as medium size and large when production cycle increases as El-Safty *et al.* (2006) reported the egg weights of 49.7 and 50.6 g in naked neck and normal plumage hens respectively during the mid-laying period and egg weights of 53.0 and 55.3 g in the same ecotypes later on. Egg length and width also increased consistently with increase in production phase and this confirms the observations made by John-Jaja *et al.* (2016) in Nera black chickens raised in Nigeria. Though, egg shape index reduced as production cycle increased, no variation existed between the first two cycles (PC1 and PC2). Johnston and Gous (2017) suggested that variation in the geometry of the egg with increase in laying cycle of the hen could be linked with the changes in the weight/size of oviduct.

Egg shell quality improved significantly (P < 0.05) as laying phase increased. Shell thickness is important in egg grading since cracked or broken shells are of economic concern because they are often unacceptable by consumers and the hatchery industry resulting in huge monetary losses. Some authors inferred that eggs with shell thickness of less than 0.33 mm are subject to easy entrance of microbes and also breakage. This study indicates that by PC3, the eggs had achieved shell thickness of 0.32 mm. This implies that more loss will be encountered when eggs from early production phases are set for hatching or handled roughly due to poor shell quality.

Effect of Seasons on External Egg Quality Traits of Nigerian Indigenous Normal Feathered Chickens

Where significant (P < 0.05) differences were observed, the external egg quality characteristics were highest in wet season, followed by dry cold and then lowest for dry hot season. The dry cold and wet seasons were in some cases similar in egg quality traits. Fred (1994) found an increase in egg weight from December to February (dry cold season). From March to May (dry hot season), there was gradual decrease; and from July to September (wet season), the egg weight was higher, which increased again in October. Parmar *et al.* (2006) reported that there was a high tendency for domestic chickens to lay larger eggs during natural breeding season (wet season) if they were hatched during the previous dry cold months. Warren (2003) noted a sharp decline (35 %) in egg number during the dry hot season when the temperature was above 30^0 C. Momoh (2005) reported a seasonal trend in egg shell thickness. The percentage of shell in relation to whole egg decreased from February through May, with an upward trend from July to the end of wet season. Similarly, in accordance with the findings of this work, Johnston and Gous (2017) observed seasonal trend in shell weight. Shell weight declined in drier months (0.25 mm), but were higher in the wet season (0.30 mm).

Conrad (2000) found that when the temperature was raised from 28^0 C to above 32^0 C, the blood calcium level decreased by 25 to 30 %. It was concluded therefore, that the decreased blood Ca was the chief cause of thinner egg shells during the dry hot season. Wang *et al.* (2011) found an association between thin shells and high ambient temperatures and showed that egg shells became thicker following a force moult. This improvement was due to the rest provided by the moult. It was concluded that thinning of egg shells could also be caused by metabolic changes in the birds itself. It has been observed that eggs with good external qualities are obtained during the wet season. Thick egg shell is a desirable characteristics during this season because the egg can withstand externally applied forces during egg handling, thus preventing breakage of egg and this is an economic indicator for commercial poultry producer and consumers.

Relationship between FGFBPI Gene and External Egg Quality Traits in Nigerian Normal Feathered Indigenous chickens

Relationship between FGFBPI Gene and External Egg Quality Traits in Nigerian Normal Feathered Indigenous chickens is presented in Table 2. There were significant (P < 0.05) differences among the three genotypes (GG, GA and AA) with respect to all the external egg quality traits. Individuals with heterozygous GA genotype generally, had the highest followed by GG, while AA genotype had the lowest values. In contrast with the findings of the present study, Mehdi and Reza (2012) in their study on FGFBP 1 gene using RLFPs, identified three genotypes (AG, AA and GG) and found that GG genotype had higher egg production, egg weight and egg length, compared to AA and AG genotypes. Similarly, Khao *et al*. (2013) in their studies on FGFBP 2 in Mia chicken breeds of Vietnam using SNP analysis observed that individuals with GG genotype had higher egg weight, egg length and egg shell qualities compared to AG genotype and AA genotype. Fan *et al.* (2013) reported that the Sodium Channel (SCNN1) gene family inclusive of FGFs and IGFs were significantly associated with egg shell traits in chickens, especially egg shell strength and egg shell thickness.

Table 2: Relationship between FGFBPI Gene and External Egg Quality Traits in Nigeria Normal Feathered Indigenous chickens

	Traits						
Genotypes	**EN**	**EW**	**EL**	**EWD**	**SI**	**SW**	**STH**
GG	9.40 ± 0.26^b	39.30 ± 0.91^b	5.21 ± 0.07^b	3.88 ± 0.06^a	76.10 ± 0.58^a	3.71 ± 0.08^a	0.33 ± 0.01^a
GA	10.00 ± 0.45^a	41.03 ± 0.91^a	5.51 ± 0.06^a	3.90 ± 0.04^a	71.72 ± 0.41^c	3.80 ± 0.20^a	0.32 ± 0.03^a
AA	7.84 ± 0.21^c	34.30 ± 0.95^c	4.71 ± 0.09^c	3.70 ± 0.04^b	78.54 ± 0.98^a	3.53 ± 0.07^b	0.28 ± 0.01^b

a,b,c: means within columns with different superscripts are significantly (P<0.05) different, EN: Egg Number, EW: Egg Weight, EL: Egg Length, SI: Shape Index, SW: Shell Weight, STH: Shell thickness. EWD: egg width

Conclusion

This study therefore concludes that External egg quality traits of Nigerian normal feathered local chickens were significantly affected by season and clutch. The traits improved with clutch across ages. However superior characteristics of egg were obtained in the wet season. Three genotypes identified with respect to all the external egg quality traits on FGFBP 1 gene polymorphism showed that individuals with heterozygous GA genotype generally, had the highest values followed by GG, while AA genotype had the lowest values. This shows that GA individuals are ideal for selection for external egg quality traits.

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