PHENOTYPIC CORRELATION BETWEEN MORPHORMETRIC BODY PARAMETERS AND CARBORNIC ANHYDRASE TYPE IN ADULT NIGERIAN LOCAL CHICKENS

BY

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CERTIFICATION

This is to certify that this project was carried out by ADEPOJU Grace Temilade (18/10AC087) in the department of Animal Production, Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria.

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DEDICATION

This research project is dedicated to Almighty God and to my beloved parents.

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My appreciation goes to God Almighty, the beginning and the end, the ruler of all, from whom all strength and help comes from, for He is my source of knowledge and confidence. My gratitude to my admirable supervisor, Prof. F.E Sola-Ojo for her patience, motherly care, advice and support, you are simply the best. I commend your effort, ma. God will bless and reward you. And also, to all my lecturers, thank you all for the moral lessons and knowledge that you have instilled in me, thank you Sir/Ma.

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ABSTRACT

Carbonic anhydrase (Ca) polymorphism in Fulani and Yoruba adult local chickens of both sexes comprising of 50 Fulani Ecotype chicken from Gaa Fulani of Irepodun Local Government in Kwara State and 50 Yoruba Ecotype Chickens collected from backyard poultry farmer in Ilorin South Local Government of Kwara State, Nigeria was determined using cellulose acetate electrophoresis. Blood samples were collected from one hundred local chickens. Population parameters were estimated using PopGen 32.0 software. The result obtained indicated that all the protein markers were polymorphic revealing three distinct genotypes of Ca (FF, FS and SS). There were significant differences between Carbonic anhydrase and Body Weight, Body Girth and Wing length. CA was positively significantly correlated with breed type, KL and BG whereas; it was negatively significantly correlated with BW, BL, BW, SKL, WL, BKL, DML and BL. In Function1, the best discriminant contributes to overall body parameters measured is BH with a value of 0.433 followed by KL 0.325 and Bill length 0.588. In Function2, the best discriminant contributes to overall body parameters measured is BG with a value of 0.4601 followed by SL 0.489 and SKL 0.339. Morphological differentiation on cluster classification as displayed by function 1 showed that carbonic anhydrase genotype FF was separated from genotype FS and SS. Prevalence of genotype FF, FS and SS at their respective locus is suggestive of their relevancies to the survival and adaptability of the Fulani and Yoruba chicken studied to its natural habitat.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

There has been a call for substantial increase in the intake of protein of animal origin in developing countries like Nigeria. This can be achieved through the production of animals that are prolific and have short generation interval (Abeke et al., 2003).

The local or indigenous chickens (Gallus gallus) is a general term given to animals or birds kept in the wide-ranging or scavenging in the free-range. They are multipurpose unimproved birds with no identified description (Mengesha, 2012). Farmers in Africa gave these chickens names like; bush chickens or African hen (Gueye, 2009).

They require little care and adapt well to rural conditions than exotic chickens (Gueye, 2010). They appear to be generally heterogeneous with no specific colour pattern, comb types, feather patterns, body sizes, and no descriptive both in phenotype and genotype also reflecting the genetic diversity and unique characteristics specific to each breed and region. The native chickens constitute about 80 % of the 120 million poultry birds (Zuber, 2010). They are known for their adaptation superiority in terms of their resistance and tolerance to common endemic poultry diseases and fluctuations in both feed quality and quantity, hence requiring minimum or no input (Desta and Wakeyo, 2012).

The productivity of indigenous chicken is low compared to the exotic strains as they lay less than 100 eggs per hen per year and weigh between 0.80 and 1.80 kg at maturity which is not sufficient to meet the needs of the growing population (Mahoro et al., 2017). Their low productivity has hindered their potential to improve the livelihood of small holder farmers, thus failing to contribute considerably to eradication of rural poverty (Habimana et al.,2019).

However, improvement of so economic traits of these birds would go a long way to arrest the situation. According to a recent study (Duenk et al., 2023), traits within livestock populations are frequently interconnected rather than independent, exhibiting various correlations.

The Yoruba ecotype and Fulani ecotype are the two notable indigenous chicken ecotypes in Nigeria. They demonstrate broodiness and take care of their chicks after hatching. There is limited research on welfare, productivity, and maternal behaviors of these two ecotypes. Yoruba has the potential to be selected as an egg-type chicken because of its morphology (Osaiyuwu et al., 2009).

Ige (2013) described high genetic variation in this chicken, which sometimes reflects the ability to adapt to environmental changes and stress. The Fulani ecotype is found in the dry savannahs (Guinea and Sahel savannah) while the Yoruba ecotype is found in the forest zones. Both Yoruba and Fulani are good scavengers and possess excellent resistance against endemic diseases. They are recognized for their hardiness, adaptability and survivability in various environmental conditions. Fulani ecotype is known to be superior in body weight, body length and height at withers compare to any other chicken ecotype in Nigeria. (Chineke, 2017). Fulani ecotype are domestic birds, and they are mostly reared around the home of Fulani people. They are good layer hen and their meat quality is rated higher when compared to other ecotype chicken present in Nigeria, and they are also not prone to diseases.

Phenotype is defined as all the observable characteristics of an organism that result from the interaction of its genotype (total genetic inheritance) with the environment. (Britannica, 2023). The phenotypic expression offers an immediate look into the genetic potential of the animal, phenotypic together with genetic and environmental trend in body weight and body size are important because they allow for the evaluation of the efficiency of selection and management schemes. Phenotypic correlation is the correlation between records of two traits on the same

animal and is usually estimated by correlation statistic. According to Ikpeme et al. (2016), the importance of estimating correlation between characters is to measure the extent of association between one trait and the other.

In their investigation of phenotypic correlations among morphological traits in animals, Jeffrey et al. (2014) found similar mean correlations around 0.5 in vertebrates and hemimetabolous insects, contrasting with higher correlations (mean 0.84) observed in holometabolous insects. According to Murari et al. (1991), Phenotypic correlation is shaped by both genetic (genotypic) and environmental factors. It characterizes the relationship between observable traits (phenotypes) of organisms, which are subject to influences from genetic and environmental interactions. Estimation of phenotypic correlations often relies on data from randomized complete block designs, where genotypic effects and plot errors conform to a bivariate normal distribution. The resulting phenotypic correlation coefficient serves as a measure of the strength of association between two traits within a population of genotypes. Analysis of genetic markers based on protein variants detected by electrophoretic method has been a tool for studying genetic differentiation among populations or phylogenic studies. It had become equally important in biosystematics and evolutionary studies.

Carbonic anhydrase is an enzyme that plays a crucial role in various physiological processes. Carbonic anhydrase helps speed up the conversion of carbon dioxide into bicarbonate ions, thereby aiding in the regulation of pH levels in the body.

According to Claudiu and Supuran (2016), the structure and function of carbonic anhydrases (CAs) are essential for various biological processes, including the interconversion between carbon dioxide (CO2) and bicarbonate (HCO3-) as well as other hydrolytic reactions. CAs are a superfamily of enzymes classified into six genetic families: α-, β-, γ-, δ-, ζ-, and η-CAs. These enzymes play crucial roles in pH regulation, ion transport, respiration, and biosynthetic

processes. The active site architecture of CAs is characterized by a metal ion (usually zinc) coordinated by specific amino acid residues, such as histidine (His) and cysteine (Cys). The metal hydroxide nucleophilic species within the active site facilitates the catalytic conversion of CO2 to HCO3-, contributing to the efficiency of CAs as catalysts. CAs can be activated or inhibited through various mechanisms. Some classes of CAs incorporate moieties in their molecules that participate in proton transfer processes, affecting the enzyme's activity. Additionally, inhibitors targeting the zinc ion in the active site can modulate CA activity (Claudiu & Supuran, 2016).

Different classes of CA inhibitors target specific regions of the enzyme to disrupt its activity and modulate physiological processes. Overall, the diverse genetic families of CAs, their unique active site architectures, and regulatory mechanisms highlight the importance of these enzymes in biological systems and their potential as therapeutic targets (Claudiu & Supuran, 2016).

1.2 PROBLEM STAEMENT

There is growing interest in the Yoruba and Fulani ecotype chicken as a genetic resource for poultry breeding programs aiming to develop more resilient and sustainable production systems. This selection of FEC for breeding and research purposes is hindered by the unavailability of sufficient data about their blood protein polymorphism which is a useful tool for studying genetic variations of the breed.

1.3 JUSTIFICATION:

Examining the Phenotypic correlation between morphometric body parameters and carbonic anhydrase type in Nigerian local chickens can provide valuable insights into the physiological adaptation also health status, metabolic efficiency of these indigenous poultry breeds, adaptations to their local environment and management conditions. The detection of variation at protein loci will provide immense contribution to the characterization of the Yoruba and Fulani and would also provide insights into the genetic diversity existing among them.

1.4 OBJECTIVES OF THE STUDY

The objective of the study was to:

- Characterize Fulani and Yoruba chicken using Carbonic anhydrase blood protein loci
- Determine the phenotypic correlations between specific morphometric traits and carbonic anhydrase type to elucidate potential relationships and their implications for the physiological performance of Fulani and Yoruba chicken.
- Determine morphometric traits that differentiate the Fulani and Yoruba chicken using discriminant analysis

CHAPTER TWO

LITERATURE REVIEW

2.1 ORIGIN OF CHICKEN

Chickens are the most popular poultry worldwide and are now used for both meat and egg production. This includes birds such as chicken, turkey, duck, goose, ostrich, quail, pheasant, guinea fowl, and peafowl. The origin of domestic chickens is a topic of great interest and has been studied extensively. The domestication of chickens is believed to have originated from the red junglefowl (Gallus gallus) native to regions spanning from Southeast Asia to Southwest China. Chicken domestication was previously thought to have occurred in the Indus Valley around 2000 BC (Hata et al., 2021).

Several studies have contributed to our understanding of the origin and genetic diversity of domestic chickens and their wild progenitors. For example, a study by Wang et al. (2020) analyzed 863 genomes to reveal the origin and domestication of chickens. Another study by Liu et al. (2006) suggested multiple maternal origins of chickens, indicating a complex domestication history.

In Thailand, where chicken domestication may have occurred, red junglefowl populations exhibit high genetic diversity, and Thai indigenous chickens are reared under free-range conditions by small-scale farmers. A study by Hata et al. (2021) aimed to elucidate the origin of domestic chickens and their evolutionary history by investigating the genetic diversity of wild red junglefowl and indigenous chickens in Thailand.

Overall, the domestication of chickens is a significant milestone in human civilization, with the process likely occurring independently across multiple regions in Southeast Asia. The genetic studies provide valuable insights into the ancestral populations, genetic diversity, and

evolutionary history of domestic chickens, shedding light on their fascinating origin (Hata et al., 2021).

2.2 LOCAL CHICKEN BREEDS

An Ecotype is a population that has evolved to fit into a particular environment. Ecotypes of chickens refer to genetically distinct populations adapted to specific environmental conditions and production purposes. These adaptations manifest in various phenotypic traits, including growth rate, egg production, disease resistance, and behavioral attributes.

Local or indigenous chickens are more common particularly in developing and underdeveloped countries. Local chickens are preferred over exotic chicken breeds because of their juicy meat. Local Chickens helps to diversify income, provides high quality protein contribute to fertilizer production, and acts as form of household financial security and insurance (Besbes, 2009). A study in the Niger delta showed that family poultry husbandry contributes 35% of the income of household's women and it is estimated at about 25% and 50% of Nigerian minimum wage and per capita income, respectively, underscoring significance of indigenous poultry husbandry practices (Branckaert et al., 2000). They also sell at a cheaper price (Liswaniso et al., 2020, Mengesha, 2012). Therefore, there is a high demand for indigenous chicken products, including eggs and meat. It is estimated that local chickens constitute 80% of poultry production in sub-Saharan countries (Desha et al., 2016) with Nigeria known to have the highest number of local chickens with an estimated population of 180 million (Pym, Guerne-Bleich, & Hoffmann, 2006).

Though local chickens are slow grower and poor layers of small sized eggs they are, however, they excel as nurturing mothers and diligent sitters (Tadelle, 2003), proficient foragers, and hardy, and possess natural resistance against common diseases (Mtambo, 2000; Dessie et al.,

2011). The small body size of native chickens is considered advantageous in tropical and subtropical environment. These chickens typically scavenge around the homestead during day time, where they eat kitchen waste, leftover cereal like rice, wheat, pulses, green vegetation, insects, and other available feed stuff. These scavenged feedstuffs contribute to the production of high quality and cheap source of animal protein of native birds. Throughout the world indigenous/native breeds of chicken are reported. (Adeleke et al., 2011) reported the performance of Nigerian local chicken which consist of normal feathers, frizzle, and Naked neck. (Sola-Ojo & Ayorinde, 2011) documented the Fulani ecotype of Nigeria.

The Fulani ecotype chicken, native to the drier regions of Nigeria such as the Savannahs, Montane areas, and cattle Kraals in the North and South-West, weighs approximately 1.75-2.5 kg when mature and is raised extensively (Mathew et al., 2021). This breed is renowned for its resilience, disease tolerance, and adaptability to harsh climatic conditions, suggesting potential for genetic enhancement in both meat and egg production. Although its exact origin remains uncertain, it's speculated that the breed may have emerged from a crossbreeding initiative involving native fowls and Rhode Island Red chickens. The nomadic lifestyle of the Fulani people has played a pivotal role in preserving the genetic legacy of this chicken variety.

Research indicates a broad phenotypic diversity within Fulani chickens, reflecting a reservoir of valuable genetic traits essential for enhancing breeding initiatives. Traits like plumage color, body weight, and semen quality are recommended for consideration in breeding schemes to optimize characteristics like semen motility and concentration (Mathew et al., 2021). Particularly, selecting lighter plumage Fulani cocks with specific traits could prove beneficial for multiplication programs.

Given the unique attributes and genetic potential of the Fulani ecotype, conservation efforts are imperative to safeguard its genetic inheritance. Preserving the breed's genetic quality amidst evolving environmental dynamics and modern agricultural practices is critical for its sustained viability (Mathew et al., 2021). The observed morphological variations offer substantial opportunities for refining breeding practices and bolstering conservation endeavors. By leveraging these variations effectively, breeders and conservationists can contribute significantly to enhancing the genetic diversity and overall sustainability of the Fulani ecotype chicken breed.

The study comparing the genetic characteristics of the Fulani and Yoruba ecotypes in Nigeria utilized protein markers, specifically haemoglobin (HB) and carbonic anhydrase (CA), to assess their genetic relatedness (Ige et al., 2013). Findings revealed a close genetic affinity between the two populations, albeit with some disparities in allele frequencies. Further investigation into additional protein markers and molecular-level analysis is recommended for a comprehensive understanding of their genetic makeup and relationship.

Frizzle Feather (Asa) and Necked neck (Abolorun)

The Frizzle is also a breed of chicken that is characterized by its unique curled or frizzled plumage. While the frizzle gene can be seen in various breeds, like the Pekin and Polish. The African Naked Neck is thought to have originated in Malaysia (Khobondo et al., 2015) and two types exist. The first is considered purebred with a complete naked neck, while the second is considered to be not of purebred, showing a tassel at the front of its neck. For frizzle feather, the Frizzle is distinguished as a distinct breed in several European countries and Australia. In the United States, frizzled chickens are not classified as a distinct breed, instead they are assessed based on the standards of the breed they belong to (Ekarius, 2007).

Necked neck, Turkan is a breed that undeniably lives up to its name. It has no feathers on the neck, vent area or face, however, it leaves a feathered crown giving it a very unusual appearance which is caused by a genetic mutation (incompletely dominant gene). Naked Neck breed is less advantageous in cool or colder environments (Rajkumar et al. 2011). Both chickens are docile in temperament and are easy to handle. Frizzle Feather chickens are not renowned for their egg-laying capabilities compared to some other breeds. However, they still produce a reasonable number of eggs, making them suitable for backyard poultry farming. Due to Abolorun reduced feathers, they are not particularly effective brooders. However, meat (the breed is known for its flavorful meat) and eggs (the hens are good layers and produce brown eggs) from indigenous naked neck chickens are sometimes prescribed by traditional healers (Siripurapu and Das n.d.). Indigenous naked neck chickens in Nigeria (Fayeye et al.,2006) are smaller than normally feathered

2.3 YORUBA ECOTYPE CHICKENS

Yoruba ecotype chickens are indigenous to the southwestern region of Nigeria and are renowned for their adaptability to the humid tropical climate and traditional farming systems. These chickens play a vital role in the socio-economic fabric of rural communities, providing a source of protein, income, and cultural significance. Understanding the genetic diversity, phenotypic characteristics, and production performance of Yoruba ecotype chickens is essential for sustainable utilization and conservation efforts.

Genetic diversity is a cornerstone of Yoruba ecotype chickens, their resilience and adaptability to diverse environmental conditions. In a study, genetic diversity of Fulani and Yoruba Nigeria indigenous chickens was studied. High genetic diversity, low differentiation between populations in savannah zone, influenced by historical migration patterns, selection pressures, and environmental factors (Ige et al., 2017). Microsatellite marker analyses have identified

distinct genetic clusters and population substructures, indicating the presence of unique genetic signatures and potential for selective breeding (Fayeye et al., 2010).

Yoruba ecotype chickens exhibit a wide range of morphological and behavioral traits adapted to their local environment and production systems. They are characterized by medium to large body sizes, with variations in plumage colors and patterns, including black, brown, and speckled (Olawumi et al., 2015). They are also known for their dual-purpose attributes, combining moderate egg production with acceptable meat quality, making them well-suited for backyard farming and free-range systems.

Despite their adaptive traits, Yoruba ecotype chickens generally exhibit lower productivity compared to commercial breeds. However, studies have shown considerable variability in production performance among Yoruba chicken populations, suggesting potential for improvement through selective breeding and management interventions (Rasheed et al., 2023).

Picture of Yoruba Ecotype cock

2.4 FULANI ECOTYPE CHICKENS

Fulani Ecotype Chickens are mostly from the Sahel and Guinea savannah regions of Nigeria; however, others are from the mountainous areas (Adeleke et al., 2022). The Fulani ecotype chicken is indigenous to Nigeria's Middle Belt and Northern regions. They are recognized to

outperform (they are good layer hen and their meat quality is rated higher) when compared to other ecotype chicken available in Nigeria, also in terms of body weight, body length, and wither height (Chineke, 2017). Fulani Ecotype chickens are a unique breed native to West Africa, particularly prevalent in the Fulani ethnic group's regions. The heavy weight or hefty Ecotype Chicken is the common name for Fulani Ecotype chickens (Sola-Ojo and Ayorinde, 2011). These chickens play a crucial role in the agricultural and economic livelihoods of rural communities, serving as an important source of protein, income, and cultural significance.

Fulani Ecotype chickens are characterized by their adaptive traits, resilience to harsh environmental conditions, with the ability to thrive under extensive management systems. They typically exhibit a range of plumage colours, including black, brown, and white, with variations in feather patterns. These chickens are well-adapted to scavenging for food, where there are nutritional advantages with the exposure to sunlight (Vitamin D) and pasture; good source of carotene also have access to insects, which are good sources of protein contributing to their sustainability in low-input farming systems and veterinary care and treatment as they are not prone to diseases and have a higher chance of living long. Fulani ecotype chickens possess desirable traits that make them valuable. They exhibit high levels of disease resistance, tolerance to heat stress, and reproductive efficiency which are advantageous in resource-limited environments. Their ability to utilize locally available feed resources and minimal management inputs makes them cost-effective and sustainable for smallholder farmers.

Fulani ecotype chickens also have the potential to contribute to genetic diversity conservation and preservation of indigenous poultry breeds.

Picture 2: Chickens exposed to sunlight (Vitamin D)

Picture: Hen with chicks seen in a Fulani settlement scavenging for food.

2.5 PHENOTYPIC CORRELATION

Phenotype is defined as all the observable characteristics of an organism that result from the interaction of its genotype (total genetic inheritance) with the environment. (Britannica, 2023). The phenotype of an animal is often used in its characterization, it is usually a yardstick to rank livestock into grades. The phenotypic expression offers an immediate look into the genetic

potential of the animal, phenotypic together with genetic and environmental trend in body weight and body size are important because they allow for the evaluation of the efficiency of selection ` and management schemes. Phenotypic correlation is the correlation between records of two traits on the same animal and is usually estimated by the product- moment correlation statistic (Searle, 1961).

Animals are commonly assessed based on their morphology, blood protein profiles, and molecular characteristics. Phenotypic characters are those characters/traits that can be viewed and measured externally in animal species which includes visual appraisal and measurements of body dimensions. Genetic characterization is the embodiment of all available knowledge both published and unpublished which contributes to reliable prediction of genetic performance of an animal species in a defined environment.

2.6 FACTORS INFLUENCING BODY CHARACTERISTICS OF CHICKENS

The genotype-phenotype (GP) relationship is best seen as a connection between two differences, one at the genetic level and one at the phenotypic level. This perspective allows for a deeper understanding of how genetic variations manifest as phenotypic differences and how these differences can be analyzed and interpreted in the context of biological diversity. By considering genotypes and phenotypes as distinct entities with their own variations, researchers can better grasp the complexity of the relationship between genetic information and observable traits, shedding light on the mechanisms underlying biological diversity and evolution (Orgogozo, Morizot, & Martin, 2015).

Phenotypic traits are the external features of living organisms which could be metric or non-metric. Non-metric traits are measured subjectively i.e visual assessment relies on subjective evaluation, whereas metric traits necessitate precise measurements of parameters such as height, length, and width. Phenotypic expression is the result of the genotype, environment and

interactions between genotype and environment. Animals are often characterized based on their phenotypic characters which include coat colour, horns (shape and size), hair, live weight and body measurements. Physical characteristics such as fur or coat colour, horn shape, hair texture and other observable traits.

Body weight is a function of size of the animal and its condition. It is an important attribute of farm animals for making management, health, production and marketing decisions. Smallholder farmers in the developing world are characterized by poor resource investment, therefore management decisions at this level are based on trial and error or on visual appraisal.

2.7 SIGNIFICANCE OF WEIGHT IN ADULT NIGERIAN LOCAL CHICKENS

The weight of adult chickens plays a vital role in determining their commercial value. Larger and heavier chickens often fetch higher prices in the market. The weight of adult chickens directly influences the yield of meat obtained from each bird. Heavier chickens usually result in more substantial meat portions, which is crucial for meeting market demands and maximizing profits in the poultry industry. While egg production is primarily focused on the number of eggs laid, the overall health and body condition of the hen, including weight, can impact egg quality and production efficiency. A healthy weight in adult hens can contribute to optimal egg production and quality. The weight of adult chickens is a critical factor in selecting breeding stock for desirable traits such as growth rate, feed conversion efficiency, and overall productivity. Breeding heavier birds can lead to offspring with improved growth potential. Monitoring the weight of adult chickens is essential for assessing their overall health and welfare. Sudden weight loss or gain can indicate underlying health issues, nutritional deficiencies, or environmental stressors that need to be addressed promptly to ensure the well-being of the birds.

The weight of adult chickens is closely related to their feed efficiency, as heavier birds may require more feed to maintain their body condition and support growth. Monitoring weight can help optimize feed management practices for efficient poultry production.

In conclusion, the weight of adult Nigerian local chickens serves as a valuable indicator of their commercial value, productivity potential, health status, and overall performance in poultry farming operations. Monitoring and managing the weight of adult chickens are important for successful poultry production and ensuring profitability in the industry.

BODY LINEAR MEASUREMENT can be used in assessing growth rate, weight, feed utilization and carcass characteristics of farm animals (Alderson 1999). Linear body measurement is necessary in the formulation of programmes for selection and improvement of livestock and in predicting the direct and correlated responses due to selection (Alade et al., 1999). Body measurements, because of their correlation with live weight, gives the livestock traders and buyers a fair idea of the value of their livestock in the absence of scales which is the case in most livestock markets in Nigeria.

The relationship between traits may be genetic, environmental or phenotypic. The relationship between traits calculated from observed or measurable values, is called phenotypic correlations. Relationships between body weight and linear body measurements are important in genetic improvement strategies. In an organized livestock marketing system, weight ought to be taken to determine the market prices of animals. This requires the use of weighing scales which, quite often may not be available to the rural livestock and poultry farmers/traders. There are other indirect methods of assessing body weights in animals without recourse to the use of weighting scale

2.8 CARBONIC ANHYDRASE TYPE

Carbonic anhydrase (CA) is a family of metalloenzymes (the active site: zinc ion) that catalyze the rapid inter-conversion reversible hydration of carbon dioxide. Into bicarbonate and proton that occurs slowly in the absence of a catalyst. (Badger and Price, 1994). They are involved in various physiological processes, including respiration, pH regulation, and electrolyte transport.

Carbonic anhydrase plays a crucial role in maintaining the acid-base balance in the body and facilitating important processes like respiration and kidney function.

Without carbonic anhydrase, chickens would have difficulty regulating pH levels, which could lead to disruptions in various bodily functions. It could affect their ability to efficiently exchange gases, like oxygen and carbon dioxide, during respiration. Additionally, the kidneys might struggle to properly filter and regulate electrolytes in the body.

The reaction of carbonic anhydrase is one of the fastest of all enzymes, and its rate is typically limited by the diffusion rate of its substrates. Typical catalytic rates of the different forms of this enzyme range between $10^4$and 10^6 reactions per second (Lindskog, 1997). The reverse reaction is relatively slow in the absence of a catalyst.

An anhydrase is defined as an enzyme that catalyzes the removal of a water molecule from a compound, and so it is this "reverse" reaction that gives carbonic anhydrase its name, because it removes a water molecule from carbonic acid.

2.9 HOW CARBONIC ANHYDRASE INFLUENCE THE BODY PHYSIOLOGY OF CHICKENS

1. Acid-Base Imbalance: Carbonic anhydrase plays a crucial role in maintaining the acid-base balance in chickens. Inhibition of carbonic anhydrase can disrupt this balance, leading to an

accumulation of carbon dioxide and a decrease in bicarbonate ions. This can result in metabolic acidosis, which can have detrimental effects on various physiological processes.

2. Impaired Gas Exchange: Carbonic anhydrase is involved in the conversion of carbon dioxide into bicarbonate ions, which aids in gas exchange during respiration. Inhibition of carbonic anhydrase can reduce the efficiency of this process, leading to difficulties in removing carbon dioxide and obtaining oxygen.

3. Kidney Dysfunction: Carbonic anhydrase is necessary for the reabsorption of bicarbonate ions in the kidneys. Inhibition of carbonic anhydrase can impair this process, affecting the regulation of acid-base balance and electrolyte levels in chickens.

According to Das and Deb (2008) the transport of CO2, Heamoglobin utilization for controlling pH of body fluids and selection for the production of carbonate ions are facilitated by carbonic anhydrase. They observed six phenotypes viz. AA, BB, CC, AB, AC and BC controlled by three co-dominant alleles (CA-1A, CA-1B and CA-1C) located at an autosomal locus CA-1. They discovered no significant differences between various biochemical types and economic traits. However, they reported that the activity of CA has been positively correlated with egg shell thickness.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL LOCATION

The research will be carried out at the Biotechnology Laboratory of the Faculty of Agriculture, University of Ilorin, Ilorin Kwara State Nigeria.

3.2 MATERIALS

Centrifuge machine, Micro-pipette, Nose mask, Vortex machine, Beaker, Funnel, Nurse cap, Electrophoresis machine, stirring rod, Laboratory coat, Gloves, Filter paper, Electrophoresis tank, Eye shade, Marker, Blade, Cellulose acetate paper, Ruler, EDTA bottles, Pen, Keyes tray feeder, feeding trough, Distilled water, Scissors, Glass rod, Paper tape, Pipette tube, PH meter, Weighing scale, Conical flasks, Beaker, Drinker, Bulb, Syringe.

3.3 DATA COLLECTION

3.3.1 QUANTITATIVE TRAITS

Quantitative traits:

A total of 100 adult Nigerian Local Chickens of both sexes comprising of 50 Fulani Ecotype chicken from Gaa Fulani of Irepodun Local Government in Kwara State, and 50 Yoruba Ecotype Chickens collected from backyard poultry farmer in Ilorin South Local Government of Kwara State, Nigeria.

MEASUREMENT OF BODY PARAMETERS

Data was collected from the chickens on body weight and other morphometric body parameters according to standard procedures. Body parameters that were taken include: Body weight (BW), Body length (BL), Body girth (BG), Shank length (SL), Drumstick length (DL), Wing length (WL), Keel length (KL) and Shank diameter (SD), Wing feather length, (WFL), Longest toe length (LTL), Head length (HL), Beak length (BKL), Neck circumference (NC),

- **BODY WEIGHT:** Body weight of individual birds will be taken with the aid of a digital weighing scale calibrated in grams (g).
- **BODY LENGTH:** Length between the tip of the beak and that of the caudal tail without feathers by the use of a measuring tape in centimeter (cm).
- **BODY GIRTH:** The circumference of the breast region by the use of a measuring tape in centimeter (cm).
- **SHANK LENGTH:** Distance from the shank joint to the extremity of the digital pedis by the use of a measuring tape in centimeter (cm).
- **DRUM STICK:** Distance between the thigh and tarsus by the use of a measuring tape in centimeter (cm).
- **WING LENGTH:** Wing length will be taken by stretching the wing from the humerus joint to the tip of the wing by the use of a measuring tape in centimeter (cm).
- **KEEL LENGTH:** This will be taken from the breastbone which runs axially along the midline of the sternum and extends outward, perpendicular to the plane of the ribs by the use of measuring tape.
- **SHANK DIAMETER:** Circumference of the shank region using measuring tape (cm).
- **WING FEATHER LENGTH:** Wing length down to the tip of the longest wing feather using measuring tape(cm).

- **NECK CIRCUMFERENCE:** Circumference of the neck region using measuring tape (cm).
- **LONGEST TOE LENGTH:** Length of the longest toe using a measuring tape (cm).
- **BEAK LENGTH:** Length of the beak using a measuring tape (cm).
- **HEAD LENGTH:** Length of the head using a measuring tape (cm).

3.3.2 BLOOD COLLECTION AND PREPARATION

I collected 2ml of blood from a chicken's wing vein using a needle and syringe. To prevent contamination, I followed proper procedures like wearing gloves, a nose mask, and using a clean needle and syringe for each bird. I poured the blood into EDTA bottles with anticoagulant and labeled each bottle. After drawing the blood, I cleaned the puncture site with a cotton wool soaked in spirit to help with clotting. Finally, the blood sample was packed and taken to the Biotechnology Laboratory at the University of Ilorin for Blood Protein analysis using Cellulose acetate Electrophoresis procedure.

3.3 SAMPLE PREPARATION

LABORATORY PROCEDURE TO DETERMINE THE CARBONIC ANHYDRASE OF BLOOD.

A Gene Buffer (DANSUTECH Genotype Buffer) containing Tris EDTA and Boric Acid (Manufactured by Dansutech Resources Nig. Enterprises) was prepared by dilution with 100ml of distilled water. Cellulose Acetate paper strip was soaked in the buffer for 30 minutes and bloated with filter paper to remove excess buffer; 0.1 µ1 of the haemolysed blood was carefully loaded to the acetate paper and placed on the bridge in an humified electrophoresis tank that has been connected to a power supply (Model DY-300 for electrophoresis) and set to run for 5 minutes at 300 volts. The sample end was placed at the anode point and the movement of the

protein type towards the cathode was observed for 15 minutes to note a clear separation of the carbonyl band; after which the acetate paper was removed with forceps and soaked in Ponceau S (Acid Red 112) solution for 5 minutes for perfect staining of the band, then washed in glacial acetic acid solution for another 5 minutes and gently stirred in 95% absolute methanol for clearer observation of the band. Movement of the carbonyl bands towards the cathode was interpreted as described below:

A single faster band was identified to be FF Homozygote.

A single slower band was identified to be SS Homozygote.

A double band containing both faster and slower band was identified to be FS Heterozygote.

Frequencies of Carbonic anhydrase type observed in all samples was calculated.

3.4 TEST OF HYPOTHESIS

If the test value $(O-E)^2/E$, is higher than the Chi-square, it shows the population is due to chance, as it has not been selected, therefore accept H_0, but if the addition of the test value is below the Chi-square, it shows that the population have been tampered with so therefore, accept H_1.

Ho: The population was due to chance

H_1: The population was not due to chance

3.5 STATISTICAL ANALYSIS

Albumin types of bands obtained from gel electrophoresis were directly entered in GenAIEx 6.5 and exported to Pop gene 32.0 to determine their frequencies and genetic variation among and within the population of chickens studied based on plumage types.

CHAPTER FOUR

RESULT

TABLE 4.1. EFFECT OF CARBONIC ANHYDRASE ON MORPHOMETRIC BODY PARAMETERS OF FULANI AND YORUBA CHICKENS

Table 4.1 shows the mean values of the quantitative traits of carbonic anhydrase variant FF, FS and SS and it varied significantly ($p<0.05$). The result showed that significant differences existed in Body Weight, Body Girth and Wing length while there is no significant different in the Shank Length, Keel Length, Shank diameter, Body Height, Thigh Length, Beak Length, Drum Stick, Length and Body Length across the variants of carbonic anhydrase variant FF, FS and SS. Chicken having SS had higher significantly ($P<0.05$) values in Body girth and Wing length, Chicken with FF bands had the lowest significantly ($P<0.05$) Body girth and chicken with FS bands had the lowest significantly ($P<0.05$).

Table 4.1 shows the Carbonic anhydrase distribution for Fulani and Yoruba chickens

Traits	FF	FS	SS
BW	1.21	1.21	1.25
BG	29.51[b]	31.10[ab]	33.15[a]
SKL	9.55	9.17	9.87
KL	13.62	13.79	13.38
WL	17.56[ab]	15.66[b]	18.24[a]
SHD	4.21	5.45	4.01
BH	30.78	28.79	30.00
THL	11.18	12.08	12.35
BKL	3.96	3.60	2.65
DML	12.63	12.35	12.84
BL	37.00	36.00	36.81

[a, b] means with different superscript along the same row are significantly different.

BW, Body Weight; BG, Body Girth; SKL, Shank Length; KL, Keel Length; WL, Wing Length; SHD, Shank Diameter; BH, Body Height; THL, Thigh Length; BKL, Beak Length; DML, Drumstick Length; BL, Body Length.

4.2 Correlation coefficients of carbonic anhydrase, breed type, sex and morphometric parameters of Fulani and Yoruba chicken

Table 4.2 reveals the Pearson correlation coefficient of Carbonic anhydrase (CA) with sex and morphometric parameters in chicken. CA was positively significantly correlated with breed type, KL and BG whereas, it was negatively significantly correlated with BW, BL, BW, SKL, WL, BKL, DML and BL. BW was moderately correlated and significantly $p<0.05$ with BG and Bl and negatively correlated with CA, Sex and BKL. BG was moderately correlated with THL and negatively correlated with sex, SHL and DML. SKL was moderately and significantly $p<0.05$ with BW. The highest correlation was in this study was between KL and THL with the

value 0.551. Bh was negatively correlated with sex and positively correlated with BW but was not significant $p<0.05$.

Table 4.2: Pearson's Correlation coefficients of carbonic anhydrase and body measurements of Fulani and Yoruba chicken

	CA	SEX	BW	BG	SKL	KL	WL	SHD	BH	THL	BKL	DML	BL
CA	-	-0.273*	-0.351*	0.203	-0.232*	0.134	-0.410*	-0.052	-0.317*	0.146	-0.283*	0.055	-0.027
SEX	-0.273*	-	-0.308*	-0.128	-0.010	0.032	0.294*	-0.127	-0.162	-0.175	0.189	-0.246*	0.038
BW	-0.351*	-0.308*	-	0.354*	0.429*	0.079*	0.128*	0.244*	0.355*	0.246*	-0.041	0.223	0.318
BG	0.203	-0.128	0.354*	-	0.198	0.280*	0.035	-0.043	0.170	0.459*	-0.079	0.149	0.403*
SKL	-0.232*	-0.010	0.429	0.198	-	0.289	0.328	0.119	0.399	0.208	-0.206	0.186	0.165
KL	0.134	0.032	0.079	0.280*	0.289*	-	-0.062	0.000	-0.028	0.548*	-0.172	-0.215*	0.268*
WL	-0.410	0.294	0.128	0.035	0.328	-0.062	-	-0.017	0.401*	-0.077*	0.012	0.089	0.204
SHD	-0.052	-0.127	0.244*	-0.043	0.119	0.000	-0.017	-	0.029	-0.118	-0.013	0.002	-0.089
BH	-0.317*	-0.162	0.355*	0.170	0.399*	-0.028	0.401*	0.029	-	-0.089	0.066	0.344*	0.392*
THL	0.146	-0.175	0.246*	0.459	0.208	0.548*	-0.077*	-0.118	-0.089	-	-0.205	-0.211*	0.136
BKL	-0.283*	0.189	-0.041	-0.079*	-0.206	-0.172	0.012	-0.013	0.066	-0.205	-	-0.255*	0.022
DML	0.055	-0.246	0.223	0.149	0.186	-0.215	0.089	0.002	0.344	-0.211	-0.255	-	0.117
BL	-0.027	0.038	0.318	0.403	0.165	0.268	0.204	-0.089	0.392	0.136	0.022	0.117	-

ion is significant at the 0.05 level ($P<0.05$)

RFT, Rare Feather Type; BW, Body Weight; BG, Body Girth; SKL, Shank Length; KL, Keel Length; WL, Wing Length; SHD, Shank Diameter; BH, Body Height; THL, Thigh Length; BKL, Beak Length; DML, Drumstick Length; BL, Body Length.

4.3 DISCRIMINANT ANALYSIS

4.3.1 Summary of Canonical Discriminant Function of Fulani and Yoruba chicken

Results of the stepwise discriminant analysis showing eigen values, variance proportion, canonical correlation and Wilk's Lambda values is presented in Table 3. Two discriminant functions or variables were extracted, the significance of the discriminant function tested with Wilks Lambda (0.766, 0.899) and Bartlett's test (Chi-Square 21.297 P<0.726, 8.531 P<0.742) for the two functions, this provided validity for the canonical discriminant analysis.

Table 4.30 Canonical Discriminant Function of Fulani and Yoruba chicken

Function	Eigen Value	% of Variance	Cumulative %	Canonical Correlation	Chi-square	Sig.	Wilks' Lambda
1	0.173[a]	60.6	60.6	0.384	21.297	0.726	0.766
2	0.113[a]	39.4	100	0.318	8.531	.742	0.988

4.4 Standardized Canonical Discriminant Function of Fulani and Yoruba chicken

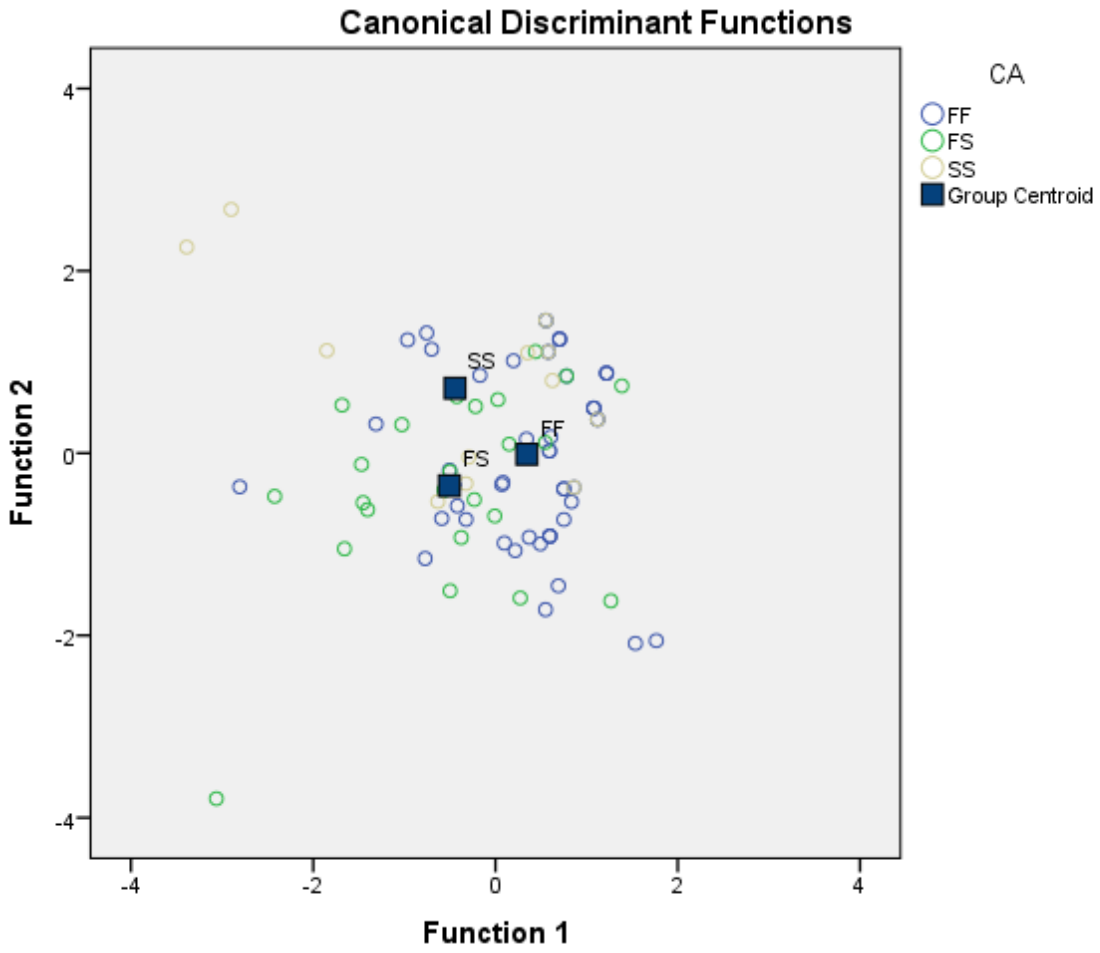
Table4.3 showed the standardized canonical coefficients and total variation as explained by canonical variables. For all the variables, each corresponding value indicates the original contribution of each parameter to the overall variation among the population, reflecting its discriminatory power. Generally, any variable with a loading of 0.30 (or higher) is considered to contribute significantly as a discriminating variable. In Function1, the best discriminant contributes to overall body parameters measured is BH with a value of 0.433 followed by KL 0.325 and Bill length 0.588, while BW, SKL, WL, BKL, DML and BL had least value 0.023-0.201. BG, SHD and THL had a negative value of -0.299 TO – 0.751. In Function2, the best discriminant contributes to overall body parameters measured is BG with a value of 0.4601 followed by SL 0.489 and SKL 0.339, while DML have least value 0.071. BW, KL, SHD, BL, BKL and THL had a negative value ranging from of -0.042 to 0.201.

Table 4.4 shows the Standardized Canonical Discriminant Function of Fulani and Yoruba chicken

PARAMETERS	FUNCTION1	FUNCTION2
BW	0.139	-0.204
BG	-0.753	0.612
SKL	0.023	0.384
KL	0.325	-0.264
WL	0.133	0.498
SHD	-0.339	-0.339
BH	0.433	-0.166
THL	-0.299	-0.042
BKL	0.052	-0.214
DML	0.029	0.071
BL	0.201	-0.060

4.5. CANONICAL REPRESENTATION OF CARBONIC ANHYDRASE FOUND IN FULANI AND YORUBA CHICKEN

The cluster analysis is necessary for identification of breed of chicken with respect to their carbonic anhydrase genotypes and this could be useful in any improvement program designed for chicken as well as their selection. Morphological differentiation on cluster classification as displayed by function 1 showed that carbonic anhydrase genotype FF was separated from genotype FS and SS. Function 2 show that carbonic anhydrase genotype FF and SS are separated from genotype FS as shown in figure 1 below.



CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

Blood protein polymorphism have been used by researchers as a marker to study evolutionary relationships in genotypes of mammals and between different breeds of sheep, deer, goat, chickens and rabbit (Kalab et al., 1990; Emerson and Tate, 1993; Buchanan et al., 1994; Guney et al., 2003; Malan et al., 2003 and Chineke et al., 2007). The transport of CO2, haemoglobin utilization for controlling pH of body fluids and selection for the production of carbonate ions are facilitated by carbonic anhydrase.

The three carbonic anhydrase genotypes observed in this study were the same with the observation of Ige *et al.,* 2013, Oguntunji, *et al.,* (2004) and Folorunsho, *et al.,* (2018) who studied blood protein characterization of Fulani and Yoruba chicken , blood polymorphism of locally adapted Muscovy ducks and genetic diversity between exotic and Nigeria indigenous turkeys respectively, but the highest observed frequency in this study is not in alignment with CA^{FF} that was predominant in the report of Oguntunji, *et al.,* (2004) and Folorunsho, *et al.,* (2018).The genotype obtained in this study is genetically controlled by two codominant alleles CA^{F} and CA^{S}. The prevalence of the three-genotype obtained in this study states its relevance to the yet-to be-known physiological advantage it confers on this bird in its natural habitat.

Correlation analysis is estimated to measure the extent of association or connection between one trait and the other. The results of correlation analysis are essential in determining the degree of relationship between the studied morphometric traits. Most of the quantitative traits are naturally correlated due to geneticm (pleiotropy and linkage disequilibrium) and non-genetic environment-related effects (Rosario *et al.*, m2008). Thus, understanding the association between quantitative

traits is of paramount importance in designing sustainable genetic improvement programs through selection within the local animal populations. In the current study, the correlation coefficients revealed a strong positive and negative correlation between Carbonic anhydrase and Fulani and Yoruba morphometric traits. The varying positive estimates of inter-correlation among traits could be attributed to the variations in traits of sampled animal was not taken into cognizance to ensure uniformity of sample. Observed negative correlation between certain morphometric traits indicates that selection on the basis of any of these traits will lead to a decrease in its associated trait. Discriminant analysis is one of the most promising tools for predicting and solving problems of discriminating between groups. The discriminating power of Body height was highest under function 1 and Wing length under function 2. Wilk's lambda test also confirmed that all the selected variables in the stepwise discriminant analysis had a highly significant contribution to discriminate the total population into separate groups. However, based on the values of Wilk's lambda and the average squared canonical correlation, BH has shown the highest level of significant discriminating power while SML had the least in differentiating the chicken populations. Most of the discriminating variables (LW, BL, BC, SL, NL, KL) in the present study are similar to those reported by Daikwo *et al.* (2015) for Nigerian indigenous chickens and that of Ajayi *et al.* (2012) for Algerian chickens. Consistent with the current findings, Neto *et al.* (2019) reported that BL, WS, and LW showed the greatest variability to discriminate between the Brazilian fighting cocks and naturalized roosters. On the contrary, Getu *et al.* (2014) reported SL as the most important variable to discriminate among three chicken ecotypes reared in North Gondar of Ethiopia. Such variations among different studies may arise from the sample size and birds' age considered under individual study. The use of canonical discriminant analysis in evaluating morphometric traits of Fulani and Yoruba chicken will help in understanding the

genetic relatedness of Carbonic anhydrase genotypes. The canonical plot representation revealed that Carbonic anhydrase genotypes FF, FS and SS are distinctive pointer for chicken fitness, health and adaption at their different environment.

5.2 CONCLUSION

It is evident that Carbonic anhydrase genotypes typed are heterozygotes in Fulani and Yoruba chicken. The prevalence of certain genes at their respective locus is suggestive of their importance to the adaptability and survival of these chicken in its natural harsh tropical environment. The performance of these strains revealed that some morphometric (traits) (i.e. linear measurements) can be tapped in order to make strategic improvement programme in the poultry industry and also help in designing a long-term genetic improvement programme for indigenous chicken in Nigeria

5.2 RECOMMENDATION

Besides, application of DNA technologies such as microsatellite markers would help a lot to elucidate further genetic diversity and provides deeper insight to Fulani and Yoruba chicken inherent genetic difference

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APPENDIX

EFFECTS OF CARBONIC ANHYDRASE DISTRIBUTION ON GROWTH TRAITS AND THEIR PHENOTYPIC CORRELATION IN NIGERIAN LOCAL CHICKENS

```
ONEWAY BREED SEX BW BG SKL KL WL SHD BH THL BKL DML BL BY CA
  /MISSING ANALYSIS
  /POSTHOC=DUNCAN ALPHA(0.05).
```

Oneway

Notes

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	Cases Used	Statistics for each analysis are based on cases with no missing data for any variable in the analysis.

Syntax		ONEWAY BREED SEX BW BG SKL KL WL SHD BH THL BKL DML BL BY CA /MISSING ANALYSIS /POSTHOC=DUNCAN ALPHA(0.05).
Resources	Processor Time	00:00:00.09
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[DataSet0]

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
BREED	Between Groups	2.192	2	1.096	1.631	.202
	Within Groups	57.808	86	.672		
	Total	60.000	88			
SEX	Between Groups	.432	2	.216	.871	.422
	Within Groups	21.343	86	.248		
	Total	21.775	88			
BW	Between Groups	.021	2	.011	.105	.900
	Within Groups	8.792	86	.102		
	Total	8.814	88			
BG	Between Groups	148.384	2	74.192	3.631	.031
	Within Groups	1757.105	86	20.431		
	Total	1905.489	88			
SKL	Between Groups	4.546	2	2.273	1.199	.306

	Within Groups	163.024	86	1.896		
	Total	167.570	88			
KL	Between Groups	1.406	2	.703	.022	.978
	Within Groups	2767.702	86	32.183		
	Total	2769.108	88			
WL	Between Groups	77.234	2	38.617	3.214	.045
	Within Groups	1033.429	86	12.017		
	Total	1110.662	88			
SHD	Between Groups	28.929	2	14.465	.961	.386
	Within Groups	1293.842	86	15.045		
	Total	1322.771	88			
BH	Between Groups	71.520	2	35.760	1.963	.147
	Within Groups	1566.685	86	18.217		
	Total	1638.205	88			
THL	Between Groups	21.966	2	10.983	1.529	.222
	Within Groups	617.573	86	7.181		
	Total	639.540	88			
BKL	Between Groups	18.148	2	9.074	.409	.666
	Within Groups	1907.962	86	22.186		
	Total	1926.110	88			
DML	Between Groups	2.249	2	1.124	.284	.754
	Within Groups	340.869	86	3.964		
	Total	343.118	88			
BL	Between Groups	16.890	2	8.445	.483	.619

Within Groups	1503.514	86	17.483		
Total	1520.404	88			

Post Hoc Tests

Homogeneous Subsets

BREED

Duncan[a,b]

CA	N	Subset for alpha = 0.05
		1
FF	52	100.8846
SS	13	101.0000
FS	24	101.2500
Sig.		.169

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 21.767.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

SEX

Duncan[a,b]

CA	N	Subset for alpha = 0.05

		1
FS	24	106.4583
FF	52	106.6154
SS	13	106.6154
Sig.		.332

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 21.767.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

BW

Duncan[a,b]

CA	N	Subset for alpha = 0.05
		1
FF	52	1.2096
FS	24	1.2104
SS	13	1.2538
Sig.		.671

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 21.767.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

BG

Duncan[a,b]

CA	N	Subset for alpha = 0.05	
		1	2
FF	52	29.5385	
FS	24	31.1042	31.1042
SS	13		33.1538
Sig.		.256	.138

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 21.767.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

SKL

Duncan[a,b]

CA	N	Subset for alpha = 0.05
		1
FS	24	9.1708
FF	52	9.5577
SS	13	9.8692
Sig.		.118

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 21.767.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

KL

Duncan[a,b]

CA	N	Subset for alpha = 0.05
		1
SS	13	13.3846
FF	52	13.6288
FS	24	13.7917
Sig.		.826

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 21.767.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

WL

Duncan[a,b]

CA	N	Subset for alpha = 0.05	
		1	2
FS	24	15.6667	
FF	52	17.5615	17.5615
SS	13		18.2462
Sig.		.075	.516

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 21.767.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

SHD

Duncan[a,b]

CA	N	Subset for alpha = 0.05
		1
SS	13	4.0077
FF	52	4.2115
FS	24	5.4458
Sig.		.254

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 21.767.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

BH

Duncan[a,b]

CA	N	Subset for alpha = 0.05
		1
FS	24	28.7917
SS	13	30.0000
FF	52	30.8712
Sig.		.133

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 21.767.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

THL

Duncan[a,b]

CA	N	Subset for alpha = 0.05
		1
FF	52	11.1846
FS	24	12.0833
SS	13	12.3538
Sig.		.179

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 21.767.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

BKL

Duncan[a,b]

CA	N	Subset for alpha = 0.05
		1
SS	13	2.6538
FS	24	3.6042
FF	52	3.9683
Sig.		.391

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 21.767.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

DML

Duncan[a,b]

CA	N	Subset for alpha = 0.05
		1
FS	24	12.3542
FF	52	12.6250
SS	13	12.8462
Sig.		.447

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 21.767.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

BL

Duncan[a,b]

CA	N	Subset for alpha = 0.05
		1
FS	24	36.0000
SS	13	36.8077
FF	52	37.0096
Sig.		.458

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size =

21.767.

b. The group sizes are unequal. The

harmonic mean of the group sizes is used.

Type I error levels are not guaranteed.

```
DISCRIMINANT
  /GROUPS=CA(103 105)
  /VARIABLES=BREED SEX BW BG SKL KL WL SHD BH THL BKL DML BL
  /ANALYSIS ALL
  /PRIORS EQUAL
  /STATISTICS=COEFF RAW CORR COV GCOV TCOV
  /PLOT=COMBINED SEPARATE
  /CLASSIFY=NONMISSING POOLED.
```

Discriminant

Notes

Analysis Case Processing Summary

Group Statistics

CA		Valid N (listwise)	
		Unweighted	Weighted
FF	BREED	52	52.000
	SEX	52	52.000
	BW	52	52.000
	BG	52	52.000
	SKL	52	52.000
	KL	52	52.000
	WL	52	52.000
	SHD	52	52.000
	BH	52	52.000
	THL	52	52.000
	BKL	52	52.000

	DML	52	52.000
	BL	52	52.000
FS	BREED	24	24.000
	SEX	24	24.000
	BW	24	24.000
	BG	24	24.000
	SKL	24	24.000
	KL	24	24.000
	WL	24	24.000
	SHD	24	24.000
	BH	24	24.000
	THL	24	24.000
	BKL	24	24.000
	DML	24	24.000
	BL	24	24.000
SS	BREED	13	13.000
	SEX	13	13.000
	BW	13	13.000
	BG	13	13.000
	SKL	13	13.000
	KL	13	13.000
	WL	13	13.000
	SHD	13	13.000
	BH	13	13.000
	THL	13	13.000
	BKL	13	13.000
	DML	13	13.000
	BL	13	13.000
Total	BREED	89	89.000

SEX	89	89.000
BW	89	89.000
BG	89	89.000
SKL	89	89.000
KL	89	89.000
WL	89	89.000
SHD	89	89.000
BH	89	89.000
THL	89	89.000
BKL	89	89.000
DML	89	89.000
BL	89	89.000

Pooled Within-Groups Matrices[a]

		BREED	SEX	BW	BG	SKL	KL	WL	SHD	BH	THL	BKL	DML	BL
Covariance	BREED	.672	-.105	-.092	.774	-.239	.615	-1.102	-.243	-1.047	.308	-1.122	.112	-.046
	SEX	-.105	.248	-.050	-.298	-.022	.100	.468	-.212	-.399	-.227	.455	-.258	.055
	BW	-.092	-.050	.102	.511	.189	.145	.138	.311	.485	.213	-.060	.141	.424
	BG	.774	-.298	.511	20.431	1.224	7.198	.475	-.703	3.260	5.582	-1.665	1.330	7.621
	SKL	-.239	-.022	.189	1.224	1.896	2.313	1.431	.777	2.250	.810	-1.325	.481	.877
	KL	.615	.100	.145	7.198	2.313	32.183	-1.156	-.073	-.616	8.322	-4.610	-2.419	6.427
	WL	-1.102	.468	.138	.475	1.431	-1.156	12.017	.284	5.573	-.613	.315	.488	2.711
	SHD	-.243	-.212	.311	-.703	.777	-.073	.284	15.045	.887	-1.314	-.303	.109	-1.246
	BH	-1.047	-.399	.485	3.260	2.250	-.616	5.573	.887	18.217	-.937	1.429	2.863	6.844
	THL	.308	-.227	.213	5.582	.810	8.322	-.613	-1.314	-.937	7.181	-2.603	-1.109	1.585
	BKL	-1.122	.455	-.060	-1.665	-1.325	-4.610	.315	-.303	1.429	-2.603	22.186	-2.380	.474
	DML	.112	-.258	.141	1.330	.481	-2.419	.488	.109	2.863	-1.109	-2.380	3.964	.919
	BL	-.046	.055	.424	7.621	.877	6.427	2.711	-1.246	6.844	1.585	.474	.919	17.483
Correlation	BREED	1.000	-.258	-.351	.209	-.212	.132	-.388	-.076	-.299	.140	-.290	.069	-.013
	SEX	-.258	1.000	-.314	-.132	-.032	.035	.271	-.110	-.187	-.170	.194	-.260	.026
	BW	-.351	-.314	1.000	.354	.430	.080	.125	.251	.355	.248	-.040	.221	.317
	BG	.209	-.132	.354	1.000	.197	.281	.030	-.040	.169	.461	-.078	.148	.403
	SKL	-.212	-.032	.430	.197	1.000	.296	.300	.145	.383	.220	-.204	.175	.152
	KL	.132	.035	.080	.281	.296	1.000	-.059	-.003	-.025	.547	-.173	-.214	.271
	WL	-.388	.271	.125	.030	.300	-.059	1.000	.021	.377	-.066	.019	.071	.187
	SHD	-.076	-.110	.251	-.040	.145	-.003	.021	1.000	.054	-.126	-.017	.014	-.077

	BH	-.299	-.187	.355	.169	.383	-.025	.377	.054	1.000	-.082	.071	.337	.384
	THL	.140	-.170	.248	.461	.220	.547	-.066	-.126	-.082	1.000	-.206	-.208	.141
	BKL	-.290	.194	-.040	-.078	-.204	-.173	.019	-.017	.071	-.206	1.000	-.254	.024
	DML	.069	-.260	.221	.148	.175	-.214	.071	.014	.337	-.208	-.254	1.000	.110
	BL	-.013	.026	.317	.403	.152	.271	.187	-.077	.384	.141	.024	.110	1.000

a. The covariance matrix has 86 degrees of freedom.

Covariance Matrices[a]

CA		BREED	SEX	BW	BG	SKL	KL	WL	SHD	BH	THL	BKL	DML	BL
FF	BREED	.810	-.143	-.094	.583	-.278	1.611	-1.763	-.199	-1.201	.455	-1.236	.025	.040
	SEX	-.143	.241	-.066	-.534	-.066	-.306	.363	-.046	-.590	-.310	.517	-.343	-.526
	BW	-.094	-.066	.101	.475	.220	-.035	.245	.118	.620	.170	-.234	.245	.496
	BG	.583	-.534	.475	16.920	.792	3.221	-.021	-.549	5.119	3.256	.067	2.539	6.588
	SKL	-.278	-.066	.220	.792	2.212	1.331	1.288	.408	2.376	.434	-1.827	1.051	.406
	KL	1.611	-.306	-.035	3.221	1.331	29.758	-4.339	-.106	-4.460	4.652	-5.858	-.840	3.545
	WL	-1.763	.363	.245	-.021	1.288	-4.339	8.630	.681	3.996	-1.472	-.137	.777	1.615
	SHD	-.199	-.046	.118	-.549	.408	-.106	.681	.454	1.135	-.210	-.025	-.003	.488
	BH	-1.201	-.590	.620	5.119	2.376	-4.460	3.996	1.135	16.485	-1.271	.677	3.665	6.037
	THL	.455	-.310	.170	3.256	.434	4.652	-1.472	-.210	-1.271	5.215	-2.935	-.975	.117
	BKL	-1.236	.517	-.234	.067	-1.827	-5.858	-.137	-.025	.677	-2.935	27.858	-3.083	.377
	DML	.025	-.343	.245	2.539	1.051	-.840	.777	-.003	3.665	-.975	-3.083	4.352	1.131
	BL	.040	-.526	.496	6.588	.406	3.545	1.615	.488	6.037	.117	.377	1.131	11.662
FS	BREED	.370	.011	-.079	.516	-.053	-1.076	-.239	-.373	.011	-.196	-1.327	.386	.565
	SEX	.011	.259	-.070	-.572	-.134	.100	.746	-.687	-.531	-.453	.476	-.278	.087
	BW	-.079	-.070	.073	.167	.058	.109	-.075	.808	.352	.060	.320	.057	.146
	BG	.516	-.572	.167	16.021	1.410	10.523	.243	-1.579	4.664	6.219	-5.690	-.821	8.239
	SKL	-.053	-.134	.058	1.410	1.245	2.446	1.942	1.772	1.900	.966	-.982	-.281	.754
	KL	-1.076	.100	.109	10.523	2.446	32.629	4.069	-.164	.889	13.051	-4.749	-5.075	3.217
	WL	-.239	.746	-.075	.243	1.942	4.069	18.580	-1.282	13.362	-.591	2.008	.841	7.446
	SHD	-.373	-.687	.808	-1.579	1.772	-.164	-1.282	54.883	.769	-4.450	-.955	.618	-5.439

	BH	.011	-.531	.352	4.664	1.900	.889	13.362	.769	21.824	-.351	2.947	3.403	6.033
	THL	-.196	-.453	.060	6.219	.966	13.051	-.591	-4.450	-.351	10.232	-3.009	-1.118	2.446
	BKL	-1.327	.476	.320	-5.690	-.982	-4.749	2.008	-.955	2.947	-3.009	20.973	-2.165	-.259
	DML	.386	-.278	.057	-.821	-.281	-5.075	.841	.618	3.403	-1.118	-2.165	3.815	-.109
	BL	.565	.087	.146	8.239	.754	3.217	7.446	-5.439	6.033	2.446	-.259	-.109	18.152
SS	BREED	.667	-.167	-.108	2.083	-.433	-.375	.058	-.183	-2.417	.650	-.242	-.042	-1.583
	SEX	-.167	.256	.056	1.231	.379	1.827	.378	-.005	.667	.556	.147	.144	2.462
	BW	-.108	.056	.163	1.324	.311	.978	.095	.178	.167	.685	-.049	-.141	.649
	BG	2.083	1.231	1.324	43.808	2.705	17.728	3.026	.324	-7.333	14.249	-1.309	.317	10.824
	SKL	-.433	.379	.311	2.705	1.797	6.234	1.060	.435	2.383	2.108	.151	-.484	3.114
	KL	-.375	1.827	.978	17.728	6.234	41.631	2.360	.243	12.833	14.857	.961	-4.040	24.830
	WL	.058	.378	.095	3.026	1.060	2.360	13.829	1.600	-2.658	2.994	-1.009	-1.421	-1.707
	SHD	-.183	-.005	.178	.324	.435	.243	1.600	.701	.058	.003	-.234	-.390	-.582
	BH	-2.417	.667	.167	-7.333	2.383	12.833	-2.658	.058	18.667	-.642	1.717	-1.583	11.833
	THL	.650	.556	.685	14.249	2.108	14.857	2.994	.003	-.642	9.688	-.412	-1.662	6.174
	BKL	-.242	.147	-.049	-1.309	.151	.961	-1.009	-.234	1.717	-.412	.403	.196	2.295
	DML	-.042	.144	-.141	.317	-.484	-4.040	-1.421	-.390	-1.583	-1.662	.196	2.599	1.989
	BL	-1.583	2.462	.649	10.824	3.114	24.830	-1.707	-.582	11.833	6.174	2.295	1.989	40.939
Total	BREED	.682	-.114	-.090	.864	-.260	.613	-1.206	-.153	-1.165	.362	-1.121	.091	-.114
	SEX	-.114	.247	-.049	-.317	-.007	.091	.521	-.247	-.330	-.243	.447	-.242	.084
	BW	-.090	-.049	.100	.517	.187	.140	.142	.301	.472	.213	-.065	.139	.414
	BG	.864	-.317	.517	21.653	1.254	6.966	.415	-.560	2.494	6.066	-2.205	1.342	7.198
	SKL	-.260	-.007	.187	1.254	1.904	2.233	1.605	.638	2.337	.775	-1.334	.506	.937

	KL	.613	.091	.140	6.966	2.233	31.467	-1.235	-.011	-.657	8.127	-4.472	-2.384	6.246
	WL	-1.206	.521	.142	.415	1.605	-1.235	12.621	-.255	6.147	-.774	.242	.622	3.025
	SHD	-.153	-.247	.301	-.560	.638	-.011	-.255	15.031	.404	-1.144	-.290	.021	-1.459
	BH	-1.165	-.330	.472	2.494	2.337	-.657	6.147	.404	18.616	-1.289	1.570	2.895	7.077
	THL	.362	-.243	.213	6.066	.775	8.127	-.774	-1.144	-1.289	7.267	-2.739	-1.095	1.393
	BKL	-1.121	.447	-.065	-2.205	-1.334	-4.472	.242	-.290	1.570	-2.739	21.888	-2.354	.515
	DML	.091	-.242	.139	1.342	.506	-2.384	.622	.021	2.895	-1.095	-2.354	3.899	.954
	BL	-.114	.084	.414	7.198	.937	6.246	3.025	-1.459	7.077	1.393	.515	.954	17.277

a. The total covariance matrix has 88 degrees of freedom.

Analysis 1

Summary of Canonical Discriminant Function

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	.173[a]	60.6	60.6	.384
2	.113[a]	39.4	100.0	.318

a. First 2 canonical discriminant functions were used in the analysis.

Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	.766	21.297	26	.726
2	.899	8.531	12	.742

Standardized Canonical Discriminant Function Coefficients

	Function	
	1	2
BREED	-.001	-.262
SEX	.139	.102
BW	.139	-.204
BG	-.753	.612
SKL	.023	.384
KL	.325	-.264
WL	.133	.498
SHD	-.339	-.339
BH	.433	-.166

THL	-.299	-.042
BKL	.053	-.214
DML	.029	.071
BL	.201	-.060

Structure Matrix

	Function	
	1	2
BG	-.593*	.458
BH	.477*	.237
THL	-.443*	.120
BREED	-.418*	-.261
BL	.216*	.168
WL	.370	.674*
SKL	.142	.466*
SHD	-.239	-.333*
SEX	.257	.280*
DML	.068	.227*
BKL	.171	-.198*
BW	-.056	.130*
KL	-.007	-.067*

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions

Variables ordered by absolute size of correlation within function.

*. Largest absolute correlation between each variable and any discriminant function

Canonical Discriminant Function

Coefficients

	Function	
	1	2
BREED	-.001	-.319
SEX	.280	.204
BW	.436	-.637
BG	-.167	.135
SKL	.016	.279
KL	.057	-.046
WL	.038	.144
SHD	-.088	-.087
BH	.101	-.039
THL	-.112	-.016
BKL	.011	-.045
DML	.015	.036
BL	.048	-.014
(Constant)	-30.062	4.618

Unstandardized coefficients

Functions at Group Centroids

	Function	
CA	1	2
FF	.345	-.013
FS	-.506	-.358
SS	-.444	.712

Unstandardized canonical discriminant functions evaluated at group means

Classification Statistics

Classification Processing Summary

Processed		89
Excluded	Missing or out-of-range group codes	0
	At least one missing discriminating variable	0
Used in Output		89

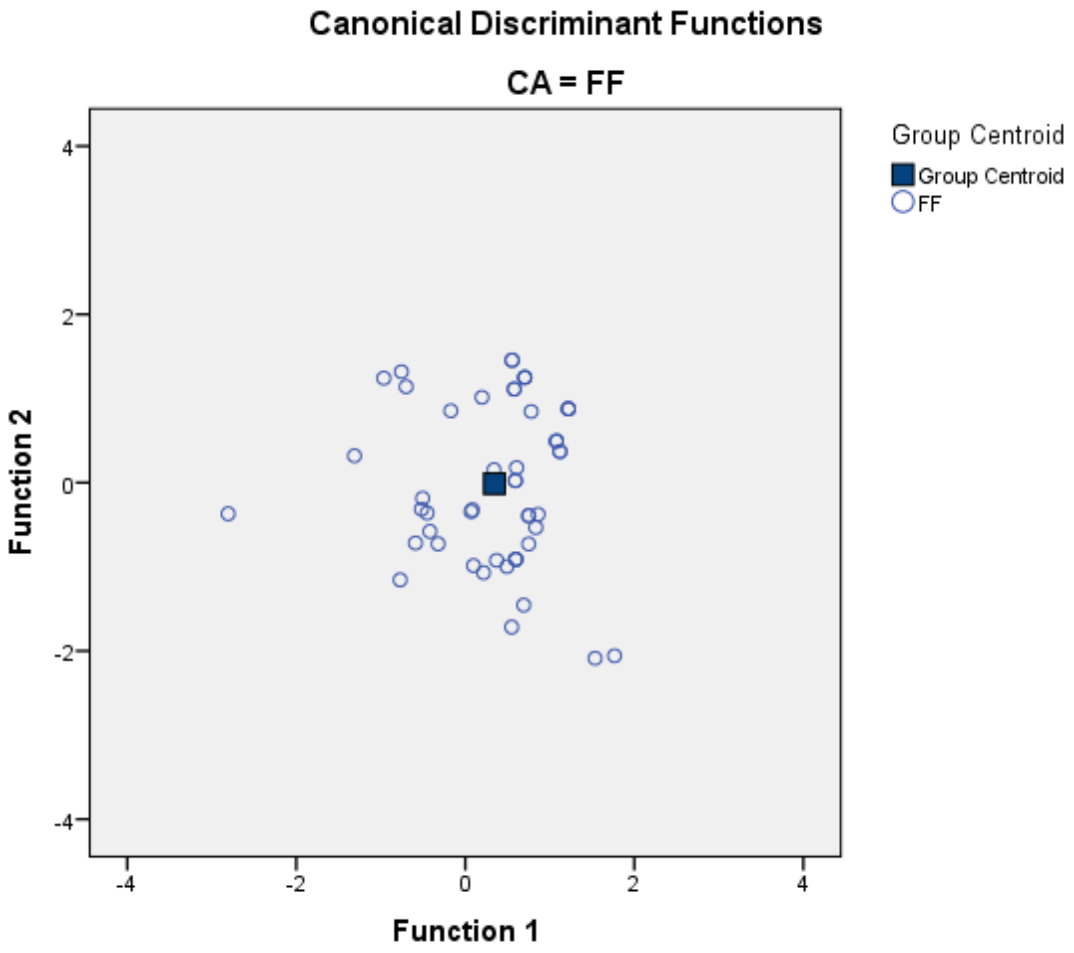
Prior Probabilities for Groups

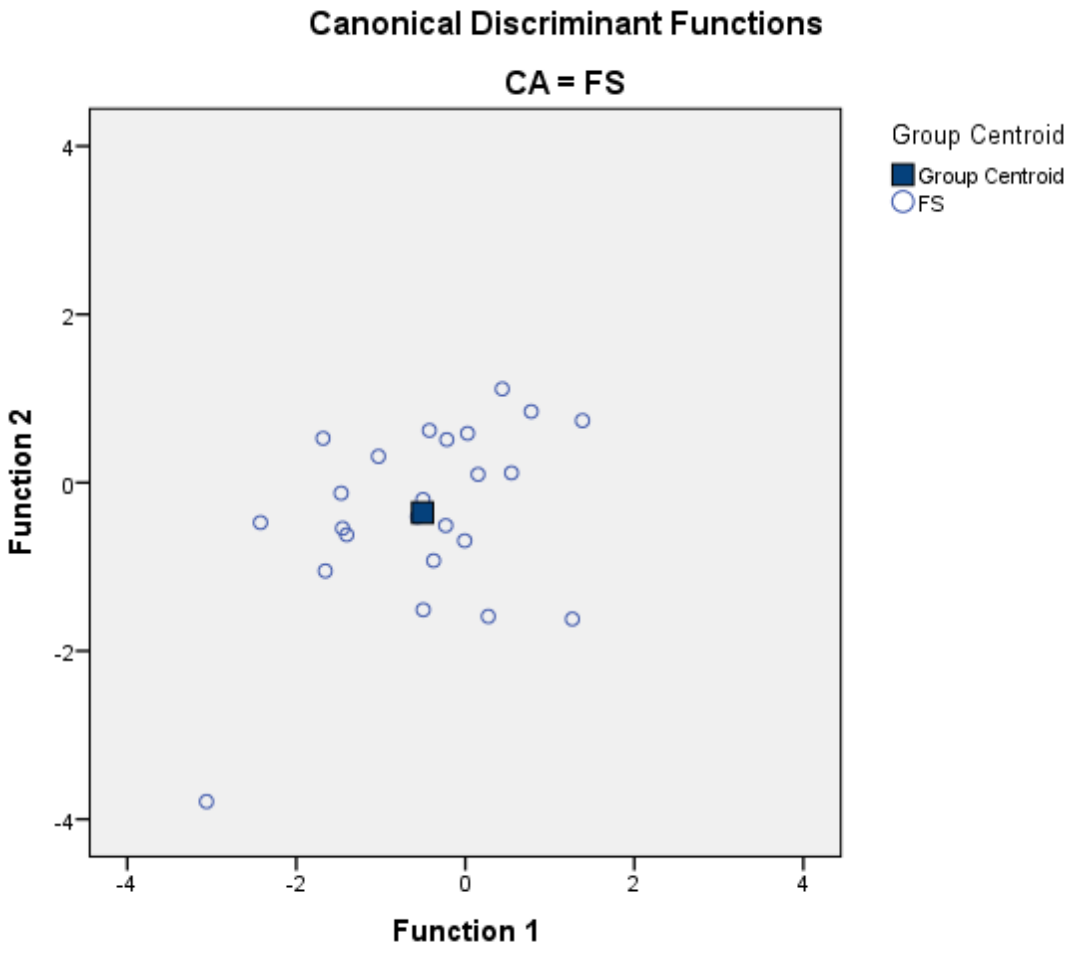
CA	Prior	Cases Used in Analysis	
		Unweighted	Weighted
FF	.333	52	52.000
FS	.333	24	24.000
SS	.333	13	13.000
Total	1.000	89	89.000

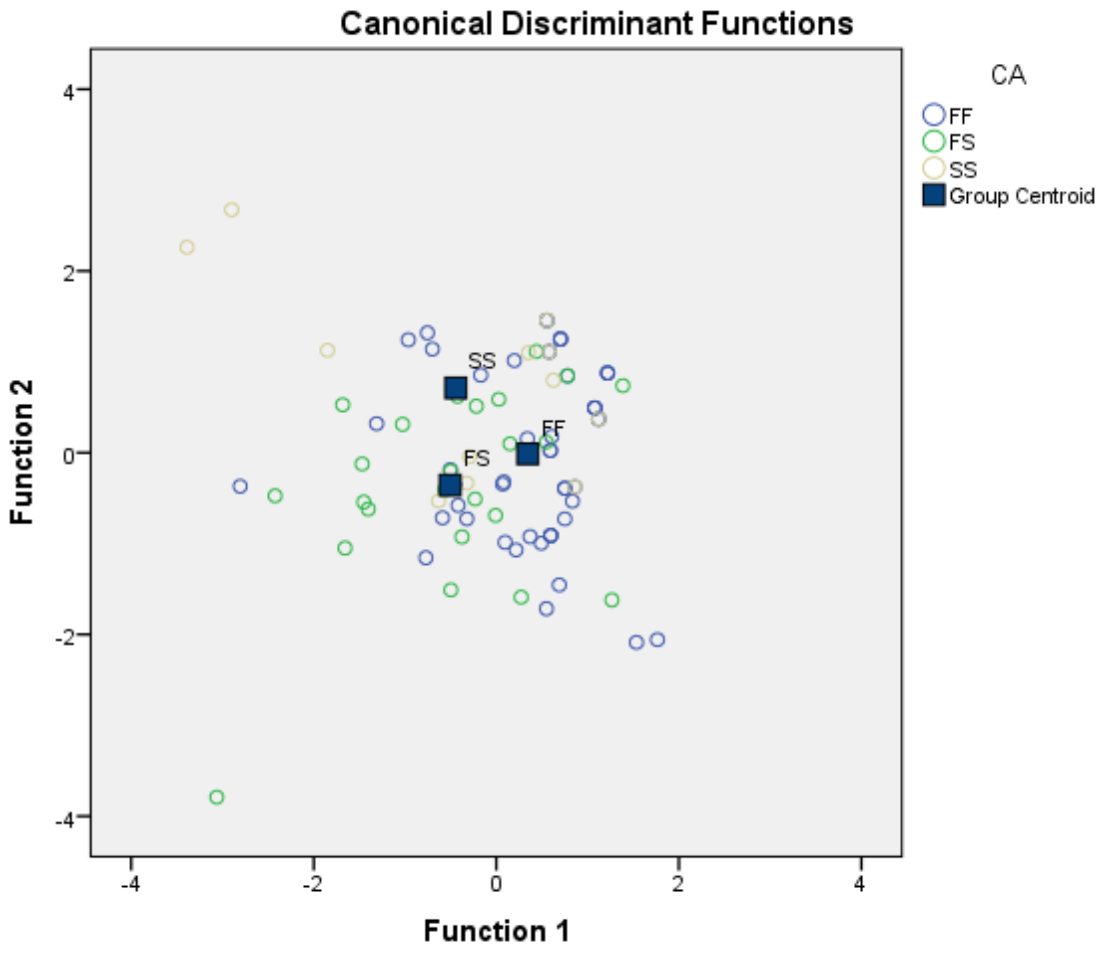
Classification Function Coefficients

	CA		
	FF	FS	SS
BREED	507.961	508.072	507.730
SEX	936.186	935.878	936.114
BW	974.490	974.339	973.685
BG	-30.215	-30.120	-29.985
SKL	-37.262	-37.372	-37.072
KL	-8.291	-8.324	-8.370
WL	-9.942	-10.024	-9.868
SHD	.155	.260	.161
BH	47.489	47.416	47.380
THL	32.477	32.577	32.553
BKL	5.630	5.636	5.588
DML	10.054	10.029	10.068
BL	-25.684	-25.720	-25.733
(Constant)	-75865.313	-75841.469	-75838.554

Fisher's linear discriminant functions


Canonical Discriminant Functions
CA = FF
Group Centroid
Group Centroid
FF
Function 2
Function 1
4
2
0
-2
-4
-4
-2
0
2
4


Canonical Discriminant Functions
CA = FS
Group Centroid
Group Centroid
FS
Function 2
Function 1
4
2
0
-2
-4
-4
-2
0
2
4

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PARTIAL CORR
  /VARIABLES=BREED SEX BW BG SKL KL WL SHD BH THL BKL DML BL BY CA
  /SIGNIFICANCE=TWOTAIL
  /STATISTICS=DESCRIPTIVES
  /MISSING=LISTWISE.
```

Partial Corr

Notes

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	Cases Used	Statistics are based on cases with no missing data for any variable listed.
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Descriptive Statistics

	Mean	Std. Deviation	N
BREED	101.0000	.82572	89
SEX	106.5730	.49744	89
BW	1.2163	.31648	89
BG	30.4888	4.65331	89
SKL	9.4989	1.37993	89
KL	13.6371	5.60956	89
WL	17.1506	3.55263	89
SHD	4.5146	3.87705	89
BH	30.1831	4.31462	89
THL	11.5978	2.69583	89
BKL	3.6781	4.67842	89
DML	12.5843	1.97461	89
BL	36.7079	4.15660	89
CA	103.5618	.73786	89

Correlations

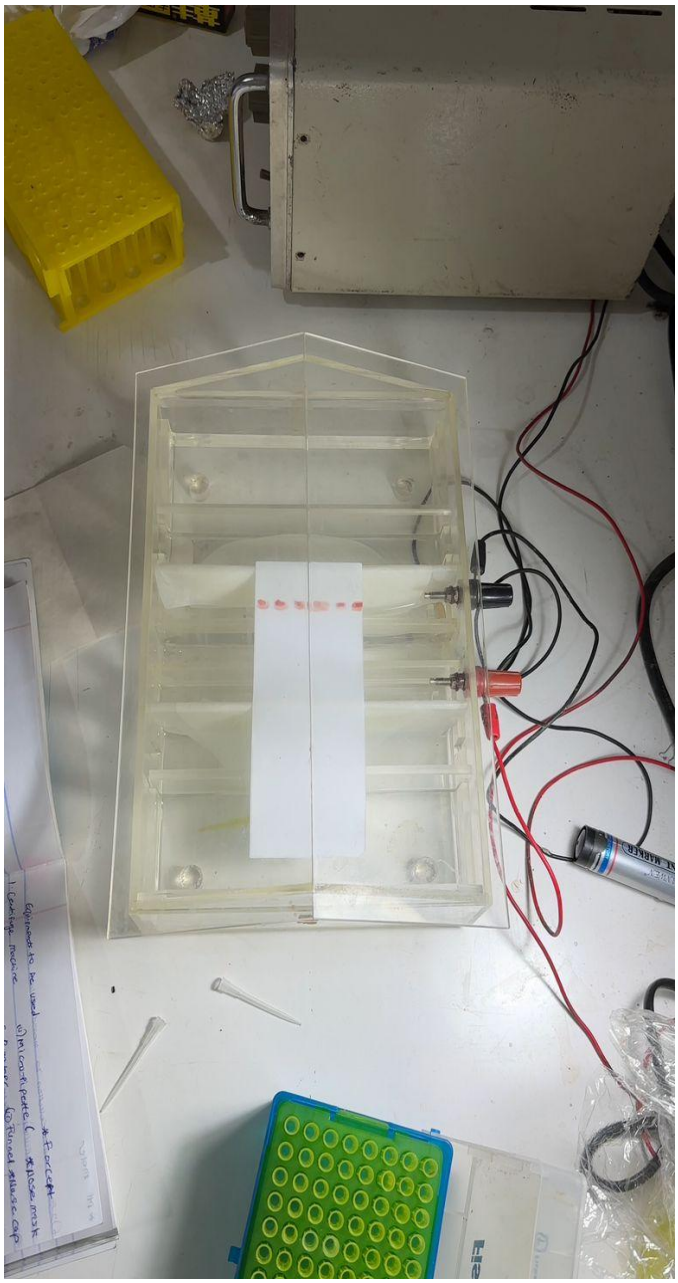
Control Variables		BREED	SEX	BW	BG	SKL	KL	WL	SHD	BH	THL	BKL	DML	BL
BREED	Correlation	1.000	-.273	-.351	.203	-.232	.134	-.410	-.052	-.317	.146	-.283	.055	-.027
	Significance (2-tailed)	.	.010	.001	.058	.030	.213	.000	.628	.003	.174	.008	.611	.800
	df	0	86	86	86	86	86	86	86	86	86	86	86	86
SEX	Correlation	-.273	1.000	-.308	-.128	-.010	.032	.294	-.127	-.162	-.175	.189	-.246	.038
	Significance (2-tailed)	.010	.	.004	.234	.929	.765	.006	.240	.130	.103	.079	.021	.725
	df	86	0	86	86	86	86	86	86	86	86	86	86	86
BW	Correlation	-.351	-.308	1.000	.354	.429	.079	.128	.244	.355	.246	-.041	.223	.318
	Significance (2-tailed)	.001	.004	.	.001	.000	.464	.235	.022	.001	.021	.707	.037	.003
	df	86	86	0	86	86	86	86	86	86	86	86	86	86
BG	Correlation	.203	-.128	.354	1.000	.198	.280	.035	-.043	.170	.459	-.079	.149	.403
	Significance (2-tailed)	.058	.234	.001	.	.065	.008	.745	.691	.113	.000	.466	.165	.000
	df	86	86	86	0	86	86	86	86	86	86	86	86	86
SKL	Correlation	-.232	-.010	.429	.198	1.000	.289	.328	.119	.399	.208	-.206	.186	.165
	Significance (2-tailed)	.030	.929	.000	.065	.P	.006	.002	.271	.000	.052	.054	.083	.125
	df	86	86	86	86	0	86	86	86	86	86	86	86	86
KL	Correlation	.134	.032	.079	.280	.289	1.000	-.062	.000	-.028	.548	-.172	-.215	.268
	Significance (2-tailed)	.213	.765	.464	.008	.006	.	.565	.998	.792	.000	.109	.044	.012
	df	86	86	86	86	86	0	86	86	86	86	86	86	86
WL	Correlation	-.410	.294	.128	.035	.328	-.062	1.000	-.017	.401	-.077	.012	.089	.204
	Significance (2-tailed)	.000	.006	.235	.745	.002	.565	.	.872	.000	.478	.913	.410	.057
	df	86	86	86	86	86	86	0	86	86	86	86	86	86

SHD	Correlation	-.052	-.127	.244	-.043	.119	.000	-.017	1.000	.029	-.118	-.013	.002	-.089
	Significance (2-tailed)	.628	.240	.022	.691	.271	.998	.872	.	.786	.274	.907	.983	.411
	df	86	86	86	86	86	86	86	0	86	86	86	86	86
BH	Correlation	-.317	-.162	.355	.170	.399	-.028	.401	.029	1.000	-.089	.066	.344	.392
	Significance (2-tailed)	.003	.130	.001	.113	.000	.792	.000	.786	.	.411	.541	.001	.000
	df	86	86	86	86	86	86	86	86	0	86	86	86	86
THL	Correlation	.146	-.175	.246	.459	.208	.548	-.077	-.118	-.089	1.000	-.205	-.211	.136
	Significance (2-tailed)	.174	.103	.021	.000	.052	.000	.478	.274	.411	.	.056	.049	.206
	df	86	86	86	86	86	86	86	86	86	0	86	86	86
BKL	Correlation	-.283	.189	-.041	-.079	-.206	-.172	.012	-.013	.066	-.205	1.000	-.255	.022
	Significance (2-tailed)	.008	.079	.707	.466	.054	.109	.913	.907	.541	.056	.	.017	.842
	df	86	86	86	86	86	86	86	86	86	86	0	86	86
DML	Correlation	.055	-.246	.223	.149	.186	-.215	.089	.002	.344	-.211	-.255	1.000	.117
	Significance (2-tailed)	.611	.021	.037	.165	.083	.044	.410	.983	.001	.049	.017	.	.278
	df	86	86	86	86	86	86	86	86	86	86	86	0	86
BL	Correlation	-.027	.038	.318	.403	.165	.268	.204	-.089	.392	.136	.022	.117	1.000
	Significance (2-tailed)	.800	.725	.003	.000	.125	.012	.057	.411	.000	.206	.842	.278	.
	df	86	86	86	86	86	86	86	86	86	86	86	86	0

PICTURE GALLERY

Micro pipette

Electrophoreses machine



Electrophoreses tank acetate.