PHENOTYPIC CORRELATIONS BETWEEN BIOLOGIC MARKERS AND QUANTITATIVE TRAITS IN F_1 NIGERIA LOCAL TURKEY

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Abstract
The study was conducted using data on 210 F_1 progeny consisting of 70 Poults each of three phenotypic classes (Black, White and Spotted) of the Nigerian local turkey to determine phenotypic associations between body weight (BWT), linear body measurements (quantitative traits) and biologic markers. Linear body measurements studied were body length (BDL), shank length (SHL), keel length (KLL), breast width (BW), wing length (WGL) and drumstick length (DSL). Biologic markers measured include packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), total blood protein (TBP), blood glucose (BGC) and rectal temperature (RT). The result showed significant ($p<0.05$, $p<0.01$), positive and moderate correlations between the biologic markers (PCV, WBC, RBC, Hb, BPT and BGC) and all the quantitative traits in the Black variety. The White phenotype had moderately positive and significant ($p<0.05$, $p<0.01$) correlations between the quantitative traits and PCV, WBC, Hb and BGC only. In the Spotted variety, WBC had significantly ($p<0.01$) positive and high associations with all the quantitative traits. The other markers (RT, PCV, RBC and Hb) however showed positively significant but moderate correlations with the quantitative traits. Generally, the positive and significant correlations indicate that increase in the biologic markers will lead to a concomitant increase in the quantitative traits and vice versa. It then implies that the quantitative traits can be genetically improved by selecting for the biologic markers studied if the latter is more difficult to measure and if environmental influence is negligible.

Keywords: Local turkey, markers, quantitative traits and phenotypic correlations

Introduction
The problem of protein shortage especially that of animal origin is a perennial one which is common throughout the country (Ojewola, 1993). As the demand for animal protein in Nigeria increases, the need to improve animal protein production becomes necessary. Local turkey is becoming few in Nigeria and are predominantly the bronze-black type raised extensively (Oluyemi and Roberts, 2000). Turkey is one of the poultry species that is declining in Nigeria due to the importation of frozen turkey from Europe and America despite its genetic resource base. Then it is imperative that the study of the native (local) turkey be an urgent necessity (SAGARPA, 2003) since there is possibility of extinction of native turkey (Aquino *et al.,* 2003). For example, to date, many genomic investigations have been conducted to characterize genetic relationships between commercial and non- commercial chicken breeds, whereas the nature of the genetic or phenotypic relatedness between commercial and heritage (local) turkeys remains unknown. Therefore, the local turkey strains in Nigeria deserve improvement in their genetic profile and physiological status (Nosike *et*

al., 2013). The main purpose of animal breeding practices is to improve traits of economic value (Mendes *et al.,* 2005) and body weight is one of those important economic traits in the selection of animals.

One plausible approach to genetic improvement of animals is selection of individuals based on the presence or absence of genetic and biologic markers which have definite association with quantitative trait loci (QTLs) such as for body weight and growth rate. This is the concept of Marker-Assisted Selection (MAS), which is the process of using marker information in the selection of individuals to become parents for future generations. Actually, many characters in domestic animals are not independent of each other; rather they tend to be associated (Ibe, 1998). The measurements of the amount of various biochemical constituents of blood have been used in the evaluation of the physiological status of animals (Solomon *et al*., 2005). Many characters in farm animals are found to associate with one another such that the improvement of one may lead to the improvement or otherwise of the other. This leads to the concept of correlation, which is a measure of the degree to which some traits vary together or measure of the intensity of linear association among traits (Singh and Kumar, 1994). The study was therefore conducted to determine relationship between biologic markers and quantitative traits in the Nigeria local turkeys.

Materials and Methods

The study was conducted at the Poultry Unit of the Teaching and Research Farm of the Michael Okpara University of Agriculture, Umudike, Abia State. Umudike is located on latitude 05^0C 28′ North and 07^0C 32′East and lies at an altitude of 122m above sea level. This area is situated within the tropical rainforest zone of West Africa which is characterized by long duration of rainfall (April - October) and short period of dry season (November-March). Average rainfall is 2169.8mm in 148 – 155 rain days. Average ambient temperature is 26^0C with a range 22^0C and 30^0C. Its relative humidity ranges from 50 to 90%.

Management of experimental birds

A total of 210 day-old F_1 Poults hatched in different batches were used in the study. The three phenotypic groups namely (Black, White and Spotted) local turkey contributed 70 straight-bred Poults each for the study. They were brooded in three small metal cages for each hatch for a period of 2 weeks after which they were transferred to small compartments according to their phenotypic group for a period of 4 weeks and finally to deep litter pens at 6 weeks of age. Dry wood shavings were used as litter material. Routine management operations such as washing of the water and feed troughs were carried out on daily basis. The birds were given routine vaccination during the period of the experiment. Prophylactic antibiotics and anticoccidial drugs were administered to the birds periodically via drinking water. The birds were also dewormed and acaricide sprayed to check worms and ectoparasites. The brooding period lasted for 6 weeks. From brooding through rearing, maximum comfort for the poults was ensured. Feed was provided in adequate quantity to the poults twice a day, namely 8.30am and 2.30pm.Poults (0-6 weeks) was fed *ad libitum* with starter mash containing 28% crude protein and 2800kcal ME/kg. Growing turkeys (7-24 weeks) were fed growers mash (20% crude protein and 3000 kcal ME/kg). Rearing was between 7 and 24 week

Data collection

Quantitative traits

All surviving healthy birds in the various phenotypic groups were further identified using indelible markers which were re-enforced fortnightly for proper identification. Parameters measured include: Body weight (BWT) (g): Body weight was measured weekly using a top loading 20kg-CAMRY scale with a sensitivity of 10g.Body length (BDL): the distance between the base of the neck and pygostyle. Shank length (SHL): length of the tarso-metatarsus from the hock joint to the metatarsal

pad. Keel length (KLL): the length of the keel bone from the V-joint to the end of the sternum. Wing length (WGL): distance between the tip of the phalanges and the coracoids-humerus joint. Breast width (BW): region of the largest breast expansion when positioned ventrally. Drumstick length (DSL): length of the femur bone. The above parameters, with the exception of BWT, were measured weekly using a tailor's 'cm' tape. The measurements were taken on the birds before feeding in the morning.

Biologic markers

A total of 72, 7-week-old local turkeys of different phenotypic groups (Black, White and Spotted) comprising 24 per phenotypic class were randomly selected and used for biologic marker studies.

Collection of blood samples

Blood samples (2ml) were collected aseptically with sterile syringe and needle from the wing vein of the turkeys into labeled test tubes, containing anti-coagulant (heparin) and another test tube with no anti-coagulant for determination of biochemical markers. It was done immediately after the skin had been damped with alcohol to disinfect the area and expose the vein. Determination was done bi-weekly for 20 weeks.

Determination of biologic markers

The following biologic markers were determined: Packed cell volume (PCV) was determined of the micro haematocrit method by Dacie and Lewis (1999). It was measured by using capillary tubes. The tubes were filled with blood to 3/4 and sealed with crystaceal. The tubes were then centrifuged at 3000rpm for five minutes in microhaematocrit (Model EBA 20) centrifuged and Packed Cell Volume was estimated from the reader. Haemoglobin Concentration (Hb) was determined using the cyanomethaemoglobin method as described by Jain (1986). Haemoglobin concentration was determined using Spin-react haemoglobin drabkin kit. In this method haemoglobin was oxidized by potassium ferric oxide into cyanomethaemoglobin by potassium cyanide. The intensity of absorbance of cyanomethaemoglobin is proportional to hemoglobin concentration. White blood Cell (WBC) was estimated using a microscope with improved Neubauer haemacytometer as described by Jain (1986). White blood cell (WBC) count was determined by diluting 0.02 ml of blood sample with physiological solution (0.38 ml Turks) and loaded on to the Neubauer counting chamber, and all cells on the four corner squares were counted using a light microscope at x10 magnifications. The number of cells counted for each blood sample was multiplied by 50 to obtain the total white blood cell count per microlitre of blood and the number of WBC counted using an improved Neubeur haematocytometer with the aid of a microscope.Red Blood Cell (RBC) was determined using a microscope with improved Neubauer haemacytometer as described by Jain(1986). Red blood cell (RBC) count was determined by diluting 0.02ml of blood sample with physiological solution (4ml Heyem's) in a clean test tube to make a 1:200 dilution of the blood sample. The diluted blood sample was loaded on to the Neubauer counting chamber, and all red cells on the five corner groups of 16 small squares in the central area of the Neubauer chamber was counted using a light microscope at x40 magnifications. The number of cells counted for each blood sample was multiplied by 10,000 to obtain the total red blood cell count per microlitre of blood. The total plasma protein was measured by using the standard Biuret method as described by Lawrence (1986), which is based on the reaction between the peptide bonds of protein and Cu^{2+} (from copper sulfate solution) that produces a blue-violet colored complex in alkaline solution. The measurements were done using the Biuret method (CHRONOLAB) where 100 ml of blood plasma and standard protein solution were diluted into 500 ml of the Biuret reagent in a test tube. The Biuret reagent without a sample being added was used as a blank. After mixing, the test tubes were left to stand for 30 minutes and thereafter the absorbance was read through spectrophotometer (Cecil 2000, UK) at a wavelength of 540 nm.

The calculation of the total protein was done using the following formula.

Concentration of protein (g/100ml) =

$$\frac{\text{Absorbance of sample-Absorbance of Blank}}{\text{Absorbance of Standard-Absorbance of Blank}} \times \text{Concentration of Standard (g/100ml)}$$

The values of total plasma proteins obtained were expressed in g/dl.

Blood glucose (BGC) determination was by the process described by Barker and Silverton (1976). The serum glucose is determined based on the type of colour the product of hydrolysis emits. Hydrolysis of serum glucose produces bright coloured substances. The intensity of the colour is proportional to the concentration of glucose in the blood. The colour principle leads to the calculation of glucose as follows:

$$\text{Glucose (g/dl)} = \frac{\text{Absorbance of sample-Absorbance of Blank}}{\text{Absorbance of Standard-Absorbance of Blank}} \times \text{Concentration of Standard}$$

The rectal temperature of the turkeys was measured via the rectum using a digital thermometer (0.1^0C) which was inserted into the rectum of the birds for a minute as previously described by Yahav and McMurty (2001).

Statistical analysis

Data obtained were statistically analyzed with SPSS (2011) package. Phenotypic correlations between quantitative traits and markers were determined using the Pearson's Product-Moment Correlation Coefficient (r) as described by Snedecor and Cochran (1980).

Results and Discussion

The result of the phenotypic correlations between quantitative traits and markers in the black variety is presented in Table 2.All correlations were positive. Except correlations between RT and BWT, BDL, WGL, KLL, SHL, BRW and DSL, and between BGC and BDL, all other correlations were significant ($p<0.05$) and moderate. The positive phenotypic correlations indicate that an increase in value of one quantitative trait is associated with an increase in value of each marker and vice-versa. The significant correlations indicate that the affected markers could be used in selection studies instead of the corresponding quantitative traits if the latter are more difficult to measure and if environmental influence is negligible. The phenotypic correlations between quantitative traits and markers in the white turkey are given in Table 3. All correlations were positive, except correlations between RT and each of BWT, BDL, WGL, KLL, SHL, BRW and DSL. This result is in agreement with the report of negative correlations between RT and quantitative traits in domestic rabbits (Nosike, *et al*., 2013). Except correlations between RT, RBC, BPT and the quantitative traits, all other correlations were significant ($p<0.05$). Positive phenotypic correlations indicate that an increase in value of one quantitative trait is associated with an increase in value of each marker and vice-versa. On the other hand, negative correlations indicate that an increase in value of one quantitative trait is associated with a decrease in value of each corresponding marker. As a result of some negative correlations, none of the respective markers could be used in MAS for the improvement of the quantitative traits. The significant ones indicate that the affected markers could be used in selection studies instead of the corresponding quantitative traits if the latter are more difficult to measure and if environmental influence is negligible.

The phenotypic correlations between the quantitative traits and markers in the spotted turkey are given in Table 4. All correlations were positive. Except correlations between BPT and BGC, and each of the quantitative traits and between RBC and each of WGL and DSL, all other correlations were significant ($p<0.05$). Positive phenotypic correlations indicate that an increase in value of one

quantitative trait is associated with an increase in value of each marker and vice-versa. Also, as in the case of black and white turkeys, although the correlations were not high, the significant ones indicate that the affected markers could be used in selection studies instead of the corresponding quantitative traits if the latter are more difficult to measure and if environmental influence is negligible (Ng, 1988). Generally, the correlations between RT and the quantitative traits studied were not significant ($p>0.05$) in both black and white varieties, but significant correlation exist between RT and quantitative traits in spotted variety. On the other hand, there was no significant ($p>0.05$) correlation between BPT and all the quantitative traits, in the spotted and white varieties, but significant correlation exist between BPT and all the quantitative traits in black variety. The non-significant associations on some of the traits could be due to management practices and environmental and negligible genetic influences (Obike *et al*., 2013).All correlations between PCV, WBC, Hb and all the quantitative traits studied were positive and significant ($p<0.05$) in the three varieties. This trend reveals the stability and strength of the association between these markers and the quantitative traits. However, this result has shown that improvement in animals differs among genetic or phenotypic groups. Since more positive significant correlations occurred between quantitative traits and markers in Black phenotype. Black phenotypes should therefore be involved in the improvement during marker assisted selection (MAS) programmes.

Conclusion

This study has revealed that phenotypic correlation between the quantitative traits and some markers were moderate, positive and significant in the three phenotypic groups. However, a meaningful indirect selection can be achieved by improving the quantitative traits using the markers due to significant correlations. The attendant effect will be an increase in the number of quality birds, thereby assisting in bridging the animal protein gap in poor developing countries. In view of the results obtained in this study, it is recommended that: the black phenotype should be selected by breeders to achieve improved production, using the quantitative traits BDL, WGL, KLL and SHL and the markers RT, PCV, HB, WBC and RBC.

Table 1: Distribution of local turkey poults hatched per phenotypic class

		Hatch					
Mating	**Phenotypic class**	**1**	**2**	**3**	**4**	**5**	**Class total**
Black x Black	Black	3	11	10	36	26	**86**
White x White	White	2	6	6	28	30	**72**
SpottedxSpotted	Spotted	2	7	8	26	31	**74**
Total hatch		**7**	**24**	**24**	**90**	**87**	**232**

Table 2: Phenotypic correlations between quantitative traits and markers in the black variety

*Trait BWT *Marker	BDL	WGL	KLL	SHL	BRW	DSL
RT	0.09^{NS}	0.12^{NS}	0.09^{NS}	0.13^{NS}	0.13^{NS}	0.13^{NS}	0.12^{NS}
PCV	0.48**	0.33*	0.37*	0.30**	0.41**	0.42**	0.39**
WBC	0.45**	0.40**	0.38*	0.43**	0.42**	0.45**	0.42**
RBC	0.46**	0.50**	0.45**	0.55**	0.50**	0.51**	0.53**
Hb	0.57**	0.41**	0.49**	0.48**	0.51**	0.52**	0.48**
BPT	0.32*	0.50**	0.30*	0.44**	0.38**	0.42**	0.46**
BGC	0.45**	0.23^{NS}	0.37*	0.30*	0.38**	0.35*	0.32*

NS = Not Significant **Correlation is significant ($p<0.05$)* *** Correlation is significant ($p<0.01$)* *BWT=Body weight, BDL=Body length, SHL=Shank length, KLL=Keel length, BRW=Breast width, WGL=Wing length, DSL=Drumstick length, RT = Rectal Temperature, PCV = Packed Cell Volume, WBC = White Blood Cell, Hb =Haemoglobin Concentration, RBC = Red Blood Cell, BPT = Blood Protein, BGC=Blood Glucose.*

Table 3: Phenotypic correlations between quantitative traits and markers in the white variety

Trait Marker	BWT	BDL	WGL	KLL	SHL	BRW	DSL
RT	-0.27 ^{NS}	-0.14 ^{NS}	-0.21 ^{NS}	-0.18 ^{NS}	-0.22 ^{NS}	-0.19 ^{NS}	-0.19 ^{NS}
PCV	0.42**	0.36*	0.32*	0.34*	0.35*	0.38**	0.36*
WBC	0.38*	0.38**	0.37*	0.38*	0.37*	0.41**	0.39**
RBC	0.21 ^{NS}	0.17 ^{NS}	0.14 ^{NS}	0.18 ^{NS}	0.17 ^{NS}	0.17 ^{NS}	0.15 ^{NS}
Hb	0.43**	0.38**	0.39**	0.39**	0.41**	0.44**	0.39**
BPT	0.16 ^{NS}	0.23 ^{NS}	0.18 ^{NS}	0.18 ^{NS}	0.18 ^{NS}	0.19 ^{NS}	0.22 ^{NS}
BGC	0.49**	0.43**	0.44**	0.42**	0.46**	0.45**	0.44**

NS = Not Significant
**Correlation is significant (p<0.05)*
*** Correlation is significant (p<0.01)*
See Table 2 for meaning of trait abbreviations

Table 4: Phenotypic correlations between quantitative traits and markers in the spotted variety

Traits Markers	BWT	BDL	WGL	KLL	SHL	BRW	DSL
RT	0.35*	0.44**	0.45**	0.40**	0.47**	0.44**	0.40**
PCV	0.38*	0.40**	0.40**	0.41**	0.39**	0.42**	0.40**
WBC	0.57**	0.61**	0.57**	0.59**	0.58**	0.61**	0.60**
RBC	0.31*	0.32*	0.25 ^{NS}	0.31*	0.28	0.31*	0.29 ^{NS}
Hb	0.37*	0.41**	0.40**	0.41**	0.38*	0.42**	0.40**
BPT	0.08 ^{NS}	0.17 ^{NS}	0.08 ^{NS}	0.17 ^{NS}	0.01^{NS}	0.10 ^{NS}	0.14 ^{NS}
BGC	008^{NS}	0.12^{NS}	0.06^{NS}	0.12 ^{NS}	0.03 ^{NS}	0.08 ^{NS}	0.11 ^{NS}

NS = Not Significant
**Correlation is significant (p<0.05)*
*** Correlation is significant (p<0.01)*
See Table 2 for meaning of trait abbreviations

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