



Genetic Parameters of Growth Traits of Indigenous Guinea Fowls (*Numida meleagris galeatea*) from Northern Ghana

A. A. Agbolosu^{1*}

¹Department of Animal Science, Faculty of Agriculture, University for Development Studies, P.O.Box TL 1882, Tamale, Ghana.

Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

This study was carried out to estimate some genetic parameters of growth traits of indigenous guinea fowls in northern Ghana. Forty-six-week-old guinea fowls were selected at random from a randomly mating breeding population consisting of birds from Northern, Upper East, Upper West and Volta regions in 2010 and raised at the Livestock and Poultry Research Center of the University of Ghana until 2015. Parameters taken were hatch weight (BW0), weight at 2 weeks (BW2), BW4, BW6, BW 8, BW10, BW12, BW16, BW20, BW24, BW28, BW32, BW36, brooding daily gain (BDG0-4), post brooding daily gain from 4 weeks to 8 weeks (PBDG4-8), PBDG8-12, PBDG12-20, PBDG20-24 and PBDG8-20. Estimation of the heritability of body weight was carried out using the Mixed Model Methodology. The sire model and known genetic relationship in single trait analysis was carried out using the ASREML 3 statistical program. Estimation of heritabilities for the growth rates was done using Becker (1984) procedure. Estimates of genetic and phenotypic correlations were obtained using appropriate expressions involving the estimated variance components according to Becker (1984). Generally, the heritability (h^2) estimates of body weight for all guinea fowls in this study were low (0.06 for BW36) to moderate (0.51 for BW32). Estimates of heritability for growth rates were medium (0.39 for PBDG4-8) to high (0.78 for PBDG8-12) except PBDG8-20 which had a low heritability value (0.22). With respect to phenotypic correlations, the

*Corresponding author: E-mail: aagbolosu@uds.edu.gh;

least coefficient of -0.007 was between BW0 and BW10 whereas the highest of 0.979 was between BW24 and BW28. The heritability estimates of body weight and growth rates were low to high i.e., 0.08-0.70 and 0.22-0.78, respectively. Body weight at two weeks of age served as an indicator for the early selection of guinea fowls based on body weight. It is recommended that the results obtained could be included in the breeding objectives of any upcoming guinea fowl improvement program.

Keywords: Guinea fowl; heritability; growth traits; correlation; variance.

1. INTRODUCTION

The indigenous guinea fowl are very important Animal Genetic Resources predominantly found in the northern parts of Ghana. They are hardy, nervous, wild, and act as good watchdogs and provide meat that has a gamey taste [1]. Unlike other livestock species whose ownership is limited to particular gender and social groups, guinea fowls can be owned by individuals of all ages and gender [2]. As such, they represent a great asset for poverty reduction [3]. Apart from their importance as a source of income and protein for families, they also play other important social roles in certain ceremonial and festive rites [4]. Guinea fowls have the ability to scavenge for insects and grains, protect themselves against predators and are tolerant to common parasites and diseases. These make them outstanding species for rural small-scale poultry farming [5]. Generally, there are little or no cultural barriers to the consumption of guinea fowl products.

In Ghana, the livestock policy of the Animal Production Directorate (APD) of the Ministry of Food and Agriculture (MOFA) seeks to establish breed improvement schemes to enhance the performance of indigenous livestock and poultry species which includes guinea fowls [6].

There is no known genetic selection improvement program for guinea fowls in Ghana [7]. There are no established breeding stations undertaking genetic improvement programs in the industry and almost every farmer is a breeder, selling breeding stock (live birds) or eggs. Farmers wanting new genetic material buy their eggs or chicks from other farmers or purchase the breeding stock from the market. These exchanges are inappropriate since inbreeding might become a problem in the future. There is therefore the need to design some appropriate guinea fowl breed improvement program in Ghana. However, before any breeding objectives can be defined for the guinea fowl, its phenotypic and genetic parameters need to be established, and this is the main objective

of this research work. The main objective is to characterize indigenous guinea fowls genetically through estimation of genetic parameters.

2. MATERIALS AND METHODS

2.1 Study Area

The quantitative genetic characterization studies were carried out at the Livestock and Poultry Research Centre (LIPREC) of the University of Ghana between September 2012 to June 2015. It is located on Latitude $05^{\circ} 40'N$ and Longitude $00^{\circ} 16'W$ in the Accra Plains which forms part of the Coastal Savannah. The area had an average annual rainfall of about 800 mm. The long rainy season occurs between March and July with a peak in June and the short rainy season occurs between August and November with a peak in October. Mean monthly temperature was about $26.1^{\circ}C$. The area is gently rolling with low elevation and covered by natural grassland of medium tussock growth with scattered fire-resistant trees and shrubs [8].

2.2 Experimental Birds

Forty-six (46) week old guinea fowls were selected at random from a randomly mating breeding populations obtained from eggs from local guinea fowls sampled and purchased from the Northern, Upper East, Upper West and Volta regions in 2010 and raised at LIPREC. The eggs were hatched using an APPE incubator (A. P. Poultry Equipment, India) at LIPREC. Pedigree records were available on the birds from which selection was done. Selected birds were weighed and grouped by regions; into a total of 25 families consisting of 125 local guinea fowls (25 males and 100 females) in a sex ratio of 1 cock to 4 hens per pen. Commercial (Exotic) day old guinea keets purchased from Belgium were also raised at LIPREC and later grouped into families at maturity as was done for the local guinea fowls. All the birds in the families were identified with wing tags purchased from Japan. The sexes of the birds were determined using a combination of vent sexing as described by Teye *et al.* [9].

2.3 Management of Birds

The birds were confined and reared in pens of size 21m x 15m x 15m in a deep litter housing structure. The top and sides of the cages were covered with wire mesh to prevent birds from flying out. Medication, routine vaccination and other management practices were carried out. Water was offered *ad libitum*. Sick birds were isolated and treated. Dead birds were sent for postmortem examination at the clinic at LIPREC to ascertain the cause of death. All keets were fed starter ration containing 16.3% crude protein (CP) for 6 to 8 weeks, grower ration containing 13% CP from 6/8 to 20/24 weeks and layer ration containing 15.6% CP after 20/24 weeks. Diets were formulated at LIPREC feed mill using specified mixtures. Feeding and other management practices were the same for all groups and routine vaccination and medication schedule were carried out.

2.4 Egg Management (Collection, Incubation and Hatching)

The collection of fertile eggs began after three weeks in order to purge any residual semen being carried by the hens. Eggs were collected from the various families daily, labeled and weighed (using an OHAUS Explorer electronic scale, UK scale with precision up to 0.001g) before incubation. Eggs were selected for physical quality and fumigated with 17% potassium permanganate in 20% formalin and incubated. Table-top and walk-in incubators were used to hatch eggs. The hatchery was cleaned and disinfected with 1% formalin spray before the setting of the eggs. Two hours before transfer of eggs from the setter to the hatcher and before each candling, 1% formalin was sprayed in the hatchery room to disinfect and avoid infection of the ova with pathogens while in the hatchery. Egg candling was done at 10 and 20 days after incubation. After hatching, the keets were reared separately on deep litter in the brooder and grower houses.

2.5 Data Collection and Analysis

2.5.1 Measurement of traits

Measurements were taken on body weight and growth rate as defined below:

i) Body Weight

Body weights were taken by using a KERN electronic digital scale (UK) (with precision 10g).

Body weight and growth traits were defined and/or measured as follows:

Day old weight (DOC): Weight of newly hatched keet taken within 24 hours after hatching.

Weekly and monthly weights: Weight taken weekly up to 12 weeks and monthly from 16 weeks to 36 weeks, respectively e.g., BW12 = body weight at 12 weeks; BW1 = body weight at 1 week.

ii) Growth Rate

- Brooding daily gain (*BDG0-4*): Weight gain (g) from day old to 4 weeks (1 month) of age divided by the number of days from day old to 4 weeks.
- Post brooding daily gain from 4 to 8 weeks (*PBDG4-8*): Weight gain (g) from 4 weeks to 8 weeks divided by number of days from one month to 8 weeks.
- Post brooding daily gain from 8 to 12 weeks (*PBDG8-12*): Weight gain (g) from 8 weeks to 12 weeks divided by number of days from 8 weeks to 12 weeks.
- Post brooding daily gain from 12 to 20 weeks (*PBDG12-20*): Weight gain (g) from 12 weeks to 20 weeks divided by number of days from 12 weeks to 20 weeks.
- Post brooding daily gain from 20 to 24 weeks (*PBDG20-24*): Weight gain (g) from 20 weeks to 24 weeks divided by number of days from 20 weeks to 24 weeks.
- Post brooding daily gain from 8 to 20 weeks (*PBDG8-20*): Weight gain (g) from 8 weeks to 20 weeks divided by number of days from 8 weeks to 20 weeks.

2.6 Heritability estimates of body weight traits

Estimation of the heritability of body weight was carried out on the guinea fowl records using the mixed model methodology. The sire model and known genetic relationship in single trait analysis was carried using the ASREML 3 statistical program [10]. The model used is given below:

$$y_{ijklmn} = \mu + \text{Sex}_i + \text{Region}_j + \text{Season}_k + \text{Year}_l + \text{sire}_m + \text{animal}_n + e_{ijklmn}$$

Where y_{ijklmn} is the phenotypic record of animal n, at a given age; μ is the overall mean; Sex_i is the fixed effect of the sex of animal n; Region_j is the fixed effect of the region of Ghana animal n was sampled from; Season_k and Year_l are the fixed

effects accounting for the season and year in which the phenotypic record was taken; $sire_m$ is the random additive genetic effect of the sire of animal n ; $animal_n$ is the random effect of animal n and e_{ijklmn} is the random residual error term.

The variance structure of the model was defined as $Var(sire) = A\sigma_s^2$, where A is the matrix of additive genetic relationships between individuals and σ_s^2 is a quarter of the additive genetic variance; $Var(animal) = I\sigma_{animal}^2$ and $Var(e) = I\sigma_e^2$, where I is the identity matrix, σ_{animal}^2 is the animal variance, and σ_e^2 is the residual variance.

2.7 Heritability Estimates of Growth Rates

For growth rates, heritability was estimated using Becker [11] procedure. The heritability values for the various traits were calculated using the paternal half-sib correlation. The statistical package used was the one-way analysis of variance of Genstat Discovery Edition [12]. The one-way analysis of variance (sire) model used was as follows:

$$Y_{ik} = \mu + S_i + e_{ik}$$

Where Y_{ik} is the observation on animal i_k , sired by sire i ; μ is the common mean, S_i is the effect of the i th sire; e_{ik} is the uncontrolled environmental and genetic deviations attributable to individuals within sire groups; the random error term assumed normally and independently distributed (NID) $(0, \sigma_e^2)$.

The analysis of variance table yields two sources of variance i.e. between sires and between progeny within sires with their corresponding mean square estimates. The mean square estimate for between sires (MS_s) equals the progeny within sire variance (error variance) σ_w^2 plus k times the sire variance σ_s^2 , that is $(MS_s = \sigma_w^2 + k\sigma_s^2)$, and the mean square estimates (MS_w) equals the progeny within sire variance σ_w^2 that is $(MS_w = \sigma_w^2)$,

where

k = weighted number of progeny per sire or the average number of progeny per sire

$$k = [1/s_i - 1] \times [n. - (\sum n_i^2/n.)]$$

where n_i is the number of progeny from the i th sire; $n.$ is the total number of progeny; $\sum n_i^2$ the sum of squares of progeny per sire; s_i = the number of sires. The heritability of the trait was calculated using the formula

$$h^2 = \frac{4\sigma_s^2}{\sigma_s^2 + \sigma_w^2}$$

Where h^2 = heritability of the trait, σ_s^2 = the sire variance, σ_w^2 = the progeny within sire variance.

The standard error of a heritability estimate was obtained using the formula:

$$SE(h^2) = 4 \sqrt{\frac{(1-t)^2 [1+(k-1)t]^2}{k(k-1)(s-1)}}$$

Where t is the intra-class correlation given by Becker [11] as

$$t = \frac{4\sigma_s^2}{\sigma_s^2 + \sigma_w^2}$$

Estimates of genetic and phenotypic correlations were obtained using appropriate expressions involving the estimated variance components according to Becker [11]. Heritability and correlations were categorized as follows: Heritability: low (<0.30), medium ($\geq 0.30 - <0.50$) and high (≥ 0.50) [13] Correlations: low ($0.10 - <0.30$), medium ($\leq 0.30 - <0.50$) and high ($\geq 0.50 - 1.00$) regardless of sign.

3. RESULTS AND DISCUSSION

3.1 Quantitative Genetic Characterization

The heritability estimates (h^2) of body weights and their standard errors are shown in Table 1. Generally, the heritability (h^2) estimates of body weight for all Guinea fowls in this study were low (0.06 for BW36) to moderate (0.51 for BW32). The heritability estimate for BW20 was zero.

3.2 Heritability Estimates of Growth Rates of Guinea fowls

The heritability estimates of growth rates (i.e. PBDG0-4, PBDG4-8, PBDG8-12, PBDG12-20 and PBDG8-20) are shown in Table 2 below. Estimates of heritability for growth rates were medium (0.39 for PBDG4-8) to high (0.78 for PBDG8-12) except PBDG8-20 which had a low heritability value (0.22).

Table 3 shows the phenotypic (below diagonal) and genetic (above diagonal) correlations between body weight traits of Guinea fowls. With respect to phenotypic correlations, the least coefficient of -0.007 was between BW0 and BW10 whereas the highest of 0.979 was between BW24 and BW28. Genetic correlations obtained in this study also ranged from -0.002 (BW0/BW16) to 0.968 (BW24/BW28).

Table 1. Heritability (h^2) Estimates of Body weight at different ages in Guinea fowl populations

Traits	Number of observations	h^2	s. e.
BW0	1214	0.40	0.08
BW2	967	0.32	0.09
BW4	797	0.46	0.09
BW6	696	0.44	0.09
BW8	582	0.33	0.10
BW10	492	0.18	0.10
BW12	385	0.27	0.01
BW16	270	0.29	0.09
BW20	252	0	0
BW24	256	0.09	0.01
BW28	210	0.20	0.23
BW32	137	0.51	0.13
BW36	87	0.06	0.51

Table 2. Heritability Estimates of Growth Traits at different stages in the Guinea Fowl Population

TRAITS	Sub traits	n	$h^2 + s. e.$	k	t
Growth Rates	PBDG0-4	380	0.63 ± 0.20	10.319	0.1563
	PBDG4-8	380	0.39 ± 0.17	10.319	0.0984
	PBDG8-12	283	0.78 ± 0.25	8.879	0.1962
	PBDG12-20	218	0.56 ± 0.26	8.086	0.1418
	PBDG20-24	202	0.41 ± 0.24	8.099	0.1029
	PBDG8-20	300	0.22 ± 0.16	9.381	0.0553

NB: k = maturity rate; t is the intra-class correlation

Genetic correlations between BW0 and BW2 – BW10 were lowly positive whereas between BW0 and BW12 - BW36 were low negative. Phenotypic correlations between BW0 and body weights BW2 to BW8 were lowly positive whereas they were low negative between BW0 and body weights BW10 to BW36. Low positive genetic correlations were obtained between BW2 and body weights BW6 to BW36. BW2 had a highly positive genetic correlation (0.618) with BW4. High positive phenotypic correlations were obtained between BW2 and body weights BW4 to BW36. BW4 had a high positive genetic correlation (0.814) with BW6 and medium genetic correlation with BW8 (0.341). Low positive genetic correlations were obtained between BW4 and body weights BW10 to BW36. BW4 had high positive phenotypic correlation with BW6 to BW24 but medium phenotypic correlation with BW28 to BW36. High positive genetic correlations were obtained between BW6 and BW36 (0.962), BW8 (0.937) and BW10 (0.526) whereas a medium positive estimate was obtained between BW6 and BW12 (0.331). Low

positive genetic correlations were obtained between BW6 and body weights from BW16 to BW32. BW6 had high positive phenotypic correlation with BW8 to BW36. High genetic correlations were obtained between BW8 and BW10 to BW12. BW8 had medium genetic correlations with BW16 and BW20 but low genetic correlations with BW24 to BW36. BW8 also had high phenotypic correlations with BW10 to BW36. High positive genetic correlations (range of 0.525 - 0.968) and phenotypic correlations (range of 0.668 – 0.979) were obtained among body weights BW10 to BW36.

McLennan and Lewer [14] have reported that both genetic variance and genetic coefficient of variation are measures of diversity in domestic livestock. Charlesworth [15] and Houle [16] have reported that there are two reasons for making comparisons of genetic variation for quantitative characters. The first is to respond to selection and the second is to make inferences about the forces that maintain genetic variability (diversity).

Table 3. Phenotypic (*Below diagonal*) and Genetic (*Above diagonal*) correlations between body Weights (*BW_i*) at various ages in guinea fowls

Traits	BW0	BW2	BW4	BW6	BW8	BW10	BW12	BW16	BW20	BW24	BW28	BW32	BW36
BW0		0.137	0.076	0.003	0.002	0.000	-0.001	-0.002	-0.001	-0.001	-0.001	-0.001	-0.001
BW2	0.139		0.618	0.205	0.082	0.044	0.029	0.021	0.017	0.018	0.018	0.016	0.015
BW4	0.279	0.626		0.814	0.341	0.179	0.101	0.058	0.048	0.047	0.044	0.035	0.034
BW6	0.029	0.658	0.824		0.937	0.526	0.331	0.205	0.171	0.166	0.157	0.136	0.962
BW8	0.064	0.587	0.771	0.948		0.958	0.597	0.372	0.305	0.290	0.275	0.230	0.219
BW10	-0.007	0.552	0.699	0.920	0.969		0.961	0.891	0.911	0.964	0.968	0.923	0.962
BW12	-0.068	0.554	0.605	0.890	0.928	0.973		0.891	0.727	0.692	0.662	0.557	0.525
BW16	-0.157	0.539	0.477	0.755	0.794	0.855	0.902		0.911	0.858	0.820	0.708	0.665
BW20	-0.130	0.564	0.510	0.805	0.830	0.886	0.938	0.922		0.964	0.930	0.813	0.749
BW24	-0.091	0.596	0.507	0.795	0.806	0.852	0.911	0.885	0.976		0.968	0.878	0.797
BW28	-0.072	0.613	0.464	0.749	0.758	0.802	0.865	0.840	0.935	0.979		0.923	0.842
BW32	-0.095	0.564	0.374	0.659	0.646	0.668	0.741	0.738	0.831	0.904	0.934		0.962
BW36	-0.097	0.545	0.393	0.662	0.662	0.691	0.753	0.748	0.826	0.885	0.920	0.973	

BW_i= Body weight at week *i*, where *i* = 0, 2.....36 weeks

Genetic diversity, that is, the heritable variation within populations is usually acted upon by selection, be it natural or artificial. Differential survival of individuals in a particular population in each generation due to selection ultimately results in changes in gene frequencies, hence evolution of such populations. Genetic diversity therefore allows for evolution as well as artificial selective breeding to occur. Several authors have stated that additive genetic variance is the variance of breeding values, therefore, medium to high genetic diversity in body weight and growth traits would enable selection pressure to be exerted in a breeding program to alter or improve these traits [17,18].

3.3 Heritability of Traits

It must be noted that the choice of statistical procedure for use in calculating heritability is very much dependent on the nature of the pedigree record that can be extracted from the records available [19]. If the data available allows for the possible selection of a number of procedures, then the procedure that gives the least amount of bias should be chosen and used for estimating heritability.

The mixed model procedure used here could only be applied to estimating heritability of the body weights. However, the choice of the use of Becker [11] programs procedure for estimating heritability of growth rates, as well as the phenotypic and genetic correlations was very much due to the nature of the family unit or pedigree record that could be obtained from the available record.

3.4 Heritability of Body Weights and Growth Rates

There are no reports in literature on estimates of heritability of body weight of guinea fowls in Ghana at various ages. The low to moderate estimates of body weight in this study suggests that they are not very much affected to a large extent by additive genetic effects. This may imply that the response to selection may take a longer time period.

The low to moderate h^2 estimates obtained for body weight at different stages in this study are comparable to the range of 0.28 to 0.58 reported by Singh *et al.* [20] for body weight of guinea fowls in India at various ages using the paternal half-sib analysis method. Mundra *et al.* [21] reported h^2 estimates by the paternal half-sib

method and without adjustment for full-sib relationship in guinea fowl to be 0.25 - 0.58. The findings in this study closely agrees with that of Vivek *et al.* [22] who also reported low h^2 estimates of 0.17 ± 0.12 , 0.08 ± 0.10 and 0.13 ± 0.11 for 8-, 12- and 16-week body weights in guinea fowls in India.

The results of this study are also similar to the findings by Prado-Gonzalez *et al.* [23] in Creole chicken who observed that h^2 estimates did not increase with age, but are contrary to the report by Chambers [24] that heritabilities for body weight of broilers tend to increase with age. According to Prado-Gonzalez *et al.* [23] differences in heritability estimates could be attributed to the method of estimation, breed, environmental effects and sampling error due to small data set or sample size.

Environmental factors such as high temperature and humidity as well as poor management conditions are also known to increase the residual variance and hence decrease heritability estimates. Low heritability estimates suggest that factors other than additive genetic effects, which may or may not be subject to control by producers, account for substantial variation in these traits. Prado-Gonzalez *et al.* [23] also reported that low heritabilities mean that dominance, epistatic and environmental effects are more important than additive genetic effects on body weight under the prevailing conditions. Correct estimates and use of heritability values of desired traits greatly increase precision of selecting breeding animals for the traits in question. This indicates that genetic selection will be effective in improving the performance levels of these traits [25,17]. The current results may be considered for inclusion in the breeding objectives of guinea fowl breeding programs in Ghana.

3.5 Phenotypic and Genetic Correlation between Body Weights

Generally, genetic and phenotypic correlations between hatch weight (BW0) and all other body weights ranged between lowly negative and lowly positive suggesting that there was weak (little) or no relationship between hatch weight and the other body weights. This implied that hatch weight could be used as a measure or selection criteria for other body weights. Improving the hatch weight of guinea fowls would not result in the improvement of other body weight traits. This finding closely agrees with that observed by Abdellatif [26] in Dandarawy chicken in Egypt.

He observed that the genetic correlation between hatch weight and weight at 4 weeks, 8 weeks, 12 weeks and 16 weeks were low and negative. He concluded that hatch weight had no effect on the later body weights and that body weight at hatch must be considered as a separate trait. The study showed that genetic improvement of the body weight at two weeks of age in guinea fowls will only result in a correlated response in their 4 week body weight with no effect on their body weights at later ages. This is because the genetic correlation coefficient between body weight at 2 weeks and body weight at 4 weeks is high (0.618). This may indicate that some of the same genes that influence 2 week body weight also influence 4 week body weight. Alternatively, this could mean that the genes that contribute to the two traits are co-inherited [27]. However, the 2 week body weight can be used to predict the weights at later ages. This is because the phenotypic correlation between 2 week body weight and the later traits have high positive coefficients, and these range between 0.539 to 0.658.

Selection for body weight at 4 weeks will improve the 6 week and 8 week body weights of the birds with no effect on their body weight at subsequent ages. This is because of the moderate to high positive genetic correlation between them (0.341 to 0.814). However, body weight at 4 weeks can be used to determine the weights at later ages since the phenotypic correlation between 4 body weight and body weights at later ages range between moderate (0.374) to high (0.814). The medium to high positive genetic correlations between 6 week body weight and body weights at 8 weeks, 10 weeks, 12 weeks and 36 weeks of age suggest that there will be correlated response in these body weights when the 6 week body weight of the birds are genetically improved. The 6 week body weight can be used to predict body weights from 8 weeks to 36 weeks due to the high positive phenotypic correlation between them. However, improvement in the 6 week body weight will have with no effect on 16 to 32 week body weights. The medium to high genetic correlations between BW8 and body weights BW10 to BW20 implies that correlated responses are expected in these later weights when the eight-week weight is genetically improved with no response expected in body weights at 24 to 36 weeks. Improvement in 8-week body weight will result in improvement in the BW10 to BW36.

This study showed that weight at hatch is not a good indicator trait of BW10 to BW36 and vice

versa. Generally, body weight traits except hatch weight in guinea fowls can be used as a measure for the others and improving one with result in the improvement of others.

4. CONCLUSION

From the results, the heritability estimates of body weight and growth rate in Guinea fowl populations of Ghana were found to be low to high i.e. 0.08-0.70 and 0.22-0.78, respectively. It was revealed that hatch weight cannot be used as a measure or selection criteria for other body weights; however, the body weight at two weeks of age served as an indicator for the early selection of guinea fowls based on body weight.

5. RECOMMENDATIONS

It is recommended that the results obtained could be included in the breeding objectives of any upcoming guinea fowl improvement program.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the author and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the author.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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