Taxonomic discrimination of six poultry species as influenced by haematological and serum biochemical parameters

Kareem-Ibrahim, K. O., Abanikannda. O. T. F. and Hassan, Z. D.
Department of Animal Science, School of Agriculture, Lagos State University, Epe – Lagos
Corresponding author: otfabanikannda@hotmail.com; +234 802 312 8376

Abstract

The binomial nomenclature method of classification (Taxonomy) of organisms developed by Linnaeus entails arranging animals and plants into natural, related groups based on structure, embryology, or biochemistry into kingdom, phylum, class, order, family, genus, and species. This study aimed at using haematological and biochemical parameters of blood obtained from six poultry birds in three different families: Phasinidae (Chicken, Turkey and Quail), Numidae (Guinea Fowl) and Anatidae (Goose and Duck), to investigate the haematological and biochemical parameters and then statistically classify the species into distinct clusters. Blood was collected from the wing vein of five birds each from each species. Two sets of blood samples on each of the 30 birds across the six species were evaluated for haematology and serum biochemical parameters. Data from the laboratory were statistically analyzed using Minitab® (17) statistical software for descriptive, analysis of variance, cluster analysis, discriminant analysis and principal component analysis. The method used in the cluster analysis was the complete, Pearson's distance model. Effects of species was highly significant (P<0.001) on all haematological and biochemical indices studied, with the exception of its effect on basophil and creatinine (P<0.05), providing a veritable platform for statistical discrimination across the six species investigated. However, the three clusters produced by the dendrogram had Duck and Turkey in the first cluster, Guinea Fowl was alone in the second cluster and Goose, Chicken and Quail were in the third cluster. There was no misclassification in the discriminant analysis, whereby all six species in the test data were classified appropriately with a 100 percent accuracy. However, this study calls for further investigation of the genetic similarity among the species in the light of the juxtaposition of Turkey and Goose in contrast to the conventional Linnean taxonomy currently in use.

Keywords: Taxonomy, Haematology, Serum Biochemistry, Poultry Species

Discrimination taxonomique de six espèces de volailles basée sur des paramètres hématologiques et biochimiques sériques

Résumé

La classification binominale des organismes, développée par Linné, repose sur l'organisation des animaux et des plantes en groupes naturels apparentés selon des critères morphologiques, embryologiques ou biochimiques. Ces groupes sont hiérarchisés en royaume, phylum, classe, ordre, famille, genre et espèce. Cette étude visait à explorer l'utilité de paramètres hématologiques et biochimiques sanguins pour la discrimination taxonomique de six espèces de volailles appartenant à trois familles distinctes : les Phasianidés (poulet, dinde et caille), les Numididés (pintade) et les Anatidés (oie et canard). Le sang a été prélevé sur l'aile de cinq oiseaux par espèce. Pour chaque oiseau (n=30), deux échantillons sanguins ont été analysés afin d'évaluer les paramètres hématologiques et biochimiques sériques. Les données de laboratoire ont été analysées statistiquement à l'aide du logiciel Minitab® (17) pour réaliser des analyses descriptives, des analyses de variance, des analyses de cluster, des analyses discriminantes et des analyses en composantes principales. La méthode d'analyse de cluster utilisée était la méthode de distance complète de Pearson. L'espèce a eu un effet hautement significatif (p<0,001) sur tous les indices hématologiques et biochimiques étudiés, à l'exception des basophiles et de la créatinine (p<0,05). Cela démontre la pertinence de cette approche pour la discrimination statistique entre les six espèces étudiées. Le dendrogramme issu de l'analyse de cluster a regroupé le canard et la dinde dans un premier cluster, la pintade dans un second cluster distinct, et l'oie, le poulet et la caille dans un troisième cluster. L'analyse discriminante n'a identifié aucune erreur de classification, les six espèces de l'échantillon test ayant été classées correctement avec un taux de précision de 100 %. Néanmoins, cette étude souligne la nécessité

d'approfondir l'investigation de la similarité génétique entre les espèces, notamment au regard du regroupement surprenant de la dinde et du canard dans l'analyse de cluster, ce qui diffère de la classification taxonomique linnéenne conventionnelle.

Mots-clés : Taxonomie, Hématologie, Biochimie sérique, Espèces de volaille
Running title: Statistical classification of six species of poultry

Introduction

Taxonomy, the science of classification of organisms into natural, related groups based on structure, embryology, or biochemistry into kingdom, phylum, class, order, family, genus, and specie has been used extensively used to investigate similarity or dissimilarity among different organisms.

Primordial characterization involves the identification of populations based on morphological descriptors that can also provide useful information on the suitability of breeds for selection (Ajayi *et al.,* 2012), and this methodology is applied to many livestock species for morphological characterization with the aim of comparing their various breeds (Francesch *et al*., 2010).

The rearing of birds originated many years ago by collection of their eggs and young ones from their natural habitat, and domesticating them as farm animals living in close proximity with humans. Poultry farming entails the domestication of birds such as chicken, duck, quail, turkey, and goose with intent of rearing them for meat, egg production as well as using their incidental products such as faecal droppings and feathers in industries as natural unprocessed materials (Stiles, 2017).

Poultry birds include chickens, peacocks, turkeys, ducks, geese, quails, partridges, pheasants, guinea fowls, emus, swans, pigeons etc. Linnaeus (1758) drew up rules for assigning names using binomial nomenclature principles based on morphological indices to classify organisms into their respective species. This method was used to classify organisms into their respective kingdom, phylum, class, order, family, genus, and species.

Blood acts as a pathological reflector of the status of exposed animals to intoxicant and other conditions and examining blood for their constituents can provide important information for the diagnosis and prognosis of diseases in animals. Animals with good blood composition are likely to show good performance (Isaac *et al*., 2013) and the normal levels for hematological and biochemical variables in domestic birds can be determined using blood measurements (Elagib *et al.*, 2012).

This study aimed at assessment of haematological and serum biochemical parameters of blood from six species of poultry birds from three different families [Phasinidae (Chicken, Turkey and Quail), Numidae (Guinea Fowl) and Anatidae (Goose and Duck)] among the species, develop statistical similarities based on the parameters evaluation with a view to classify the species into distinct clusters and compare the statistical classification with the traditional taxonomy based on morphological differences by Linneaus (1758). The study would also build a discriminant function based on the parameters for each species and use the values to reclassify the species.

Materials and Methods

Experimental Site

This study was conducted at the Department of Animal Science, Lagos State University, Epe Campus, lying at latitude 6.35'347°N and longitude 3.59'55.5°E.

Experimental Units

Five birds aged 12 months from each of the six species investigated (Chicken, Duck, Quail, Turkey, Geese and Guinea Fowl) were randomly sampled from three different areas (Araga – Epe, Sagamu and Ibadan), making a total of 30 birds. These birds are drawn from three different families as depicted by Linnaeus (1758) in Figure 1.

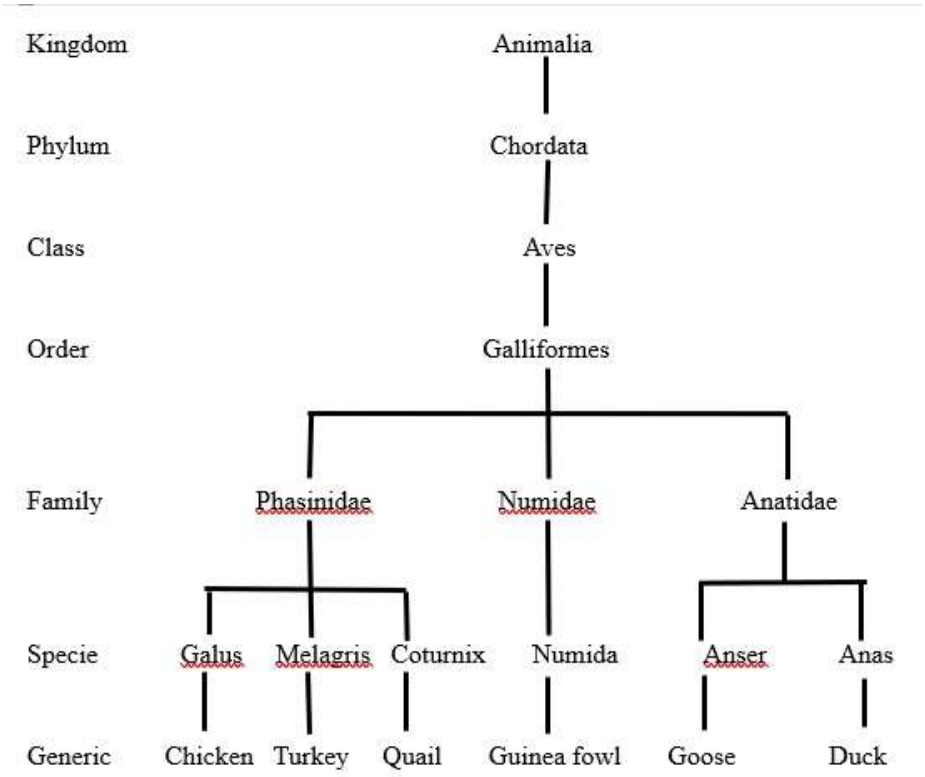

Figure 1: Taxonomy of the six species of poultry studied (Linnaeus, 1758)

After acclimatization of the birds for two weeks, two sets of blood samples (n=30) were collected from each of the birds in EDTA bottles for haematological analyses and Universal bottles for serum and biochemical analyses, making a total of 60 samples.

Experimental Design

The experiment is a completely randomized design investigating only the differences in measured parameters along species lines. It is a one-way analysis of variance with specie as the only explanatory variable on the measurements.

Experimental Procedure

Blood samples measuring 5mL were drawn from the wing vein clearly visible running between the biceps and the triceps muscle, where it forms a V (bifurcates). The area around the bleeding site was disinfected by swabbing with 70% alcohol and the needle was inserted inside the tendon, then into the vein in the direction of the flow of the blood. A 2.5 mL syringe was used for smaller birds, while the 5mL syringe was used for medium sized and larger birds. The plunger of the syringe was gently pulled as soon as the tip of the needle is in the vein, and pressure was applied to the vein for a few second to stop further bleeding after collecting sufficient blood and removing the needle. Subsequently, some of the collected blood was immediately transferred into the EDTA vacutainer bottles and kept immediately in a cooler, while the remaining blood was transferred into the vacutainer Universal bottle. The blood in the Universal bottle is allowed to stand and clot before being placed in the cooler.

Laboratory Analyses

All laboratory analyses were conducted at the laboratory of the Institute of Agricultural Research and Training (IAR&T), Moor Plantation Ibadan, Oyo State, Nigeria. The hematological tests were conducted using hematology analyzer for blood cell counts and information on size and structure using standard procedures (Scoffin, 2014; Wenhao, 2016; Palm, 2016 and Chhabra, 2018). These tests are based on electrical impedance, flow cytometry, and fluorescent flow cytometry used in hematology analyzers.

Haematological parameters investigated included; Packed Cell Volume (PCV), Hemoglobin (Hb), Red Blood Cell (RBC), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin

(MCH), White Blood Cell (WBC), Lymphocyte, Neutrophil, Basophil, Eosinophil and Monocyte.
Serum total protein (TP) was determined by Biuret method, Albumin by bromocresol green (BCG) method as described by Rodkey (1965), while Globulin concentration was obtained as the difference of subtracting albumin from total protein. Serum Urea was determined by Urease method and Creatinine by Folin-Wu filtrate methods as described by Sagar, 2021.
Serum biochemical parameters evaluated included; Glucose, Total Protein, Urea, Albumin, Globulin, Creatinine, Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT).

Statistical Analyses

Data obtained from the laboratory data were statistically analyzed using Minitab® (17) Statistical Software for descriptive statistics; including minimum, maximum, means, standard deviation and coefficient of variation for all measured variables (hematology, Serum and biochemical), further statistical tests include analysis of variance, cluster analysis and discriminant analyses.
A one-way Analysis of variance was conducted based on specie alone to test if there were differences in the mean values reported for all the measured variables. The Statistical model describing the ANOVA was given as: $Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$ where; Y_{ij} is the measured variable on the j^{th} sample within the i^{th} specie, μ is overall mean, α_i is the effect of species and e_{ij} is the residual error assumed to be random, independent and normal. After a significant ANOVA, further mean comparison procedures were conducted using the Tukey's Honestly Significant Difference (HSD) to separate the means.
To evaluate the dissimilarities among the six species, a multivariate cluster classification technique using the measured variables were conducted to produce a dendrogram of the relationship among the different species. The complete linkage, Pearson's distance method was used for the cluster analysis, and a discriminant function was generated based on haematological and serum biochemical indices for reclassification using the linear discriminant analysis. Biometrical statistical analyses involving cluster, discriminant and principal components analyses had been variously used in the past albeit on different species or breeds (Everitt, 2001; Kütting *et al*., 2017; Almeida *et al.*, 2023; Mekonnen *et al*., 2023).
A principal component analysis was conducted to weigh the influence of each haematological and serum biochemical parameters, and evaluate the loadings of the important components. All statistical analyses in the study were conducted using Minitab® 17 Statistical Software.

Results and Discussion

Haematological Parameters

Packed Cell Volume (PCV) ranged between 24.10% and 32.30% with a mean value of 28.65±0.38%. Guinea Fowl has the highest value, while Turkey had the least (Table 1a). This value obtained for Guinea Fowl is close to the reported value of 32.61±1.36 by Ali *et al*., (2019). PCV values varied widely across the species and 88.88% of variation in PCV can be adduced to the influence of species alone (Table 2a).
Across the species, haemoglobin (Hb) values ranged from 8.6 to 12.30g/dL, with a mean value of 10.41±0.17g/dL. The local chicken had the highest value while duck had the lowest value (Table 1a). Kokore *et al*., (2021a) reported mean value 11.06±0.50 g/dL for local chickens in Cote D'Ivoire, which is similar to the value obtained in this study. The effect of species on haemoglobin values is highly significant (P<0.001) accounting for about 65% of total variation in haemoglobin values (Table 2a).
Red Blood Cell (RBC) count obtained in the study ranged between $10.20 – 15.30 \times 10^{12}$/l across the six species with an overall mean value of $13.23 \pm 0.23 \times 10^{12}$/l (Table 1a). About 78% of variation in red blood cell value was explained by species differences alone (Table 2a) and the influence was highly significant (P<0.001).
The mean corpuscular hemoglobin concentration (MCHC) had average value of 33.71±0.20, with a range between 31.40 and 35.40 g/dL (Table 1a). The value of 34.74±0.31 recorded on the local chicken is in consonance with 36.45±1.20 g/dL reported by Kokore *et al*., (2021a). Specie differences alone accounted for 62.28% of total variation in MCHC across the six species studied (Table 2b), and exerted highly significant (P<0.001) influence of MCHC.

The mean corpuscular haemoglobin (MCH) had values between 5.80 and 9.10pg across the species with an overall mean of 7.24±0.15pg (Table 1a). Specie was a highly significant (P<0.001) source of variation in MCH and it alone accounted for 59.31% of variation in MCH values, with the Guinea fowl ranking highest in value (Table 2a).

White blood cell ranged from 6.10-11.10×10^9/L across the species with an overall mean of 8.42±0.22×10^9/L (Table 1a). The highest value was recorded for Guinea fowl and it was consistent with the values reported by Kokore *et al*. (2021b) who also worked on Guinea fowl. Species was highly significant (P<0.001) on WBC and it accounted for 61.61% of the differences in WBC (Table 2a).

Lymphocyte ranged between 55.10 - 63.20%, with an overall mean of 59.32±0.43% (Table 1b) across the six species. Species was a highly significant (P<0.01) source of variation in lymphocyte values, accounting for 87.39% of total variation in lymphocyte (Table 2b), with the quail recording the highest values, which is similar to the values earlier reported by Meshabaz *et al*. (2017).

The overall mean value for neutrophil was 33.35±0.29% with a range of between 30.00 and 36.10% (Table 1b). Specie alone accounted for 75.89% variation in neutrophil values (Table 2b), and was highly significant (P<0.001) on neutrophil.

Basophil ranged between 0.00 and 0.06% among the species, with an overall mean value of 0.01±0.00% (Table 1b). Specie was significant (P<0.05) on basophil values and it accounted for 40.84% of total variation in basophil values (Table 2b).

Eosinophil was highly significantly (P<0.01) impacted by species, accounting for 76.82% of total variation in eosinophil values (Table 2b). The value of eosinophil across the six species ranged between 1.10 and 4.60%, with an overall mean of 3.22±0.17% (Table 1b).

The values for monocyte in the study ranged between 1.12 and 5.28%, with an overall average of 2.01±0.16% (Table 1b). Guinea fowl and Goose both had the highest monocyte values. About 65.91% of total variation in monocyte was due to the influence of species alone (Table 2b), which exerted highly significant (P<0.001) effect on monocyte.

It was observed in the study that specie was a highly significant (P<0.001) source of variation on all haematological parameters with the exception of basophil where it was significant at 5%. The implication of this is that it promises to be a good delineating factor in building the discriminant function for statistical classification.

Table 1a: Mean ± standard error of some hematological parameters.

Species	N	PCV (%)	Hb (g/dL)	RBC (×10^{12}/L)	MCHC (%)	MCH (pg)	WBC (×10^9/L)
Duck	5	28.30±0.35^{b}	9.12±0.19^{c}	12.98±0.33^{b}	32.86±0.24^{cd}	6.98±0.25^{b}	7.74±0.39^{bc}
Guinea Fowl	5	31.44±0.30^{a}	11.20±0.25^{a}	13.78±0.24^{ab}	34.56±0.26^{ab}	8.48±0.22^{a}	9.80 ±0.42^{a}
Goose	5	29.56±0.35^{b}	10.54±0.29^{ab}	14.44±0.30^{a}	34.26±0.32^{abc}	7.46±0.24^{ab}	9.08±0.28^{ab}
Local Chicken	5	29.06±0.45^{b}	11.24±0.32^{a}	13.58±0.30^{ab}	34.74±0.31^{a}	7.20±0.23^{b}	8.88±0.33^{ab}
Quail	5	28.62±0.32^{b}	10.56±0.35^{ab}	13.66±0.31^{ab}	33.30±0.40^{bcd}	6.68±0.30^{b}	8.10±0.51^{bc}
Turkey	5	24.94±0.27^{c}	9.78±0.23^{bc}	10.96±0.28^{c}	32.56±0.41^{d}	6.64±0.30^{b}	6.92±0.26^{c}
Combined	30	28.65±0.38	10.41±0.17	13.23±0.23	33.71±0.20	7.24±0.15	8.42±0.22

PCV = Packed Cell Volume, HB = Haemoglobin, RBC = Red Blood Cell, MCHC = Mean Corpuscular Hemoglobin Concentration, MCH = Mean Corpuscular Haemoglobin, WBC = White Blood Cell. Means with different superscripts within the same Column differ significantly (p<0.05).

Table 1b: Mean ± standard error of hematological parameters

Species	N	Lymphocyte (%)	Neutrophil (%)	Basophil (%)	Eosinophil (%)	Monocyte (%)
Duck	5	57.52 ±0.39^{b}	31.38 ±0.31^{d}	0.00 ±0.00^{b}	3.32 ±0.19^{ab}	1.12 ±0.19^{b}
Guinea Fowl	5	61.36 ±0.39^{a}	34.88 ±0.43^{a}	0.02 ±0.01^{a}	4.10 ±0.19^{a}	2.84 ±0.42^{a}
Goose	5	61.20 ±0.29^{a}	34.60±0.37^{ab}	0.00 ±0.00^{b}	1.62 ±0.18^{c}	2.84 ±0.19^{a}
Local Chicken	5	57.86 ±0.44^{b}	33.18±0.40^{bc}	0.00 ±0.00^{b}	3.02 ±0.27^{b}	2.18 ±0.18^{ab}
Quail	5	61.66 ±0.5^{a}	34.24±0.41^{ab}	0.00 ±0.00^{b}	3.42 ±0.28^{ab}	1.78 ±0.20^{ab}
Turkey	5	56.30 ±0.39^{b}	31.82±0.35^{cd}	0.01 ±0.01^{ab}	3.86 ±0.20^{ab}	1.30 ±0.20^{b}
Combined	30	59.32 ± 0.43	33.35 ±0.29	0.01 ±0.00	3.22 ± 0.17	2.01 ±0.16

Means with different superscripts within the same Column differ significantly ($p<0.05$).

Table 2a: Analysis of variance of some hematological parameters based on specie.

Source	DF	PCV (%)	Hb (g/dL)	RBC ($\times10^{12}$/L)	MCHC (%)	MCH (pg)	WBC ($\times10^{9}$/L)
Species	5	22.67***	3.41***	7.29***	4.30***	2.33***	5.37***
Error	24	0.59	0.38	0.43	0.54	0.33	0.70
R-sq		88.83%	64.99%	78.00%	62.28%	59.31%	61.61%

PCV = Packed Cell Volume, HB = Haemoglobin, RBC = Red Blood Cell, MCHC = Mean Corpuscular Hemoglobin Concentration, MCH = Mean Corpuscular Haemoglobin, WBC = White Blood Cell. *** = $P<0.001$ and * = $P<0.05$

Table 2b: Analysis of variance of hematological parameters based on specie.

Source	DF	Lymphocyte (%)	Neutrophil (%)	Basophil (%)	Eosinophil (%)	Monocyte (%)
Species	5	27.66***	10.95***	0.00*	3.83***	2.76***
Error	24	0.83	0.72	0.00	0.24	0.30
R-sq		87.39%	75.89%	40.84%	76.82%	65.91%

*= $p<0.05$, ***= $p<0.001$, R-sq= coefficient of determination, DF= degrees of freedom.

Serum biochemical properties

Glucose values ranged from 48.10 to 53.70g/dL among the different species with an overall mean of 51.26±0.30 g/dL (Table 3a). Specie was highly significant ($P<0.001$) on Glucose value and accounted for 79.45% of the total variation (Table 4a).

The value for total protein was between 60.40 and 65.60 g/L among all the samples, with an overall mean of 63.13±0.23 g/L (Table 3a). Specie accounted for 68.02% of the total variation and exerted highly significant ($P<0.001$) influence on total protein values (Table 4a).

The range of values for urea was between 11.50 and 15.70 mg/dL among the samples in the study with an average of 13.92±0.20 (Table 3a). About 78.26% of variation in urea value was due to differences in species, whose influence was highly significant ($P<0.001$) on urea levels (Table 4a).

The overall mean for albumin was 31.21±0.25, with a range between 27.80 and 33.80 g/L (Table 3a). Specie was a highly significant ($P<0.001$) source of variation in albumin value and it accounted for 58.26% of the variation in albumin values.

Globulin had values that ranged between 27.80 and 35.3 g/L with an overall average of 31.79±0.36 g/L (Table 3b). Specie exerted a highly significant impact on globulin value and was responsible for 89.76% of the variation in globulin values (Table 4b).

Creatinine was significantly ($P<0.05$) impacted by specie accounting for a meagre 37.23% of the total variation in creatinine level (Table 4b). The mean creatinine value was between 0.80 and 2.50 mg/dL with an average of 1.43±0.08 mg/dL (Table 3b).

Serum glutamic oxaloacetic transaminase (SGOT) had values between 58.60 and 71.90 IU/l across all the samples with a mean of 67.77±0.72 IU/L as presented in Table 3b. Effect of specie accounted for 94.24% of total variation and its influence was highly significant ($P<0.001$) on SGOT (Table 4b).

Serum glutamic pyruvic transaminase (SGPT) values ranged between 34.40 and 47.90 IU/L, with an overall mean of 40.37±0.65 IU/L (Table 3b). Specie was a highly significant (P<0.001) source of variation in SGPT values, and accounted for 90.46% of the total variation in SGPT.

The very high influence of specie on all eight serum biochemical parameters studied also provide a veritable discriminant function for the classification of the six species based on their serum biochemical parameters. With the exception of creatinine whose effect of specie was only significant at 5%, specie exerted highly significant (P<0.001) influence on all other seven serum biochemical parameters.

Table 3a: Mean ± standard error of some serum parameters

Species	N	Glucose (mg/dL)	Total Protein (g/L)	Urea (mg/dL)	Albumin (g/L)
Duck	5	49.70 ± 0.28^{cd}	63.10 ± 0.19^{ab}	13.98 ± 0.19^{ab}	30.48 ± 0.26^{bc}
G.Fowl	5	52.24 ± 0.49^{ab}	63.72 ± 0.35^{ab}	15.04 ± 0.27^{a}	31.68 ± 0.53^{ab}
Goose	5	49.20 ± 0.40^{d}	61.30 ± 0.33^{c}	12.12 ± 0.22^{c}	32.62 ± 0.53^{a}
L.Chicken	5	52.84 ± 0.26^{a}	63.74 ± 0.37^{ab}	14.10 ± 0.27^{ab}	29.58 ± 0.50^{c}
Quail	5	52.74 ± 0.36^{a}	64.38 ± 0.50^{a}	14.76 ± 0.24^{a}	32.04 ± 0.28^{ab}
Turkey	5	50.84 ± 0.36^{bc}	62.38 ± 0.26^{bc}	13.54 ± 0.29^{b}	30.84 ± 0.40^{abc}
Combined	30	51.26±0.30	63.103 ± 0.23	13.92 ± 0.20	31.21 ± 0.25

Means with different superscripts within the same Column differ significantly (p<0.05).

Table 3b: Mean ± standard error of some serum parameters

Species	N	Globulin (g/L)	Creatinine (mg/dL)	SGOT (IU/L)	SGPT (IU/L)
Duck	5	32.56 ± 0.33^{bc}	1.22 ± 0.16^{ab}	69.98 ± 0.50^{ab}	39.60 ± 0.87^{bc}
G.Fowl	5	34.34 ± 0.31^{a}	1.94 ± 0.17^{a}	71.14 ± 0.29^{a}	40.54 ± 0.63^{bc}
Goose	5	28.60 ± 0.29^{e}	1.52 ± 0.23^{ab}	59.66 ± 0.32^{d}	46.94 ± 0.34^{a}
L.Chicken	5	30.82 ± 0.35^{d}	1.26 ± 0.12^{ab}	68.20 ± 0.52^{bc}	40.86 ± 0.34^{b}
Quail	5	33.12 ± 0.23^{ab}	1.16 ± 0.11^{b}	69.70 ± 0.29^{abc}	38.24 ± 0.38^{cd}
Turkey	5	31.32 ± 0.33^{cd}	1.50 ± 0.21^{ab}	67.92 ± 0.72^{c}	36.04 ± 0.50^{d}
Combined	30	31.79 ± 0.36	1.43 ± 0.08	67.77 ± 0.72	40.37 ± 0.65

Means with different superscripts within the same Column differ significantly (p<0.05)
SGOT = Serum Glutamic Oxaloacetic Transaminase, SGPT = Serum Glutamic Pyruvic Transaminase

Table 4a: Analysis of variance of serum parameters.

Source	Df	Glucose (mg/dL)	Total protein (g/l)	Urea (mg/dL)	Albumin (g/l)
Species	5	12.50***	6.19***	5.38***	6.22***
Error	24	0.67	0.61	0.31	0.93
R-sq		79.45%	68.02%	78.26%	58.26%

R-sq = Coefficient of determination, DF= degrees of freedom *= p<0.05, ***= p<0.001.

Table 4b: Analysis of variance of serum parameters.

Source	df	Globulin (g/l)	Creatinine (mg/dL)	SGOT (IU/l)	SGPT (IU/l)
Species	5	20.20***	0.42*	85.95***	67.31***
Error	24	0.48	0.15	1.10	1.48
R-sq		89.76%	37.23%	94.24%	90.46%

SGOT = Serum Glutamic Oxaloacetic Transaminase, SGPT = Serum Glutamic Pyruvic Transaminase. *= p<0.05, ***= p<0.001.

Cluster analyses

The cluster analysis was based on the Linnean classification of three families (Phasinidae, Numidae and Anatidae) as depicted in Figure 1.

In the cluster analysis for haematological properties (Figure 2), third cluster comprised Chicken and Quail that had a similarity of 61.52% and a distance of 2.81. This third cluster was joined by Goose that had a distance of 3.20 to the previous two occupants (Chicken and Quail) of that cluster and a similarity of 56.19%. The second cluster was solely occupied by Guinea Fowl with a similarity of 34.60% and a distance of 4.78 relative to the earlier three species in the third cluster. The first cluster is comprised of Turkey and Duck, while the two members of the cluster recorded a similarity of 60.38% and distance of 2.90 between the two, the distance between the first cluster and the other two earlier clusters was 7.31 and similarity was 0.00%.

Comparison of this dendrogram with the earlier classification of Linnaeus (1758) in Figure 1 indicated that the only difference between the two was the juxtaposition of Turkey and Goose respectively from the Phasinidae and Anatidae family in this new speciation based on haematological parameters. This calls for further investigation of the two birds at the molecular level to assess their similarities or otherwise.

Similarly, clustering of birds using serum biochemical parameters followed the same trend as classification using the haematological properties albeit with varying similarities and distances within and between the three clusters as earlier enunciated. The similarity between the third cluster that comprised Chicken and Quail (Figure 2) was 61.52% with a distance of 2.81, while the adjoining Goose in the third cluster had a distance of 3.20 and similarity of 56.19 to the previous two cluster three members. Guinea Fowl was alone in the second cluster with a similarity and distance of 34.60% and 4.78 to the third cluster respectively. Duck and Turkey both belong to the first cluster with a similarity and distance of 60.38% and 2.90 between the two bird species, but however had a similarity of 0.00% and distance of 7.31 to the other two previous clusters.

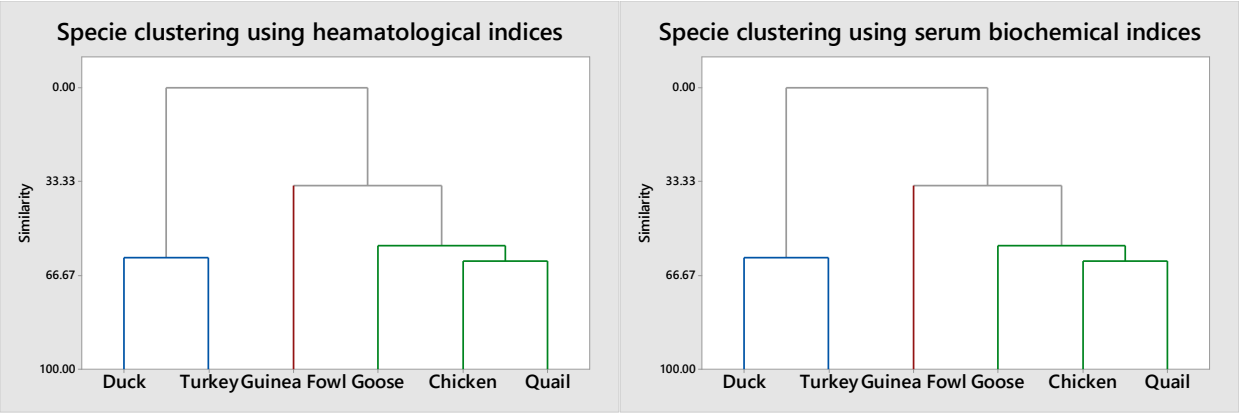

Figure 2: Species clustering of birds based on haematological and serum biochemical properties

Discriminant analysis

For haematological parameters, the largest squared distance was observed between Goose and Turkey (Table 5), while the least was recorded between Guinea Fowl and Duck. However, serum biochemical parameters recorded the largest squared distance between Goose and Guinea Fowl and the least between Turkey and Quail.

Using both set of parameters, the accuracy of reclassification was 100% in both instances, where all species in the test data were appropriately classified to their respective specie without any misclassification. This is a pointer to the fact that the differences in both haematological and serum biochemical parameters due to specie is a veritable platform for specie identification and further reinforce the need for molecular evaluation of the genetic similarities among the species, especially Turkey and Goose vis a vis their classification as

Phasinidae and Anatidae respectively by Linneaus (1758).

Table 5: Squared distance between species based on haematological and serum biochemical parameters

	Duck	Guinea Fowl	Goose	Local Chicken	Quail	Turkey
Duck		28.05	591.70	59.07	31.81	41.89
Guinea Fowl	46.27		700.36	94.46	32.09	32.85
Goose	144.47	132.36		486.89	646.08	586.02
Local Chicken	54.78	72.42	80.70		52.97	84.65
Quail	52.56	68.02	63.05	49.03		30.18
Turkey	111.67	199.33	330.51	116.39	160.11	

Values below the diagonal are for haematological, and those above the diagonal are for serum biochemical parameters

Principal component analyses

Further statistical analysis was conducted to investigate the contribution of each haematological and serum biochemical parameter to the observed differences due to species, with a view to identify the principal components with the highest loadings.

Three principal components in the haematological parameters accounted for 91 percent loading of the eigenvalue in the correlation matrix (Figure 3), with White Blood Cell (WBC), Basophil and Lymphocytes having the highest. The implication of this is that the three components are respectively responsible for defense against infection, eliciting potent effector functions in allergic diseases and type 1 hypersensitivity and make antibodies that kill tumour cells. Thus, the haematological parameters with the highest loadings are those responsible for the immunity of the animals.

The serum biochemical parameters had three principal components that accounted for 88.7 percent of the eigenvalue loadings (Figure 3). Specifically, the serum biochemical parameters were Urea, Creatinine and Glucose. Both Urea and Creatinine are markers for both liver and kidney and gave an indirect and rough measurement of renal function by measuring the amount of urea nitrogen in blood and is directly related to excretory function of kidney, while Glucose is the main source of chemical energy for cell functions, and serves as the primary metabolic fuel of mammals and the universal fuel of the fetus.

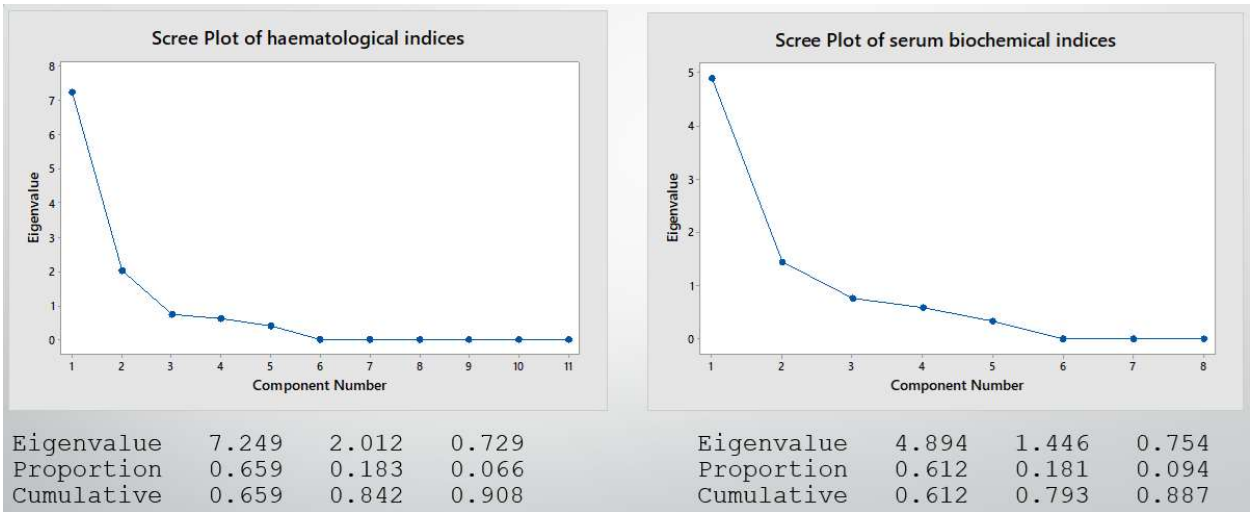

Figure 3: Principal Component Analysis of Haematological and Serum Biochemical Properties

Conclusions and Recommendations

The following can be concluded from the study:

- Effect of specie was mostly highly significant ($P<0.001$) on all haematological and biochemical parameters studied, with the exception of specie effects on Basophil and Creatinine which was significant at 5 percent. Thus, specie provided a veritable platform for statistical discrimination of the six species investigated, based on their haemotological and serum biochemical parameters.
- The cluster analysis equally produced three clusters in the dendrogram which had Duck and Turkey in cluster one, Guinea Fowl was alone in second cluster while Goose, Chicken and Quail were together in the third cluster. This was similar to the Linnean binary classification except that the positions of Turkey and Goose were juxtaposed in the statistical analysis.
- The discriminant function had a 100 percent accuracy in reclassifying the animals to their respective specie using both haematological and serum biochemistry indices.

It is thus recommended that further investigation of the genetic similarity among the species be conducted in the light of the conventional Linnean taxonomy currently in use, which classified Turkey as a member of the Phasinidae family along with Chicken and Quail on one hand and classified Goose as Anatidae family with Duck.

References

Ajayi, O. O., Adeleke, M. A., Sanni, M. T., Yakubu, A., Peters, S. O., Imumorin, I. G, Ozoje, M. O., Ikeobi, C. O., and Adebambo, O. A. 2012. Application of principal component and discriminant analyses to morpho-structural indices of indigenous and exotic chickens raised under intensive management system, *Tropical Animal Health Production,* 44: 1247–1254.

Ali, Z.I., Obese, F.Y., Naazie, A., Abdul-Rahman I.I., Ayizanga, R.A. 2019. Effect of Age and Sex on Hematological Profiles in Indigenous Helmeted Guinea Fowl (Numida Meleagris) in the Guinea Savannah Zone of Ghana. *Journal of Animal Husbandry and Dairy Science,* 3 (1): 14-19.

Almeida, E. C. de J., Carneiro, P. L. S., Nunes, L. A., Malhado, C. H. M., Campos, B. M., Figueiredo, N. E. M., Farias Filho, R. V., and de Bittencourt, T. C. B. dos S. C. 2023. Breed characterization of native chickens based on their head shape and size. *DELOS: Desarrollo Local Sostenible*, 16(45): 1876–1890. https://doi.org/10.55905/rdelosv16.n45-026

Chhabra, G. 2018. Automated hematological analyzers: Recent trends and applications. *Journal of Laboratory Physicians,* 10(1):15-16.

Elagib, H.A.A., Elamin, K.M., Ahmed, A.D.A. and Malik, H.E.E. 2012. Blood biochemical profile of males and females of three indigenous chicken ecotypes in Sudan. *Journal of Veterinary Advances.* 2(12): 568-572.

Everitt, B.S., Landau, S. and Leese, M., 2001. Cluster analysis. 4th edition, (Arnold Publisher, London).

Francesch, A., Villalba, I. and Cartañà, M. 2010. Methodology for morphological characterization of chicken and its application to compare Penedesenca and Empordanesa breeds. *Animal Genetics*. 48. 79-84. Doi: 10.1017/S2078633610000950.

Isaac, L. J., Abah, G., Akpan, B. and Ekaette, I. U. 2013. Haematological properties of different breeds and sexes of rabbits. Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria. 24-27

Kokore, B.A., Bleyere, N.M., Kamagate, S. and Yapo, P.A. 2021a. Hematological profile of broiler and local chicken in korhogo côte D'Ivoire. *International Journal of Agriculture, Environment and Bioresearch*. 16 (2): 14-23.

Kokore, B.A., Bleyere, N.M., Kamagate, S. and Yapo, P.A. 2021b. Investigation of hematological and blood biochemical properties in guinea fowl of korhogo côte D'Ivoire. *International Journal of Bioscience.* 18(6): 176-185.

Kütting, F., Schubert, J., Franklin, J., Bowe, A., Hoffmann, V., Demir, M., Pelc, A., Nierhoff, D., Töx, U. and Steffen, H.M., 2017. Insufficient evidence of benefit regarding mortality due to albumin substitution in HCC-free cirrhotic patients undergoing large volume paracentesis. *Journal of Gastroenterological Hepatology,* 32(2):327-338.

Linnaeus, C. 1758. Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata [10th revised edition], 1: 824 pp.

Meshabaz, R.A., Sulaimon, K.M., Hussein, S.H., Berwey, M.S., Salen, J.H. Ahmad, S.A. and Muhammad, N. 2017. Hematological study in three lines of quail and their crosses. *Scientific Journal of University of Zakhos.* 5(3): 249-253.

Mekonnen, K.T., Lee, D.H., Cho, Y.G., Son, A.Y., and Seo, K.S. 2023. Biometric Trait Characterization and Multivariate Discriminant Analysis of Traditionally Bred Autochthonous Chickens in Ethiopia. *Agriculture* 13: 2142. https://doi.org/10.3390/agriculture13112142

Minitab 17 Statistical Software 2000. Computer Software. Minitab, Inc., State College, PA. http://www.minitab.com/

Palm, L., Stephens, L., Bengtsson, H.L. and Broome H.E. 2016. Minimal hematology analyzer plus blood smear digital imaging/analysis provides better clinical hematology results than a complex hematology analyzer alone. *Blood,* 128(22): 4731.

Rodkey, F.L. 1965. Direct Spectrophotometric Determination of Albumin in Human Serum. *Clinical Chemistry*, 11(4): 478–487. https://doi.org/10.1093/clinchem/11.4.478

Sagar A. 2020. Difference between Serum and Plasma. Available at: https://microbiologyinfo.com/difference-between-serum-and-plasma/#

Scoffin K. 2014. Hematology Analyzers- From Complete Blood Counts to Cell Morpology. Available at: https://www.labcompare.com/10-Featured-Articles/162042-Hematology-Analyzers-From-Complete-Blood-Counts-to-Cell-Morphology/

Stiles, W. 2017. Poultry manure management. Available at: https://www.researchgate.net/publication/321001761.Poultry_manure_management/stats Accessed on the 10th June 2020

Wenhao, Z. Xudong, M., Fang-Feng, X. and Ziquan, D. 2016. Development of management software for new automatic hematology analyzer based on PC/Window. IECON, 42nd Annual Conference of the IEEE Industrial Electronics Society. 96-101.

Date received: 7th April, 2024

Date accepted: 22nd July, 2024.