UNIVERSITY FOR DEVELOPMENT STUDIES

ASSESSMENT OF THE USESFULNESS OF HETEROPHIL-LYMPHOCYTE RATIOS (H/L RATIOS) AND TEMPERAMENT SCORES AS BIOMARKERS OF DOCILITY OF THE LOCAL GUINEA FOWL (*Numida meleagris*) VARIETIES IN GHANA

DRAMANI WUMBEI

A THESIS SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT STUDIES,IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY (MPhil) DEGREE IN ANIMAL SCIENCE (ANIMAL BREEDING AND GENETICS OPTION)

2018

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SEPTEMBER, 2018

DECLARATION

Candidate

I hereby declare that this thesis is the result of my individual effort and I further declare that no part of this document has ever been presented here in this University or elsewhere for another degree. However, all work of others which I referred to as sources of information have been duly acknowledged by way of reference to the authors.

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Supervisors

We hereby declare that the preparation and presentation of this thesis were duly supervised in accordance to the guidelines on supervision of thesis laid down by the University for Development Studies.

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Co-Supervisor's Signature:………………………………..Date:……………………………….

Name: Professor S. Y. Annor

ABSTRACT

The aim of this research was to study the usefulness of the Heterophil-Lymphocyte ratios (H/L ratios) and temperament scores as biomarkers for docility in Guinea fowls. The study was carried out at the Poultry Section of the Department of Animal Science Education, University of Education, Winneba, Mampong-Ashanti campus, Ghana from May 2016 to June 2016. Docility was defined as ability of the bird to accept human presence, novel object, human contact and handling. The ability of acceptance was measured on a 4-point scale and these included: docile (1), flighty (2), restless (3) and aggressive (4). The birds were consecutively tested for temperament 2 times in a week and 8 times in all. The docility test was carried out on seventy (79) Guinea fowls comprising Pearl (37 males and 20 females), Lavender (6 males and 5 females) and White (5 males and 6 females) local varieties all of average age 10 months. The birds were tested for behavioural docility and hematological parameters which includes heterophil, lymphocytes counts and H/L ratios in a Completely Randomised Design experiment. Data were analysed using SAS and SPSS separation of means was done using LSD. Average docility score of the population was 2.15 representing 64.6% suggesting that the birds studied were generally flighty. The research revealed that the birds were consistent on this docility category. According to behavioural docility testing, sex and varieity had no significant effect ($P>0.05$) on docility. Males had a docility score of 2.2 and female 2.1 meaning both male and female Guinea fowls were flighty. From this experiment the Pearl and White had similar ability to withstand stress as indicated by their H/L ratios of 0.11 which was significantly different from that of the Lavender (0.09). The behavioural docility score and the H/L ratio of the Lavender both indicated that it was a more promising type in terms of acceptable docility score and ability to withstand stress. The cumulative effect of sex, variety and test criteria (method) had significant effect ($P<0.05$) on docility. Only 14.2% of the birds were docile. The research revealed that the birds were not generally aggressive in nature as only 1.3% of them were seen as being aggressive. There was no significant ($P>0.05$) effect of docility on feed intake and weight gain of the Guinea fowls. There was no significant ($P>0.05$) influence of docility on heterophil-lymphocyte ratio of the Guinea fowls. The variety factor had significant ($P<0.05$) effect on H/L ratios and H/L counts. The Lavendar recorded the lowest H/L ratio among the three varieties which was statistically significant ($P<0.05$). With the use of H/L ratios, the pearl would easily be stressed followed by the white and then Lavendar. The H/L ratio positively correlated with the docility but rather very weakly ($P=0.023$). The research revealed that the test criterion has significant ($P>0.05$) effect on docility. Based on this observation, the individual criteria were unique in their own right and can be used independently for the assessment of docility status of the birds. The Human Presence Test (HPT), Novel Object Test (NOT), Contact (CT), and Handling Test (HT) produced consistent docility scores across the weeks. The HPT, NOT and CT are similar in effect. The method which produces the greatest impact on the birds is HT. If the test methods are to be used individually, then HPT, NOT and Contact test will be ideal. The most suitable pair of method that assesses the underlying docility trait very effectively was the human presence test (HPT) with the novel object test (NOT) and is therefore recommended as the best suitable pair for behavioural docility scoring. The docility trait should be included in the breeding objectives of the Guinea fowls in order to achieve more acceptable docility scores.

ACKNOWLEDGMENT

I humbly extend my gratitude to the Almighty Allah for His guidance and protection throughout these years of study.

I am indebted to Mr. Jakper Naandam of the Department of Animal Science of the University for Development Studies, my principal supervisor. My profound gratitude also goes to my co-supervisor, Prof. S. Y. Annor, the Dean of the Faculty of Agriculture Education, UEW, for his unflinching support, encouragement and patience.

I am also very grateful to the staff and workers of the Department of Animal Science Farm, at Mampong-Campus, especially Mr. Hudu and Mr. Thomas, for the support they gave me during my experiment. My thanks again go to Department of Animal Science for the material support offered me to undertake the research.

Special mention must be made of Mr. Peter Birteeb, a Lecturer at the Department of Animal Science, University for Development Studies, for his support in the data analysis.

For his support, encouragement and constant reminder of my obligation as a student, a mention should be made of Mr. Shuaib Mbelayim A. Husein.

I extend my profound gratitude to my boss, Dr. Eric Obeng Bempong, Principal of Animal Health and Production College, Pong-Tamale, for the leave granted me to undertake my postgraduate studies.

My special thanks go to my lovely wife and the entire family for their immense support.

I greatly appreciate every bit of help I received either in kind or cash with which I was able to accomplish this study.

DEDICATION

This work of mine is unreservedly dedicated to my dad, Mr. Shaibu Dramani and Mum, Mrs. Wumbei Fusheina.

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ACRONYMS/ABBREVIATIONS

H/L Ratio	Heterophil/Lymphocyte Ratio
HPT	Human Presence Test
NOT	Novel Object Test
CT	Contact Test
HT	Handling Test
HPA	Hypothalamus-Pituitary-Adrenal
ACTH	Adrenocorticotropic hormone
SMS	Sympathomedullary System
CRH	Corticotropin releasing hormone
EDTA	Ethylenediaminetetraacetic acid
IVD	in vitro diagnostic

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The domesticated Guinea fowl (*Numida meleagris*) is a promising genetic resource for evolving a low-input poultry enterprise mostly in developing countries and has the potential of reducing poverty. It is very popular among smallholder farmers in Africa (Microlivestock, 1991; Nwagu and Alawa, 1995).

Guinea fowl production in Ghana is on the increase and is attracting more attention from both researchers and farmers. This is due to its acceptability, no cultural and religious barriers against its consumption as well as its comparative advantage over the chicken (Ayorinde, 1999). It is also due to its low production cost, premium quality meat, greater capacity to scavenge for insect pests and grains, better ability to protect itself against predators and its resistance to some diseases of poultry (Ayorinde, 1999). For these reasons according to Ayorinde (1999) Guinea fowl production has become a special poultry of choice. Guinea fowl can also be raised intensively and extensively where they can range extensively in the open (Nwagu and Alawa, 1995). Guinea fowls are mostly raised as scavengers together with domestic village chicken (Kashindye, 2000). The birds are usually kept in households in small numbers as a source of protein for the family.

Given these important traits in these poultry birds, the Guinea fowl equally has some behavioural problems that have to do with poor temperament/docility. Technically, *Docility* is defined as the ability of an animal to accept human presence (Annor *et al.*, 2011). The term docility is used interchangeably as Animal docility or simply as Temperament (Norris *et al.*, 2014).

Docility occurs across all types of production environments and includes maternal behaviour (Jarvis *et al.*, 2005), aggressiveness (D'Eath *et al.*, 2002a), social behaviours (Lovendahl *et al.*, 2005), reactions to humans (Barozzo *et al.*, 2012; Burrow, 1997; Fordyce *et al.*, 1988), feeding behaviours, daily activities and handling responses to new objects or situations (Yoder, 2010). Docility has been recognized as an important trait in livestock and could potentially be used as an indicator for economically important traits that are difficult to measure (Norris *et al.*, 2014). It is related to many production traits such as body weight, growth rates in cattle (Fell *et al.*, 1999) and mortality as reported in lambs by Neindre *et al.* (1998). Docility is important for animal welfare reasons, the farmer's and veterinarian's safety as well as for economic reasons (Geburt *et al*., 2015).

Research shows that intense selection towards an increase in production performance in animals is postulated to have resulted in increased problems with temperament such as increased aggressiveness during handling and more excited response to restraint (Grandin *et al.*, 1998).

Docility has influenced not only on the human safety and animal welfare but importantly also on the productivity of livestock farming enterprises. Poor temperament in livestock has been associated with reduced performance, health, and carcass quality. Docility is thus increasingly becoming a focus of many studies aiming at its inclusion in animal breeding programs (Norris *et al.*, 2014). Selection of animals with amicable temperament can result in improvements in animal management, productivity, meat quality (Burrow, 1997; Reverter *et al.*, 2003) and potentially, animal welfare.

It is difficult to handle and control aggressive animals (Fordyce *et al.*, 1998). Animals or stockmen could be subjected to injury during handling of aggressive animals. Moreover injuries increase the cost of production (Annor *et al.*, 2011). Aggressive behaviour or poor docility is considered a bad trait in farming operations and animals with such characters are usually culled (Kenttamies *et al.*, 2006). In view of this, researchers and farmers are thus increasingly paying attention to livestock reactions during handling and use these to describe animal docility (Paranhos da Costa *et al.*, 2002) especially with emerging evidence that docility is not only correlated with ease of handling but also economically important traits. Animals remaining calm and docile during handling are considered to have a good temperament.

Temperamental animals have generally been found to have reduced growth rates, poor carcass traits and poor immune function (Breuer *et al.*, 2000; Burrow, 2003; Curley *et al.*, 2006b; Kadel *et al.*, 2006; Beckman *et al.*, 2008; Burdick *et al.*, 2011; Café *et al.*, 2011). Studies by Cavigelli *et al.* (2008) in rats and Capitanio *et al.* (2011) in monkeys reported negative impacts of temperament on the immune function of animals. Furthermore, temperamental animals are more easily stressed than their calmer herd mates (Curley *et al.*, 2008) and as a consequence more prone to disease infection. Selecting livestock to improve docility has positive benefits such as improvement of animal performance in addition to improving human safety and animal welfare.

1.2 Problem Statement

There is reliable evidence that docility has moderate to large genetic variation and thus can respond to selection pressure (Annor *et al.*, 2011; Norris *et al.,* 2014). Furthermore, temperament traits have been found to be positively correlated with performance traits in many livestock species. This further indicates that while temperament can be improved through direct selection, it may also be used to indirectly improve many economically important traits in various livestock species. Consideration of docility in breeding objectives has potential to improve the welfare of both animals and humans and the performance of livestock farming enterprises (Norris *et al.*, 2014). Unfortunately, docility is often overlooked in many countries, especially, developing countries.

Besides, many studies confirmed that temperament is moderately heritable which makes it even more important to study to understand its relationship with genetic factors and productive traits. For examples, studies on the heritability of temperament in dairy cattle estimate heritabilities as 0.40 (O'Blesness *et al.*, 1960), 0.53 (Dickson *et al.*, 1970), and 0.45 (Sato, 1981).

More so, in poultry production, movement and handling of the birds is inevitable. Guinea fowls especially have the behaviour of climbing and sleeping on trees under extensive system of rearing. This behaviour could have some relationship with the docility character. The genetic characteristics of this important trait has been established in grasscutter (Annor *et al.*, 2011), using sorely an observation method based on four-point scoring system. Similar researches have been done on cattle (Norris *et al.*, 2014), pigs (Yoder, 2010), sheep (Murphy, 1999), and goat (Némethet *et al*., 2009).

The techniques for measuring docility are long since being established but which are continually being refined and improved making it possible to accurately measure docility. This important trait can either be measured on objective or the subjective analogue scales based on the systems of scoring.

Besides, a more physiological determinant is available whereby a measure of stress related hormone levels is made, because stress is reported to be linked to behaviour and by extension docility. Now and more recently, as an alternative, the ratio of Heterophils (or neutrophils in mammals) to lymphocytes in blood (H ⁄ L-ratio) has been proposed and very much reliable in assessing stress levels of avian species (Muller *et al*., 2011). In addition, it is imperative for the identification and selection of animals with a temperament that will improve their welfare and productivity within their production environment (Norris *et al*., 2014).

Moreover, the current methods developed and used in livestock are especially meant for the docility assessment of cattle, sheep and pigs and are not friendly in application in avian docility studies. Docility test experiments include, flight speed test (exit velocity), pen scores, and chute scores. These methods vary in their robustness in assessing the animal of its temperament and so new measurements began to appear including the H/L ratios and the behavioural test (cage scoring) for the avian species.

Finally, concern about animal welfare has led to greater use of production systems in which animals are kept in groups, and in such systems social behaviour can have an impact on production and health. Moreover, in modern animal production, where supervision is kept to a minimum, animals are required to behave well. The current changes in husbandry systems, such as a general reduction of labour or an increase in herd size, incur a general reduction in human time spent caring for the animals. It reduces the opportunities for animals to become familiar with humans and increases their opportunities to perceive handling as stressful. The genetics of behaviour involves genetic analysis of behavioural phenotypes (Holl *et al.*, 2010). Docility studies in the Guinea fowls are scarcity in the literature.

1.3 Objectives of the Study

1.3.1 General Objective

The general objective was to study the usefulness of the Heterophil-Lymphocyte ratios (H/L ratios) and temperament scores (i.e. the animals' behavioural response to novel object, human presence and contact and handling in standardized tests) as biomarkers for docility in Guinea fowls.

1.3.2 Specific Objectives

The specific objectives of this study were to:

1. Assess the effect of sex, variety and Test Method on docility.
2. Evaluate the relationship between docility and performance traits (weight gain, feed intake) and H/L ratios.
3. Evaluate whether there is any relationship (correlation) between docility/temperament score and some blood parameters (heterophil-to-lymphocyte ratio)
4. Determine the most suitable test methodology in measuring docility.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Origin and Species of Guinea Fowl

Guinea fowl is believed to have evolved from a francolin-like Asiatic ancestor (Ghigi, 1905; Sibley and Ahlguist, 1972) but it is believed that the evolution and development to modern forms occurred solely in Africa (Cracraft, 1973; Oslon, 1974). The Guinea fowls are indigenous in Africa of which there are still some remains of wild species (Williamson and Payne, 1998; Dei and Karbo, 2004, Annor *et. al.*, 2012). They belong to the order *Galliformes* and family *Numididae* (Payne, 1990; Annor *et al*., 2012). Of the five world Galliformes, it is only the Guinea fowl, Numidinae, which is entirely endemic to Africa (Delacour, 1977). The Guinea fowl was first discovered on the Guinea Coast of West Africa from which it derives its common name (Roy and Wibberley, 1979; Payne, 1990).

Within the family *Numidae,* there are four species of which two have been domesticated namely; *Numida meleagris* (Helmeted red-wattled Guinea fowl) and *Numida ptilorhynca* (Mongi and Plouzea, 1984; Teye, 2010). The helmeted Guinea fowl (*Numida meleagris Pallas*) is most common and native to West Africa (see Table 2.1).

The species *Numida meleagris* is the most popular one in Northern Ghana (Djang-Fordjour *et. al.,* 1998)which is proven to be highly adaptable to the drier savanna with its nests and trees roosting instincts at night (Biwas, 1999).

Table 2.1 List of Guinea fowl species in taxonomic order.

Genus	Species	Description
Numida	*Numida meleagris*	Helmeted Guinea fowl
	Numida ptilorhynca	
Agelastes	*Agelastes meleagrides*	White breasted Guinea fowl with black plumage, small bare red head, white breast, black tail greenish brown bill and greyish feet
	Agelastes niger	Black breasted Guinea fowl
Acryillium	*Acryillium vulturinum*	Vulturine Guinea fowl; round body and small head; longer on the wings, neck, legs and tail; bare blue face and black neck
Guttera	*Guttera plumefera*	Plumed Guinea fowl; has bare skin on the face and neck; dull grey-blue
	Guttera pucherani	Crested Guinea fowl

Source: Annor *et al*. (2012); Iddrisu (2014)

Guinea fowl has been classified into four genera (*Agelastes, Guttera, Acryllium* and *Numida*) (Teye, 2010). The major genera of Guinea fowls can be distinguished by the lateral views of the head, neck and collar region. The Numida is the genera of all the helmeted Guinea fowls found ubiquitously distributed throughout the African savanna outside desert, Mediterranean and montane areas. The helmeted Guinea fowl is the best known of the Guinea fowl bird family. It is a large bird (53 – 58 cm) with a round body and small head. Their weight is about

1.3 kg for both the males and females. The body plumage is gray-black spangled with white dots (Teye, 2010)

2.2 Breeds and Characteristics of Guinea Fowls

In West Africa and Ghana, there are three well-known varieties of Guinea fowls namely; Pearl (Plate 2.1), Lavender (Plate 2.2) and the White (Plate 2.3) (Payne, 1990; Teye, 2010; Annor *et al*., 2012; and Iddrisu, 2014).

The most numerous and widely distributed species is the helmeted Guinea fowl, *Numida meleagris* (Biswas, 1999). It has a purplish-gray plumage, dotted or pearled with white. It is also characterized by shanks of slate grey colour and more or less dark grey-blue plumage with many rounded small white spots (Plate 2.1).

The Lavender type (Plate 2.2) has paled purple colour with black shanks, pink slate or mixture of pink and black shanks. The White type (Plate 2.3) has the ordinary white colour with pink shanks or slate shanks and white or pink wattles (Payne, 1990; Koney, 1993; Annor *et al*., 2012).

There are also exotic breeds called "industrial" broilers or layers being imported into the country from Western countries such as France and Belgium. These exotic breeds are fast growing, heavier and lay more eggs that are bigger than the local breeds (Biswas, 1999).

Plate 2.1 Pearl Guinea fowl Plate

Plate 2.2 Lavender Guinea fowl

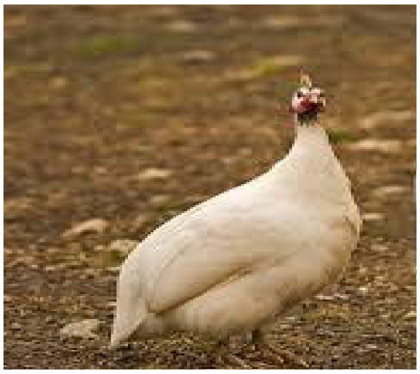

Plate 2.3 White Guinea fowl

Source: Iddrisu (2014)

The Guinea fowl is characterised by its harsh cry and agitated nature and less docile compared with other poultry. It is one of the most generally gregarious domesticated birds, but do not like confinement (Biwas, 1999; Teye, 2010).

The sexual dimorphism is not too conspicuous as compared to domestic chicken. However, the male Guinea fowl can be distinguished from the females by the harsh cry, which is a one-syllable and shriek while the female's cry is a two-syllable cry (Teye, 2010).

The size and shape of the helmet (comb) and wattles thickness and curvature and coarse head are pronounced in males. When the female is excited it never uses the two-syllable cry (Ewusie, 1964).

The egg size, as compared to domestic chicken, is generally on the reduced side with the Guinea fowl eggs being small, yellowish to brown in colour with varied mottling and weigh about 50g. The eggs keep longer than those of domestic fowls because of their unusual thicker shell (Farkas, 1965).

Even though gregarious in nature, Guinea fowls have monogamous natural instincts and under extensive systems of rearing them, they have the tendency to mate in pairs but cocks can mate with four females (Nwagu and Alawa, 1995; Biwas, 1999; Teye, 2010).

They are very secretive about their mating which is rarely observed and needs confirmation by candling of their eggs in the second week of incubation (Biwas, 1999). The female invites the male during breeding season by making intermittent squats and producing shriek noise. This signal invites the male to mount which usually go unnoticed (Djang-Fordjour *et al*., 1998).

2.3 Definition of Docility/Temperament

Temperament is the differences between individual animals in their behavioural response to alarming or challenging situations where individuals are often consistent in the way they respond when the challenge is repeated. Such differences between animals are of most importance to humans in situations that involve human interaction, like handling, or moving them. Some animals are calm and docile, while others are distressed and struggle to escape (Haskell *et al*., 2014).

Haskell *et al*. (2014)observed consistency of response within the animal, and the variation shown between individual animals or groups of animals, has historically been given a number of different labels, depending on whether the user is from a psychological, farm livestock or behavioural ecology background. In animal science, it is known as Animal Temperament or Docility. In animal husbandry settings, the term" temperament" is largely used (Tulloh, 1961; Burrow, 1997).

By way of definition, the Docility or Animal Temperament is defined as a response to environmental or social stimuli (Haskell *et al*., 2014). It is also defined as individual behavioural differences which are repeatable over time and across situations (Fordyce *et al*., 1985; Reale *et al*., 2007; D'Eath *et al*., 2002a; Sih *et al*., 2004).

This natural behaviour is often noticed when some animals like Guinea fowls, for example, employ escape and/or avoidance behaviour to minimize a threat. The expression of this natural behaviour represents the trait commonly referred to as temperament (Ferguson *et al*., 2001).

As a concept, this terminology has received an elaborated attention, and therefore, there are a number of related definitions. Debeffe *et al*. (2015) also indicated that, the term docility is an equally repeatable behaviour at both short- and long-term timescales i.e. a behavioural trait which is stable across time.

Temperament/Docility occurs across all types of production environments and includes maternal behaviour (Jarvis *et al*., 2005), aggressiveness (D'Eath *et al*., 2002a), social behaviors (Lovendahl *et al*., 2005), reactions to humans (Barozzo *et al*., 2012; Burrow, 1997), feeding behaviours, daily activities and handling responses to new objects or situations (Yoder, 2010), the ability to accept human presence (Annor *et al*., 2011) and coping behaviours (Cassady, 2007). The term *temperament* (under farm situations) is the animal's behavioural response to handling by humans and it is an inherent characteristic (Burrow, 1997).

The animals that tried to escape or move away from human contact are described as "wild," while animals that do not appear agitated by human contact are labeled as "tame" (docile) (Scott and Fredericson, 1951).

2.4 Importance of Temperament in Animal Husbandry

Temperament traits such as fearfulness or aggressiveness are important to consider as they affect how the animal responds to the husbandry and handling conditions on the farm and during procedures like transport.

Animals, like Guinea fowls, are subject to handling and management by humans on a regular basis, which can be stressful. This includes feeding, cleaning, handling and restraint, and immunizations. A negative behavioural response to handling and management procedures can greatly affect productivity. More temperamental animals have poorer growth performance, carcass characteristics, and immune responses. The obvious reason is found by Cooke *et al.*

(2009), who explained that animals with excitable temperament have altered metabolism and partitioning of nutrients in order to sustain the behavioural stress response, which results in further decreases in nutrient availability to support body functions. Also, hormones produced during a stress response, particularly cortisol, directly disrupt the physiological mechanisms that regulate reproduction in females, such as ovulation, conception, and establishment of pregnancy.

In support of this, Breuer *et al.* (2000) indicated that temperament can affect virtually all aspects of production, such as growth, reproduction, and immunity, meat quality, and milk yield.

Docility is linked to fitness (Reale *et al*., 2000, 2009), while it also influences the productivity of livestock farming enterprises (Norris *et al.*, 2014). For instance, fearful cows produce lighter calves at birth that grow less well than those produced by non-fearful (i.e. docile) cows (Turner *et al.*, 2013).

Echternkamp (1984) also found out that, in cows with calm temperament they have reduced cortisol and greater blood concentrations of luteinizing hormone, the hormone required for puberty establishment and ovulation, compared to the temperamental ones. Increased corticosterone levels have been shown to retard growth in broiler chickens (Post *et al.*, 2003). Another reason for the negative effect of temperament on productivity as presented by scientists suggest that, time spent in feeding is reduced and dry matter intake tends to reduce in animals with more excitable character (Cafe *et al.*, 2011). Therefore, the more temperamental animals tend to have lower live weights than calm animals (Fordyce *et al.*, 1985).

The importance of focusing on temperament in animal production is supported by several studies. Burrow and Dillon (1997) and Fell *et al.* (1999) found out that animals with calm temperament grew faster during fattening than nervous ones.

Temperament is being added as a new trait to the Australian beef genetic evaluation model (Breedplan) (Burrow, 2003). Experiences on commercial farms indicate that human-animal relations can have an influence on technical results such that, a more docile flock usually has better performance than a more flighty flock. Whether a flock is flighty or docile, it is, to a certain extent, genetically determined (Niekerk *et al*., 2009). In a study involving a new technique for measuring temperament in cattle, it was concluded that docile animals are more tolerant of an observer than their more temperamental contemporaries (Burrow *et al.*, 1988). Besides, empirical evidence for heritability of temperament traits has been found in many species (Boissy, 1995 and Grandin, 1998). Productivity of the animal is negatively affected when animals have poor temperaments. Burrow and Dillon (1997) indicated that animals with poor temperament may perform poorly in terms of live weight gains in feedlot than animals with good temperament. Besides the more temperamental or wild animals increase the risk of injury to facilities, workers, and other livestock, thus increasing costs (Burrow, 1997, Fordyce *et al*., 1985). Therefore, producers often select for more docile ones to reduce economic losses.

2.5 Effects of Sex, Breed, Experience and Handling

There are mixed reports of the effects of non-genetic factors on docility or temperament on animals. According to Annor *et al.* (2011), certain non-genetic factors such as litter size, sex, parity, year of birth, season of mating and season of birth and their interactions were found not to influence docility. On the other hand, another research found that, season seem to cause a decrease in docility during summer in buffaloes, as compared to other seasons. These authors are of the view that, the extent of an animal's docility to decrease and/or excitably increase is due to the onset of the hot dry season, probably resulting from increased thermal stress (Pajor *et al*., 2008; and Pajor, 2011). According to De Fries *et al.* (1978), the term temperament is a nervous system reactivity, which is determined by both genetic and environmental factors.

Another report also states that, there are a number of factors which influence docility and these include breed effect, social environment, age, sex, production system and experience (Hoppe *et al.*, 2010; Burdick *et al.*, 2011). According to Burrow (1997), temperament of livestock species can vary by age, sex, management, maternal influence, genetic factors and breed.

Yet other reports also support the claim that, temperament is determined by many factors which include, breed, sex, age, previous handling (experience), and genetics (Burrow, 1997; Grandin, 1993; Curley Jr *et. al.*, 2006; Fordyce *et al.*, 1988; Burrow, 2001). Some of these many factors that affect temperament are discussed below:

2.5.1 Breed Effects

Another factor that possibly influences docility/temperament of animals is breed effects (Norris *et al*., 2014). Hoppe *et al*. (2010) demonstrated that heritability of temperament score are affected by breed, where Herefords had a greater and Limousin had the lowest heritability estimate. It has been reported that, the pure *Bos indicus* and *Bos indicus* crosses for example appear to be more temperamental than *Bos Taurus* breeds of cattle (Burrow, 2001). Paranhos da Costa *et al.* (2002) also reported a significant effect of breed on temperament of cattle.

In a pig study by Yoder (2010), the Landrace breed was found to be more temperamental than the Duroc, Yorkshire and Chester White breeds while both Yorkshire and Chester were more temperamental when compared to the Duroc breed with the latter being the most docile amongst the studied pig breeds. In a study on two dairy cattle breeds, the Jersey was found more docile than the Holstein-Fresian (Orban *et al*., 2011). The Jersey breed had a score of 1.53 while the Holstein-Fresian had a score of 2.69. Crossbred animals have been noted to be less docile than purebreds (Schaeffer *et al*., 2011).

However, in a study by Schaeffer *et al.* (2011) this was found not to be the case as the purebreds had similar temperament as crossbreds. In goats, the least temperament breed was Sanental followed by the Alpine, with the most temperamental being the selected Hungarian (Némethet *et al*., 2009).

2.5.2 Sex Effects

An effect of sex factor on temperament is debatable. In a study, utilizing different breeds of cattle indicated an effect of sex on temperament score in which females had a greater temperament score than males (Voisinet *et al*., 1997). Another study also shows that males are calmer than females (Burrow, 1997 and Voisinet *et al*., 1997). However, studies by Burdick *et al*. (2009) and Burdick *et al*. (2011) respectively did not find an effect of sex on exit velocity. Some studies have reported a significant sex effect on temperament traits. Females are always more excitable or difficult to handle, heifers have higher temperament scores than their steers, which are more docile (Stricklin *et al*., 1984; Voisinet *et al*., 1997 a,b; Lanier *et al*., 2000; Gauly *et al*., 2001). These results clearly show that temperament traits of animals can be considered as governed by the same pool of genes between sexes, even though females may exhibit higher phenotypic and genetic variability for some traits.

There is no difference between temperament scores for the two sexes in sheep, but there are differences between different litter sizes. Twin animals have calmer temperament than singles (Pajor *et al*., 2008; and Pajor, 2011).

2.5.3 Effects of Experience and Handling

In report by McEwen *et al*. (1997), they state that the factors that contribute to whether an animal perceives situations as stressful include prior experience and development history. Both Le Neindre *et al*. (1997) and Grandin (1997) state that both genetic characteristics and prior experience influence how animals react to humans. Boandl *et al*. (1989) also found an increase in cortisol concentrations, which is a stress related hormone, in response to handling. Those investigators also found differences between the responsiveness of calves to handling, noting that calves that had greater human contact previously had a lesser cortisol response and were less responsive than those that had less human contact. Consistent handling during early life and over long periods of time seems to improve the temperament situation of an animal and perhaps prevent the negative effects of temperament (Fordyce *et al.*, 1988; Grandin, 1997; Curley Jr *et al.*, 2006; Burrow and Dillon, 1997) on productive traits. During handling, fear is a major determinant of an animal's behaviour. Genetic influences on temperament interact with early experience and learning to shape adult patterns of behaviour.

Selective breeding of rats for either high or low fearfulness clearly shows that genetics plays a major role in determining an animal's temperament (Broadhurst, 1960; Broadhurst and Eysenck, 1964; Huck and Price, 1975). Behaviours studied in the laboratory are clearly shown to be substantially influenced by genetic factors (Plomin, 1990). The degree of the effects of handling felt by animals depends on temperament of the animal. As Grandin (1997) proposed, that rough handling may be more stressful to temperamental animals than to those animals that are calmer.

Besides, the frequency of handling as it varies from one production system to another presents different effect on the animals and does not necessarily reduce an animal’s response to novel experiences but may reduce the reaction of animals to the previous exposure to those specific managerial tasks. However, this is not true for more temperamental animals as repeated handling are found not to reduce the reactivity of more temperamental cattle. In that case, more temperamental livestock may be better suited for environments where handling is limited, while calmer ones may be better suited for operations wherein intensive handling is an aspect of the production system (Grandin, 1997).

However, there are indications that consistent handling early in life and over long periods of time can improve the temperament and perhaps prevent the negative effects of temperament on carcass quality (Fordyce *et. al*., 1988; Grandin, 1997; Curley Jr. *et al*., 2006; Burrow and Dillon, 1997).

2.6 Genetic Response of Docility and Correlation with Performance of Livestock

Phenotypic and genotypic correlation of traits is very important in establishing the relationship between the traits of economic importance. Selection based on traits depends on their correlational relationship. Normally, improvement in one trait may lead to reduction in the other and the vice versa or improvement on one trait may lead to improvement in the other as well. Temperament as a genetic trait has been found to be moderately heritable. In a study by Prayaga and Henshall (2005), heritability of docility score was estimated at 0.37 in weaned Australian cattle, 0.22 in Angus heifers (Otterman *et al.*, 2013), 0.35 and 0.34 by Paranhos da Costa *et al.* (2002). Higher heritability estimates of 0.46 and 0.54 have been reported in studies by Hearnshaw and Morris (1984) and Burrow *et al*., (1988) respectively. Fordyce *et al.* (1982) reported even higher heritability (0.67) for Zebu and European breeds. All these heritabilities are indicating possibility of genetic selection for docility.

In a study by Sant'Anna *et al.* (2013) all temperament indicator traits showed large genetic variability to respond to selection for fast genetic gain in Nelore cattle. Kadel *et al.* (2006) showed that better temperament is genetically correlated with improved tenderness with moderate genetic correlations. The study by Sullivan and Burnside (1988) showed that it is feasible to identify sires with significant differences in their daughters' handling and feeding behaviour. It also further suggests that selection taken early in the heifer's lactation, would identify sires that leave predominantly quiet heifers that are easy to work within the milking and handling process.

Lennon *et al.*, (2008) found that, the heritability of ewe mothering temperament was 0.39 indicating a moderate genetic component to this behavioural trait. In another study also in sheep Murphy (1999) found a 10% higher lamb survival rate in twins for ewes selected for temperament than that of the ewes from the ‘nervous’ flock.

Aside from the positive effects on mothering ability, selection for temperament of females also affect the behaviour of the females during the mating period and in the early stages of gestation and survival of newborns (Blache and Bickell, 2010). The result of this same study also shows that females with calm temperament have a greater ovulation rate than nervous ones.

Low positive genetic and phenotypic correlations between size traits (body weight and growth rate) and docility are reported in the grasscutter (Annor *et. al.*, 2011; Yewadan, 2000) and in tropical beef cattle (Burrow, 2001). Yewadan (2000) observed low but negative genetic and phenotypic correlation between body weight and docility. Burrow (2001) observed low positive genetic and low negative phenotypic correlations between temperament (docility) and size traits.

In similar researches, the results are quite similar with respect to the phenotypic and genetic correlations between docility in terms of pre-weaning survival, and docility and post-weaning survival (Lennon *et al.,* 2008; Visscher and Goddard, 1995).

Despite these observations, the effects of temperament on body weight in animals with calmer temperament had higher weight at the end of fattening and higher average daily weight gain compared to nervous animals (Pajor *et al*., 2008).

Nkrumah *et al.* (2007) and Rolfe *et al.* (2010) found no phenotypic relationship between temperament and feed intake, and feed conversion ratio, but found low to high genetic relationships between the same traits. The conclusion is that behaviour traits may contribute to the variation in the efficiency of growth of beef cattle, and there are potential correlated responses to selection to improve efficiency.

2.7 Repeatability of Docility

The repeatability describes the proportion of the phenotypic variance explained by additive genetic and permanent environmental effects (Hohenboken, 1985). A high repeatability therefore also means that docility is influenced by permanent environmental effects, rather than temporal effects (Murphy *et al.*, 1994; Reale *et al.*, 2000; Reale and Festa-Bianchet, 2003). This observation was confirmed by Annor *et al.* (2011), when they obtained moderate proportion of phenotypic variance due to permanent effects of dam for docility.

Repeatability of a trait estimates the correlation between repeated measurements of the same individual. This parameter indicates the gain in accuracy to be expected from multiple measurements. By repeating the measurement on the same individual, the variance due to temporary environmental differences is reduced; therefore, total variance decreases leading to an increase in the heritability. Docility has been reported to be a repeatable behavioural trait in several studies, both in captivity (David *et al.*, 2012; Mazurek *et al.*, 2011) and in the field (Ferrari *et al.*, 2013; Petelle, *et al.*, 2013; Reale *et al.*, 2000, 2009).

A high value of repeatability of docility was obtained in study of docility of grasscutter which indicates that measurement of docility for the same individual at different times was highly consistent (Annor *et al.*, 2011). Also Re´ale *et al.* (2000) noted a high repeatability (r=0.81) estimate of docility in the Ram Mountain bighorn population. They therefore suggested that the trait should be heritable. It is worth noting that high repeatability of temperament in this population may also be caused by maternal effects or permanent environmental effects (Re´ale *et al.*, 2000).

This means that few records on the animal are enough to make selection decision (Hohenboken, 1985). Similar results were obtained by Reale *et al.* (2000) in wild bighorn sheep. They measured temperament for the same individual at different captures, and obtained a repeatability of 0.86, which was highly consistent. Hearnshaw and Morris (1984) also obtained high repeatability of temperament of 0.67 and 0.82, respectively for calves and cows on measurements taken the same day. They concluded that the high repeatability estimates obtained indicate that the 0-5 temperament score used was effective in describing cattle behaviour in the crush and bail.

Docility is a commonly measured behavioural trait (Benhajali *et al.*, 2010; Martin and Reale, 2008a; Reale *et al.*, 2000) often associated with the shy-bold behavioural gradient (Reale *et al.*, 2007).

2.7.1 Docility and Habituation

There is a possibility of individuals habituating to a behavioural test when they are measured several times as purported by Martin and Reale (2008a). Such conditions may lead to a decrease in the power of the behavioural response in many trials culminating in bias repeatability estimates (Debeffe *et al.*, 2015). Le Neindre *et al.* (1997), indicated that previous experience with handling has the potential to increase docility during subsequent captures or decrease the intensity of behavioural response (Martin and Reale, 2008a; David *et al.*, 2012).

2.7.2 Age and Sex Effects on Repeatability

Behavioural repeatability generally decreases as the time elapses between successive measurements (Bell *et al.*, 2009; David *et al.*, 2012; Gifford *et al.*, 2014) since with time there is more opportunity for individuals to experience developmental modifications and fluctuating environments (Stamps and Groothuis, 2010), and also because their phenotype may be influenced by different genes at different ages (Charmantier *et al.*, 2006).

Repeatability does not differ markedly between age and sex categories but tend to be higher in young males than in young females. The individual variation in the repeatability of docility is not correlated with individual body mass (Debeffe *et. al.*, 2015). Schuett *et al.* (2010) however, indicated that behavioural repeatability may differ between the sexes due to the action of sexual selection.

Contrarily to the above, Stamps and Groothuis (2010) and De Kort, *et al.* (2009) say repeatability varies in relation to age because behavioural repeatability seem to have an ontogenetic component i.e. it develops progressively over a lifetime, with the effect that, the behaviour of older individuals are more repeatable than that of younger ones. Roberts and Del-Vecchio (2000) indicated that, behavioural repeatability in humans increases with age up to 50 years old before reaching a plateau. On this score, higher repeatability of docility is expected among adults.

2.8 Measurement of Docility

In previous experiments involving other animals, the average docility score was 2.6, obtained in the study of cage-reared grasscutters in the tropical climate, where the assessment was based on four-point score (i.e. 1 – 4). The score 1 referred to docile animals and which adapt well to life in confinement and become accustomed to man quickly, whereas the non-docile (score 4) grasscutters panic when people approach and try to escape from their cages or pens (Annor *et. al*, 2011; Mensah and Okeyo, 2006).

The techniques for measuring docility are continually being refined and improved making it possible to accurately measure docility (Norris *et al*., 2014). Temperament is measured by scoring using methods developed as early as the 1960s. In temperament assessment both objective and subjective methods are utilized (Stricklin *et. al.*, 1984).

Fordyce *et al.* (1982) developed a number of temperament tests, including the flight distance test, pen scores, and chute scores, for the assessment of cattle. Burrow *et al.* (1988) later developed the flight speed test, more commonly known as exit velocity, as a more effective assessment of cattle temperament. The exit velocity is an objective assessment method while pen score and chute scores are subjective in nature.

However, Cooke and Bohner (2010b), stated that, there are a number of methods for evaluating docility and these ranges from simple visual observations to assessments that require computerized techniques. That, these methods can be divided into restrained techniques, non-restrained techniques, and phenotypic evaluations. The phenotypic evaluations account for external features of the animal that have been associated with temperament and are usually indirect measures of docility. They explained that, the restrained techniques evaluate temperament when animals are physically restricted, such as in the squeeze chute (chute scoring) while the non-restrained techniques evaluate animal docility based on their fear or aggressive response to humans when they are free to move within the evaluation area (flight speed and exit velocity).

Currently, chute scores, pen scores, and exit velocities remain the most common measurements of animal temperament. As the new measurements began to appear, more physiological and performance associations with temperament have been elucidated.

In the most recent study on the docility of German Simmental and Charolais heifers by Geburt *et al.* (2015), eye temperature appears to be another suitable indicator of stress and docility. They observed that, more stressed animals were less docile and more difficult to handle. Hence for the past several years objective and subjective methods have been utilized.

2.9 Measurement Methodology of Docility/Temperament

Temperament can be measured using a range of different tests that objectively or subjectively assess the animal's behavioural response to a fear-eliciting situation. These tests largely characterize escape and/or avoidance behaviour and range from simple subjective assessments of agitation in the cage to more complex tests (Ferguson *et. al.*, 2001).

2.9.1 Flight Speed/Time

The flight speed or flight time assessment originally used by Burrow *et al*. (1988) is now widely used by several groups in the livestock industry. The assessment typically takes place as part of a routine weighing or handling procedure, where the animal is held in a handling system, such as a race or chute. Once the procedure is complete, the animal is released from the chute. The time it takes to cover a set distance along a raceway is calculated. This distance is typically short to capture the immediate response to release (e.g., 1.7m: Burrow and Dillon, 1997; Café *et al*., 2011; 1.83 m: Curley *et al*., 2006). This can be presented as a velocity (e.g., "exit velocity"; Curley *et al*., 2006) or as a "flight time" for a set distance (e.g., Fell *et al*., 1999).

2.9.2 Chute Test

The chute test assesses the strength of response to confinement, whilst the animal is inside the chute. It is made on a categorical scale (typically1–5), with qualitative or descriptive definitions given to states of increasing agitation, from no response, docile or calm through to vigorous, wild or violent response (Tulloh, 1961; Hearnshaw *et al*., 1979; Grandin, 1993).

Similar categorical scoring systems have been used to quantify the response to confinement in cages (Annor *et al*., 2011).

There is usually a human observer who approaches the animal to observe the relative movements and reactivity of the animal. The reactions are matched with scores between 1 and 4 based on the animal reaction to the observer. The docile animals do not react to the observer, and allow the observer to approach and are given a score of 1. An animal is given a score of 2 if it is slightly aggressive, is aware of the observer, and likely stands in a corner away from the observer. Animals that move away from the observer hardly stand and move away alongside the fence/cage, fully aware of the position of the observer, are given a score of 3. A score of 4 is given to animals that are aggressive. They are aware of the observer, may run continuously along the cage or even with attempt to escape and sometime with continuous cry. Based on temperament score, the animal is ranked into temperament groups; Docile (calm), flighty (intermediate in behaviour) and temperamental (restless or aggressive), (Burdick *et al.*, 2011).

2.9.3 Docility Test

This is a main type of unrestrained test in which the animal is separated from its group mates and moved to another pen. After a short period, the handler tries to drive the animal to a corner of this pen and hold it there for a predetermined period of time without physical aids. The responses to all parts of the test are integrated in to a single score, but scores for the component parts can also be analyzed (Boivin *et al*., 1994; Le Neindre *et al*., 1997). Some authors also score response to human approach in a pen on a categorical scale (King *et al*., 2006, Annor *et al*, 2011). Similar to this is an assessment of flight distance, which is the distance at which an animal starts to move away from an approaching human (Fisher *et al.*, 2001). This is similar to the approach/avoidance distance assessments used in dairy cattle (Waiblinger *et al.*, 2003; Gibbons *et al.*, 2009b). Animal responses to each of these measures of temperament have been shown to be repeatable over time (Hearnshaw and Morris, 1984; Grandin, 1993; Burrow and Dillon, 1997; Gibbons *et al.*, 2009; Turner *et al.*, 2011). It is of interest to understand whether these different tests measure the same underlying trait. A number of studies have found a significant relationship between the measures. In beef cattle, flight speed and chute test score have been found to be significantly moderately correlated (Fell *et al.*, 1999; Olmos and Turner, 2008; Hoppe *et al.*, 2010; Café *et al.*, 2011) and positive correlations between chute score and flight speed, and chute score and docility have also been shown (Turner *et al.*, 2011). Grignard *et al.* (2001) found a significant relationship between the docility test and the chute test in Limousin cattle, with and without a human present in front of the chute. Additionally, Curley *et al.* (2006) found a moderate relationship between chute scores and response to confinement in a pen. These relationships are not found universally; others have reported weaker correlations (Burrow and Corbet, 2000), or variations in strength of the correlations between breeds (Cafe *et al.*, 2011). Overall, this would suggest that these tests are assessing similar if not identical underlying traits.

For the sake of accuracy, usually multiple methods are often employed, with the three most common measurements being chute score, pen score/docility score, and flight speed/ flight time (Grandin, 1993). According to Burdick *et al.* (2011), it is appropriate to utilize different but related methods to assess temperament and then find the average score as representative of the true behaviour in order to allow for more accurate temperament classification. In their recent studies, Burdick *et al.* (2011) have utilized an average of pen score and exit velocity to assign a temperament score to cattle. Also, Cafe *et al.* (2011) demonstrated that correlations between temperaments measured at 2 different time points were greater for an average of exit velocity and chute score than either measurement alone.

The appropriate and convenient life stages to subject an animal to docility test have been another angle of research interest. According to Burdick *et al.* (2011), temperament is most often measured at weaning, and that most of the published literature has focused on the effects of temperament during the early pre-weaning and post-weaning periods.

Earlier on, Burdick *et al.* (2009) demonstrated that exit velocity can be measured at an earlier age (21 to 24 days of age), yet exit velocity measurements made that early in life predicted temperament at weaning (173 ± 2 days of age) in less than 60% of the calves. An additional study showing the evolution of exit velocity in Brahman calves from 21 days of age through approximately 231 days of age demonstrated that animals classified as temperamental (based on temperament score) increase their exit velocity at a greater rate than calm and intermediate ranked ones (Burdick *et al*., 2011).

2.10 Docility and Stress Responses

It appears that Hans Selye is reported to have been the first biomedical author to use the term *stress* in biological science (Burdick *et al.*, 2011). Selye illustrated the stress mechanism of an organism in a physiological triad that includes (1) thymico-lymphatic involution, (2) adrenal enlargement, and (3) gastric ulceration (Selye, 1936). Selye alluded to the phenomenon of *homeostasis* coined by Walter Cannon in his article in which he termed the efforts of an organism to return to homeostasis as the “general adaptation syndrome” (Selye, 1936).

Selye attributes the achievement of an organism to a homeostatic state through the responses of the adrenal cortex, mainly the production of cortisol/corticosterone, while Cannon alluded to the role of the sympathetic nervous systems in the stress response (Cannon, 1932, Pac´ak and Palkovits, 2001). It is now a common knowledge that both hypothalamic pituitary-adrenal axis and the sympathetic nervous system play significant roles in the body's response to stressors.

The classical definition of stress used by Selye has seen some modification over time in order to reflect the progression in our knowledge base regarding the biology of stress. Stress is currently defined as "a state in which homeostasis is actually threatened or perceived to be so; homeostasis is re-established by a complex range of behavioural and physiological adaptive responses of the organism" (Chrousos, and Kino, 2005).

The stress response is stimulated by a stressor and affects the body through activation of the hypothalamic-pituitary-adrenal axis (HPA axis) system and the sympathetic nervous system (more specifically the sympathomedullary system) (Butcher and Lord, 2004).

Stressors are any internal or external stimuli or threat (physical, psychological, or chemical) that disrupts homeostasis (Aguilera, 1998; Black, 2002). In response to this altered state, the stress response is activated in order to help the body cope with the threat and return to or maintain homeostasis.

Now, given that temperament is a stress response trait, and that both the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system play roles in determining individual animal's responses to stress, it is appropriate to discuss the roles of each in stress response of an organism in the midst of stressors.

2.10.1 Hypothalamic-Pituitary-Adrenal (HPA) Axis

In response to stressful stimuli, the corticotropic releasing hormone (CRH) is released from the hypothalamus (Carrasco and Van De Kar, 2003; Plotsky, 1991; Gibbs and Vale, 1982). The CRH, increasing in concentration in the portal blood exposes the anterior pituitary glands to these neurohormones. These neurohormones then activates the adrenal axis to synthesize and secrete adrenocorticotropic hormone (ACTH) into circulation (Carrasco and Van De Kar, 2003; Webster-Marketon and Glaser, 2008). ACTH in circulation elicits the production of glucocorticoids namely, cortisol or corticosterone from the cortex of the adrenal gland (Pugh *et al*., 2011; Carrasco and Van De Kar, 2003; Makara *et al*., 1981).

In most mammals such as humans, cattle, and pigs the primary glucocorticoid is cortisol; however, in birds and rodents the primary glucocorticoid is corticosterone. When the adrenal cortex is stimulated by ACTH, glucocorticoids are released and distributed in blood, acting systemically to produce a variety of stressful effects in the animal (Burdick *et al*., 2011).

Glucocorticoids have been found to negatively affect growth by increasing the production of leptin which has been documented to reduce feed intake (Agarwal *et al*., 2009). Glucocorticoids break down protein, glycogen, and fat to increase the amount of circulating glucose (Pugh *et al*., 2011). An increase in circulating cortisol concentration also impairs the cell-mediated immunity of the animals by decreasing the number of macrophages, natural killer cells, T lymphoctyes, and cytokines (Jain *et al.*, 1991).

In the absence of stressor stimulation, CRH rather is released at a frequency of approximately 2 to 3 secretory episodes per hour, with greater pulse amplitudes in the early morning (Tsigos and Chrousos, 2002). The release of CRH and ACTH can be modulated or altered due to changes in lighting, feeding schedules, activity, and ultimately stress (Tsigos and Chrousos, 2002).

Glucocorticoids are transported through the circulatory system by carrier proteins that prevent degradation. Carrier proteins also allow glucocorticoids to be available quickly after induction of the stress response. Albumin is the major carrier protein for cortisol (Burdick *et al.*, 2011).

2.10.2 Sympathomedullary System (SMS)

The sympathetic nervous system is usually the first to be activated in response to many stressors. When it is stimulated, the sensitive neurons in the peripheral organs (heart, kidney, gut and adipose) secrete norepinephrine into circulation resulting in increased blood pressure, heart rate, and respiration rate (Burdick *et al.*, 2011).

In addition, nerve impulses in higher cortical centers within the brain transmit messages by release of norepinephrine, serotonin, and acetylcholine (Black, 2002). In conjunction with these actions, the adrenal medulla is also stimulated into producing and secreting epinephrine and norepinephrine via acetylcholine (Butcher and Lord, 2004).

The sympathetic nervous system regulates many functions in the body including the cardiovascular, gastrointestinal, respiratory, and renal systems, all of which can be modulated in response to SMS activation (Charmandari *et al*., 2005). An increase in epinephrine concentrations in the brain serves as an alarm system, resulting in a decrease in neurovegetative activities (e.g., eating and sleeping) and the activation of the stress response (HPA axis activation) (Tsigos and Chrousos, 2002). The secretion of norepinephrine within the brain also activates the fear behaviours and enhances long-term memory and storage of adversely charged emotions in the hippocampus (Tsigos and Chrousos, 2002, Sapolsky *et al*., 2000). Animals express fear when exposed to human contact and novel objects and/or new environment; during times of major changes to their social structure such as isolation. Fear is a typical stressful stimulus. Animals express this by changes in physiology (e.g. increase heart rate, adrenal secretion of catecholamine and/or cortisol) and behaviour.

2.11 Docility and Stress Relationship

The question of whether the degree of stress response activation correlates with temperament or not has been the bane of researches in recent years. That is, are stressful animals more temperamental? Fortunately, recent researches suggested that stress responsiveness is associated with animal behaviour, specifically temperament (Burdick *et al*., 2011).

Burdick *et al.* (2011) found that more excitable temperamental cattle, exhibit greater basal concentrations of glucocorticoids and catecholamines. They concluded thus, understanding the interrelationship of stress and temperament can help in the development of selection and management practices that reduce the negative influence of temperament on growth and productivity.

Indeed, docility has often been linked with behavioural traits such as activity and exploration (Ferrari *et al*., 2013; Martin and Reale, 2008a) and with physiological parameters such as hypothalamo-pituitary-adrenal reactivity (Montiglio *et al*., 2010) or the level of cortisol (Martin and Reale, 2008b). Usually, the hormone levels in vertebrates influence physiological stress due to cortisol levels increasing with increasing stress (Kandeepan, 2014).

These increases in the secretion of stress-related hormones in response to physical and psychosocial stressors are normally associated with livestock management procedures such as weaning, castration, transportation, and regrouping (Burdick *et. al*., 2011).Since is stress responsiveness has been associated with animal behaviour especially temperament. Stressors in the form of handling influence physiological processes. Due to interactions of the stress response and temperament, the effect even results in immune function altering in more temperamental animals.

In another finding, stress is linked with certain behaviours and conditions such as fear, anxiety, and depression (Tyrka *et. al.,* 2008), because according to Johnson *et al.* (2005), glucocorticoid receptors congregate in the lateral amygdala, a region of the brain known for detecting and storing fear memory. These finding confirmed what Grandin (1997) earlier on found out, when he stated that even though the management procedures that do not necessarily cause pain, they may act as a psychological stressor by inducing fear, which is a strong stressor. In recent years the secretion of stress hormones has been linked to temperament in many species of livestock (Curley Jr. *et al*., 2008; Sørensen *et al*., 2005). It has been found in mice that overexpression of phenylethanolamine-n-methyl transferase (PMNT), the enzyme that converts norepinephrine to epinephrine, produce greater amounts of epinephrine and are more aggressive (Sørensen *et al*., 2005). Otterman *et al*. (2013) also said that, blood cortisol is positively correlated with temperament as measured by exit velocity.

Stress not only adversely affects key physiological processes of the reproductive and immune systems, but also animal behaviour, specifically temperament. This means temperament influences the animal's stress response and has similar relationships with the HPA. In cattle it is found that those with more excitable temperaments exhibit greater basal concentrations of glucocorticoids and catecholamines (stress hormones).

A positive correlational relationship has been found to exist between temperament and cortisol levels in the blood, suggesting that more excitable animals will be easily stressed (Curley *et al*., 2008; Cooke *et al.*, 2009). A study in humans suggested that cortisol secreted in breast milk may influence infant temperament (Glynn *et al.*, 2005). In cattle, differences in temperament have been linked to stress responsiveness with more excitable (temperamental) cattle having greater basal concentrations of cortisol than calm cattle (Fell *et al*., 1999; Curley Jr. *et al*., 2006; King *et al*., 2006).

It has also been suggested that temperamental cattle display an endophenotype of chronic stress, due to the chronic nature in which cortisol concentrations are elevated (Curley Jr. *et al.*, 2008), as well as depression, as temperamental cattle display a reduced ACTH response to CRH but an enhanced response to Vasopressin (Curley *et al*., 2010).

2.12 Hematological Parameters used for Stress Assessment

2.12.1 Heterophils and Lymphocytes

Heterophils, the avian equivalent of the neutrophil, function as professional phagocytes to aid in regulation of innate host defenses (Kogut *et al.*, 2002). Heterophils are functionally equivalent to neutrophils. They actively participate in inflammatory lesions and are phagocytic. Most vertebrates have five types of WBCs: lymphocytes, neutrophils/heterophils, eosinophils, basophils and monocytes. In birds and reptiles, the neutrophil is replaced with the heterophil, which performs the same immunological function (Hawkey and Dennett, 1989; Jain, 1993).

Heterophils and lymphocytes make up the majority (i.e. nearly 80% combined) of WBCs in mammals (Jain, 1993), birds (Rupley, 1997), amphibians (Bennett *et al*., 1972; Cathers *et al*., 1997; Thrall, 2004) and reptiles (Eliman, 1997; Fisse *et al*., 2004; Werner, 2007). However, heterophils are the most abundant granulocyte in most avian species and occur alongside lymphocytes, monocytes, eosinophils and basophils in avian blood. Heterophils are the primary phagocytic leukocyte, and proliferate in circulation in response to infections, inflammation and stress (Jain, 1993; Campbell, 1995; Rupley, 1997; Harmon, 1998; Thrall, 2004). Lymphocytes are also involved in a variety of immunological functions such as immunoglobulin production and modulation of immune defense (Campbell, 1995).

All these points emphasis the primary function of the heterophil and lymphocytes, but then scientists have also established that there is a relationship between these blood parameters and stress levels in animals. Hence it is now preferred to be used in indexing stress in vertebrates (Table 2.2).

Table 2.2 Comparison of leukocyte profiles (percentage of total leukocytes) across vertebrate taxa

Taxon	**Species**	**Lym.**	**Neut/ Het**	**Eos.**	**Bas.**	**Mon.**	**Source**
Mammals	Dog (*Canis lupus familiaris*)	23·1	66·4	6·3	0·1	4·0	(Jain, 1986)
	Human (*Homo sapien*)	34·0	59·0	2-7	0·5	4·0	(Albritton, 1952)
	Horse (*Equus caballus*)	38·7	52·6	3·4	0·5	4·3	(Jain, 1986)
Birds	Chicken (*Gallus gallus*)	63·0	30·1	2·5	1·3	3·1	(Branton *et al.*, 1997)
	Great tit (*Parus major*)	68·5	19·6	5·6	5·6	1·0	(Hauptmanova *et al.*, 2002)

	Glaucous-winged gull (*Larus glaucescens*)	43·0	53·0	3·0	0·0	1·0	(Newman *et al.*, 1997)
Amphibians	Red-spotted newt (*Notopthalmus viridescens*)	63·5	24·3	6·2	3·2	2·8	(Bennett and Daigle, 1983)
	Bullfrog (*Rana catesbeiana*)	62·9	22·0	8·9	2·5	0·6	(Cathers *et al.*, 1997)
	American toad (*Bufo americanus*)	20·0	68·0	3·3	7·4	1·5	(Forbes *et al.*, 2006)
Fish	North African catfish (*Clarias gariepinus*)	58·8	39·4	0·0	0·0	2·6	(Gabriel, *et al.*, 2004)
	Channel catfish (*Ictalurus punctatus*)	43·0	3·5	0·0	0·0	1·6	(Ellsaesser and Clem, 1986)
	Tilapia (*Oreochromis mossambicus*)	69·5	7·8	1·3	0·0	21·5	(Nussey *et al.*, 1995)
Reptiles	Diamondback terrapin (*Malaclemys terrapin*)	17·7	74·6	1·1	1·6	6·1	(Werner, 2007)
	Russian tortoise (*Agrionemys horsfieldi*)	46·7	37·2	4·8	5·0	6·3	(Knotkova *et al.*, 2002)
	Inland bearded dragon (*Pogona vitticeps*)	59·0	27·0	0·0	9·0	5·0	(Eliman, 1997)

Eos = Eosinophil, Neut/Het = Neutrophil/ Heterophil, Bas = basophil, Mon = Monocyte

Besides scientists also know that elevated plasma corticosterone increases circulating heterophil/lymphocyte (H/L) ratio (Siegel, 1995). For example, Puvadolpirod *et al.* (2000) achieved a pronounced increase in H/L ratio in birds when he mediated with an exogenous stressor. Hence according Lentfer *et al.* (2015) measuring the ratio of heterophils and lymphocytes (H/L) in response to different stressors is a standard tool for assessing long-term stress hens.

Much of the early literature points to a close link between leukocyte profiles and glucocorticoid levels. Specifically, these hormones act to increase the number and percentage of heterophils in birds, while decreasing the number and percentage of lymphocytes. This phenomenon is seen in all five vertebrate taxa (Table 2.2) in response to either natural stressors or exogenous administration of stress hormones. Therefore, high ratios of heterophils to lymphocytes (‘H: L’ ratios) in blood samples reliably indicate high glucocorticoid levels.

Furthermore, this close relationship between stress hormones and H:L ratios needs to be highlighted more prominently in haematological assessments of stress, as it aids the interpretation of results. Given the universal and consistent nature of the haematological response to stress, plus the overwhelming evidence from the veterinary, biomedical and ecological literature, it can be concluded that this method can provide a reliable assessment of stress in all vertebrate taxa (Davis *et al.*, 2008*)*.

2.12.2 Heterophil/Lymphocyte Ratios in Stress Prediction

Now in order to understand the interrelationships between stress and docility, it is proper to review the methodology of indexing stress. The methodologies for stress measurement include the use of level of glucocorticoids in blood and now heterophil to lymphocyte ratio (H/L ratio). Assessment of stress on the basis of H/L ratios has several advantages over measurement of hormone levels (Davis *et al*., 2008).

Giammarco *et al*. (2012) indicated that the haematological parameters are useful in highlighting the stress condition. They observed that, stress significantly causes neutrophilia

(P<0.001) and lymphocytopaenia (P<0.001) in rabbits which they attributed to the endogenous release of corticosteroids after the animals have been exposed to transport as stressor. They also said that, N/L ratio (or H/L ratio in birds) is another measure useful to evidence the sustained effect of stress, which increases with stress. Scope *et al.* (2002) reiterated that the H: L ratio has been used as a reliable index of stress in birds.

The Heterophils-to-Lymphocytes ratio (H/L ratio) as reliable and widely used index and estimator of stress in birds and reptiles was indicated by Gross and Siegel (1983); Maxwell (1993); Aguirre *et al*. (1995); and Ots *et al*. (1998) as stated in Cirule *et al.* (2011).Vleck *et al*. (2000) confirmed the use of H/L ratio as index of stress in birds.

Gross and Siegel (1983), Gross (1989), McFarlane and Curtis (1989) recounted that exposure to novel social situations ultimately elevate the number of heterophils and depress the number of lymphocytes.

The H/L ratio as index of stress has a relationship with glucocorticoid hormone levels which is used as an estimator of stress. It is said that the leukocytes components in vertebrates an increase in glucocorticoid hormones (i.e. corticosterone in birds) cause characteristic changes in the leukocyte components of the vertebrate immune system that can be quantified and related to hormone levels (Davis *et al.*, 2008). But the leukocyte numbers change more slowly (30 min to 20 hr.) in response to stress than does corticosterone (Dein, 1986; Maxwell, 1993; Cunnick *et al*., 1994). These changes are less variable and longer lasting than the corticosterone response, and multiple stressors usually have an additive affect (McFarlane and Curtis, 1989; McKee and Harrison, 1995).

These preceding statements confirm the fact that using H/L levels as indicator variable of stress is more appropriate and reliable than the use of glucocorticoids. This even offers more advantages over direct glucocorticoid measurement. It does not require prohibitive rapid sampling since levels of plasma corticosterone rise quickly immediately following capture (Romero and Reed, 2005), thus making it difficult to obtain baseline measurements in field situations and is relatively inexpensive. Moreover, this approach to measuring stress can be applied to most vertebrates, and that results obtained from one taxonomic group should be useful for making predictions in others (Davis *et al.*, 2008).

Physiologically what stress does is that, it causes a relative increase in numbers of heterophils and a corresponding reduction in lymphocytes in the white blood cells. This results in a significant increase in the H/L ratio (Giammarco, *et al*., 2012).

Interestingly, the high H/L ratios are caused by the increased blood corticosterone levels (El Lethey *et al*., 2003; Müller *et al*., 2010; Shini *et al*., 2010). This finding of the above authors re-emphasises the relationship of H/L ratios and the glucocorticoids levels.

Krams *et al*. (2012) also adds that, the H/L ratios predict the magnitude of stress response to a novel stressor in birds. They further indicated that, H/L ratios are specifically sensitive to either natural stressors or administration of stress hormones, so that relatively high heterophil counts in relation to lymphocytes reliably indicate high glucocorticoid levels (reviewed by Ots *et al*., 1998; Davis *et al*., 2008).

Banbura *et al*. (2013) also considered Heterophil-to-lymphocyte ratio (H/L) as a reliable indicator of prolonged stress reaction in birds while these authors, Ots *et al*. (1998); Vleck *et al*. (2000); Gross and Siegel (1983); Moreno *et al*. (2002) and Davis *et al*. (2008) in theirs researches found the H/L ratio turned out to be a reliable indicator of chronic stress that develops over a longer time. Maxwell (1993) postulated that the H/L ratio is a good indicator of chronic mild to moderate stress.

2.13 The Relationship of H/L Ratios and Docility

Based on the premise that stress has a link with certain behaviours and conditions such as fear, anxiety, and depression (Tyrka *et. al.*, 2008), this parameter is based on the stress levels of the bird which associates with heterophils and lymphocytes counts in the white blood cells (WBCs). The higher the H/L ratio, the higher the stress levels and then the more hostile and aggressive is the bird.

Krams *et al*. (2011) indicated that, according to the concept of glucocorticoid induction in stress, the less stressed individuals, (i.e. the birds with low H/L ratios) are less aggressive and respond to behavioural temperamental test better than the birds with high H/L ratios. They however stated that, this prediction relies on the assumption that H/L ratios reflect the extent of chronic stress experienced previously.

Interestingly, there seemed to be a negative correlation between physiological stress and lymphocytes counts as indicated by Siegel (1962a), and Thaxton *et al*. (1968; 1974). However, this condition rather results in an increase in heterophil counts. This is confirmed by the

MedlinePlus findings that state that, an abnormal increase in one type of white blood cell can cause a decrease in the percentage of other types of white blood cells(http://www.nlm.nih.gov/medlineplus/ency/article/003657.htm; 10/05/2015).

Gross and Siegel (1983) indicated that, the number of lymphocytes in chicken blood samples decreased and the number of heterophils increased in response to stressors and to increasing levels of corticosterone. The ratio of heterophils to lymphocytes is less variable than the number of heterophil or lymphocyte cells.

2.14 The Relationship of H/L Ratios and Sex, Variety, Production Traits and Test methods

Ots *et al*. (2001) showed that birds with lost in body mass also had the highest increase in H/L ratios. This was later confirmed by Krams *et al*. (2011), who said there is a negative correlation between H/L ratio and body mass. Vleck *et al*. (2000) found that sex and handling have no effect on H/L. Burdick *et al*. (2011) in their study of the association between heterophil/lymphocyte ratio as a marker of 'resistance' to stress, and some production and fitness traits in chickens, had a mean H/L ratios for males and females, respectively, as 0·87±0·03 and 0·83±0·03 (P>0·05). This indicates that there is positive relationship between temperament and H/L ratios. But for males and females, there is no significant difference between them.

Vleck *et al*. (2000) found that repeated handling does not affect H/L of free-living Adelie Penguins but there were significant differences among individuals.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of Study

The study was conducted at the Guinea fowl Section of the Department of Animal Science Education, University of Education Winneba, Mampong-Ashanti campus, Ghana from April 2016 to June 2016. Mampong-Ashanti lies in the transitional zone between the Guinea savanna zone of the north and the tropical rain forest in the south of Ghana (Ghana Districts, 2006).

Geographically, Mampong-Ashanti lies between latitude 07° 04’ north and longitude 01° 24’ west with an altitude of 457m above sea level. Rainfall in the district is bimodal, occurring from April to July (major rainy season) and August to November (minor rainy season), with an average rainfall of about 1224mm per annum. The dry season occurs from December to March (Meteorological Service Department, 2010). The vegetation in this area is transitional savanna woodland which is suitable for livestock rearing due to prevailing conducive rearing temperatures.

3.2 Experimental Birds and Their Management

The experimental birds used were Lavender, Pearl and White Guinea fowl females and males genetic lines were selected at 8 weeks of age. A total of 79 birds comprising of Pearl (37 females and 20 males), Lavendar (6 males and 5 females) and White (5 males and 6 females) local varieties were used.

The birds were obtained as day-old keets from a local commercial hatchery (Akate Farms Ltd) in the Ashanti Region of Ghana and raised to 10 months of age for the experiment. All the birds were housed in three-tier wooden cages with each bird housed singly in a cage of size 60 cm x 50 cm x 40cm. The cages were partitioned with wire mesh. The sides and floor of the wooden cages were also covered with wire mesh. Boards of packing cases were used to bar the birds within a single three-unit tier from seeing each other. In order to ensure that birds in adjacent tiers do not see birds in other tiers, the tiers were sealed at the rear and back with packing cases. The down and middle tiers were decked with wood and lined with floor carpet to enable collection of droplets of birds and liquid from the top-tiers and also to aid in cleaning and drainage of liquid from stacks above. Cages were housed in a sandcrete house roofed with corrugated iron sheets. The birds were identified using cage numbers plus sex and breed notations.

The birds were vaccinated at 10 days of age against Infectious Bursal Disease via their drinking water. At 4 weeks of age, the keets were also vaccinated against Newcastle Disease and Infectious Bronchitis through the same route. All birds were vaccinated against second and third Newcastle Disease. Birds were de-wormed with Albendazole, 2.5% (Mobedco-Vet, Jordan), two weeks prior to the experiment.

The keets were fed starter mash containing 2,950 kcal ME/kg and 21% crude protein from 10 to 20 days of age. From 21 days of age the diets were changed to a grower diet containing 3,200 kcal ME/kg and 19% crude protein followed by a layer diet containing 3,100 kcal ME/kg and 19% crude protein during the experiment.

On each day the birds were fed in the morning at about 8:00 hours GMT, the behavioural docility tests were carried out before feeding the animals in the morning by two evaluators.

Feed and water were provided in empty tinned tomato containers. Feed intake was measured daily, where the left-over feed was weighed using a 3000g capacity Electronic Kitchen Scale and subtracted from the total amount of feed offered the previous day to get the daily feed intake.

Only one death was recorded during the experiment and postmortem examination revealed broken egg within the *infandibulum* which might have occurred during the transfer of the birds into the individual cages.

3.3 Experimental Design

Seventy nine (79) Guinea fowls comprising Pearl (37 males and 20 females), Lavender (6 males and 5 females) and White (5 males and 6 females) local strains were tested for temperament and haematological parameters in a Completely Randomised Design experiment. The birds were selected at random and placed into three-tier wooden cage units.

Each three-tier wooden cage unit housed nine (9) birds placed individually per cage. In all there were nine three-tier unit cage structures with each compartmentalized into 3 x 3 individual cage units to house a total of 81 birds. However only 79 birds were available for this experiment since before the start of the experiment one of the birds died.

Under the behavioural docility test, docility was defined as *the ability of the bird to accept human presence, novel object, human contact/touching and handling* and scored on a scale of 1 to 4 (Annor *et al.,* 2011) as shown in Table 3.1. The duration of the docility scoring experiment lasted for 4 weeks. There were four tests/treatments applied to each bird irrespective of the sex, variety and initial weight.

The docility test was systematically carried out twice every week with five days interval over a period of four weeks.

To distinguish and classify the birds in terms of the test definition (temperament of the birds), the birds in the cages were systematically exposed to the Novel object test, human presence test, handling test and Touch test (Table 3.1) by the same person one test after another at short time intervals. The test methods are independent of each other and hence were used to independently assess each bird's natural ability to accepts or react to those challenging situations.

Table 3.1 Description of the tests methods used to assess the temperament of the Guinea fowls.

Test	Description	Test Objective
Human presence/Moving person Test (HPT)	Person walks up to the cage;	To ascertain the bird's reaction to a moving person and/ or human presence.
Touch/Contact Test (CT)	Person try to make a physical contact with the bird	To test the capability of the bird to accept person making contact or touching
Novel Object Test (NOT)	Person throw a novel object in the cage of the bird	To test the ability of the bird to react to an unknown (novel) object
Handling Test (HT)	Person physically handles the bird for 5 seconds	To test the ability of the bird to accept handling

Two weeks preliminary observations was carried out on the birds in order to ascertain whether there was the need to modify the assessment criteria adopted by Annor *et al.* (2011). It was concluded that, one requires a set of descriptive factors to categorise the birds behaviour into docile, restless, flighty and aggressiveness. However, the criterion did not differ from what Annor *et al.* (2011) used.

Apart from the behavioural docility test, haematological characterization based on heterophil to lymphocyte ratio (H/L ratio) was used to confirm the docility status of each bird.

In this current study, a combination of the two assessment criteria was adopted i.e. both simple visual observation assessments (behaviour test) based on four-point scoring system and differential white blood cell count (i.e. heterophils-to-lymphocytes) in order to leverage the possibility of assessing the true docility status of the Guinea fowl.

3.4 Docility Assessment Methodology

3.4.1 Behavioural Docility Test

During the evaluations each bird's docility status (DS) was assessed by two evaluators using simple visual appraisal and the average score taken on each bird.

At the point of assessment, a fowl was assigned a subjective docility score (DS) (see Table 3.2) by the two evaluators based on a 4-point scale defined by the Annor *et al.*, (2011) with some modifications.

The DS for each point in time was averaged between the two evaluators. The average docility score for the whole period amounted to 8 individual tests on each bird. The average docility score from the 8 individual tests on each animal was then used in the analysis.

The score was based on several behavioural component measures that indexed how much resistance Guinea fowl showed during behavioural test (see Table 3.2 for more details), and was assessed by the same experienced evaluators at each behavioural test. Note that the absolute value of this score has no direct interpretation; rather it represents the judgement of the evaluators as to how stressed the bird was during the test relative to other individuals in the population.

Table 3.2 Docility Scoring codes and descriptions representing the behavioural traits of the bird.

Scale/ Score	**Code**	**Test**	**Reactions (behaviour) of Bird**
1	*Docile* (the bird is quiet, compliant, submissive, obedient, tame)	❖ HPT ❖ NOT ❖ CT ❖ HT	❖ The bird does not react to observer. ❖ Allow observer to approach. ❖ The bird maintains its proximity ❖ Bird is quiet, calm and moves way slowly ❖ Undisturbed and stands or moves slowly ❖ Allow to be picked up and handled easily
2	*Flighty* (the bird is changeable,	❖ HPT ❖ NOT	❖ Aware of an observer, the bird stands away from the observer in a corner

	undependable, inconsistent, unreliable)	❖ CT ❖ HT	❖ The bird runs/moves away from the object ❖ Constant and moderate movements ❖ Tries to escape. Struggles little and stop
3	*Restless* (the bird is impatient, agitated, unrelaxed)	❖ HPT ❖ NOT ❖ CT ❖ HT	❖ Frighten and moves away on sighting an observer and persistently looking for escape holes along the cage. The bird hardly stands at one point. ❖ The bird runs/moves away from the object, continuously moving in the cage during the time of assessment ❖ The bird jumps and makes sharp cry(s) ❖ Whiles in hand, struggles and wing flapping
4	*Aggressive* (violent, hostile, destructive)	❖ HPT ❖ NOT ❖ CT	❖ The bird begins to move vigorously and continuously along cage and attempts to escape and sometimes with sharp cry ❖ Bird jumps and raises its feet off the cage floor and making persistent cries ❖ Difficult touching the bird

		❖ HT	❖ Whiles in hand, continuously struggles throughout

HPT – Human Presence Test, NOT – Novel Object Test, CT – Contact/Touch Test, HT – Handling Test.

3.4.2 Haematological Characterisation

In the morning after behavioural assessment, all the birds were bled to obtain blood samples of about 1-2 ml from the left cutanea ulnae using a heparinized syringe and a 20 gauge needle. This was immediately transferred into EDTA-K$_3$ coated vacutainer tubes (1-4ml, IVD Sterile A; serial number LOT: 1406202 from SG Biotech).

The blood samples were analysed for lymphocyte and heterophil cell counts in a Blood Cell Counts Analyser (Cell Dyn 18200, USA). Heterophilic-lymphocyte ratios, an indicator of stress in birds (Gross and Siegel, 1983), were calculated by dividing the percentage of heterophils in 1 ml of peripheral blood by the percentage of lymphocytes in order to establish H/L ratio for each bird.

3.5 Data Collection

The haematological characteristics such as Heterophil and Lymphocyte counts (percentages) and H/L ratios were recorded on each bird. The behavioural experiment recorded 8 average docility scores on each bird. Other relevant data points recorded on each bird within the experimental period were sex, variety of bird, feed intake and daily weight gain. The docility test was carried out on the bird before feeding it each morning during the time of testing. For the weight gain, this was obtained by first taking an initial weight of each bird at the beginning of the temperament scores recording and the final weight for each bird was again recorded at the point of taking the last temperament scores. Then the weight gain was computed by subtracting the initial weight from the final weight for each bird.

The amount of feed intake for each bird for a day was obtained by subtracting the leftover feed from the amount of feed offered to the bird the previous day. This was repeated for each bird for 30 days.

3.6 Data Analysis

The MS Excel (2007) was used to summarize and organize the data for the data analysis with SAS (2008) and SPSS (version 18) software.

The effects of sex, variety and test methods on docility scores were analyzed using Generalized Linear Mixed Models (GLMM) with GLIMMIX procedure of SAS (2008). The model used for the analysis was:

$Y_{ijk} = \mu + S_i + V_j + M_k + \varepsilon_{ijk}$

Definitions of variables in this model are:

Y_{ijk} = observations (docility status) of bird;

μ = General mean (population mean);

S_i = Effect of i^{th} sex of bird on docility, i = 1, and 2; (1 = male, 2 = female)

V_j = Effect of j^{th} variety of bird on docility, j = 1, 2, and 3; (pearl = 1, white = 2 and lavender = 3)

M_k = Effect of k^{th} test method on docility; k = 0, 1, 2, 3 and 4; (Define the values as in others)

ε_{ijk}= residual effect

Also the effects of docility on production traits (feed intake, weight gain) and H/L ratio were analyzed using Generalized Linear Mixed Models (GLMM) with GLIMMIX procedure of SAS (2008). The model used for the analysis was:

$F_i = \mu + D_i + \varepsilon_i$

$W_j = \mu + D_j + \varepsilon_j$

$R_k = \mu + D_k + \varepsilon_k$

Definitions of variables in this model are:

F_i = feed intake of the bird; W_j = weight gain of the bird; R_k = H/L ratio of bird

μ = General mean (population mean);

D_i = Effect of docility on feed intake, i = 1…3 (1 = 0 – 50g, 2 = 51 – 100g and 3 = >100g)

D_j = Effect of docility on weight gain, j = 1….. 4; (1 = 0 – 1g/day; 2 = 1.1 – 2g/day; 3 = 2.1 – 3g/day; and 4 = >3g/day)

D_k = Effect of docility on H/L ratio; k = 0…….4;

ε = residual effect

The correlation analysis between docility and behavioural measures of docility were done using the SAS software (2008). The level of suitability of the pairs of test methods was measured by the correlational relationship between any pair of behavioural traits. The stronger the correlational relationship by indication of its correlational coefficient the better the suitability measure.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 The Frequency and Proportions of Observations of the Fixed Factors

Table 4.1 shows the proportions of sex, variety and test method among the experimental birds. The birds with the flighty behaviour constituted the majority among the Guinea Fowls observed whereas those with the aggressive behaviour constituted the least. There were three varieties of the birds which were Pearl, White and Lavendar with the Pearl outnumbering the rest.

The effects of sex, variety and test methods on weekly docility scores are presented in Table 4.2. Out of the 316 there were more observations recorded on males as compared to the females. Moreover, majority of the birds (64.6%) showed flighty behaviour as indicated in

Table 4.1, resulting in an average docility score of 2.15 (Table 4.2). This score represents flighty on the 1 – 4 point scale on docility (Table 3.2). Such birds accept human presence and touching but will try to escape and/or will jump when startled by the presence of a human being or a novel object. On handling, the bird will struggle a little in an attempt to escape.

Table 4.1: Proportions of the observations for sex, variety of bird, test methods and the Docility status of the birds

Criterion	Category	No. of Observations	Percent (%)
Docility Status	Docile	45	14.2
	Flighty	204	64.6
	Restless	63	19.9
	Aggressive	4	1.3
	Total	316	100
Sex	Female	120	38.0
	Male	196	62.0
	Total	316	100

Variety	Pearl	228	72.2
	White	40	12.7
	Lavendar	48	15.2
	Total	316	100

It has been observed that, in the first week of assessment (Table 4.2), the test criteria recorded a wider variability in docility as indicated by the standard deviation (0.62) of the first week mean docility score but majority of which were between flighty and restless in nature (docility score = 2.52±0.051). Soon after the first week, the birds were observed to be flighty (ranging from 2.16±0.046 to 1.89±0.039) and they remained so throughout the rest of the experimental period and only changing slightly.

From the results presented above, it was deduced that, the most probable (common) docility trait among the Guinea fowl population was the flighty as against the popular view that, they are mostly aggressive birds in captivity. This character of aggressiveness could possibly have been replaced by the more friendly characters on the temperament scale (i.e. Flighty, restless and docile) through the effect of domestication and selective breeding. The results showed there were still a significant proportion of those birds with the wild nature since only 14.2% of them were docile (Table 4.1). This finding is pointing to the fact that, the ‘aggressive’ Guinea fowls now are truly not so but are either of the flighty or restless type. The average docility score of 2.13 (Table 4.2) which was obtained from this experiment is numerically different from a score of 2.6 recorded by Annor *et al.* (2011) in the grasscutters. Annor *et al.* (2011) concluded that the grasscutters were flighty, as observed in their work.

With this finding there is therefore the need to include docility trait in the breeding programmes (Burrow, 2003) of Guinea fowls in order to produce docile birds.

It is also worth noting that in the first week of the assessment majority of the birds were observed to be flighty and restless (2.52) in nature.

The variability among the scores might be due to:

- birds being variable in docility character
- evaluators learning to stablise in their scoring
- it showed that at the beginning of the such evaluations birds will show variation in docility and to be stablised later to obey the observation that, the docility character was repeatable at different times and situations

Soon after that, due to high repeatability of docility, the birds were observed to be flighty and remained so throughout the rest of the experimental period. This observation tended to agree with Haskell *et al.* (2014) who stated that temperament in this case, docility " is the differences between individual animals in their behavioural response to alarming or challenging situations where individuals are often consistent in the way they respond when the challenge is repeated". The results were also in agreement with the works of these other researchers, Fordyce *et al.* (1985) in *Bos indicus* cross cattle and D'Eath *et al.* (2002) in dairy cattle.

Table 4.2: Least square means and standard errors for the Effects of variety, Sex and Method on Docility on weekly basis

		Weekly Docility Score				
Variable	No.	Week 1	Week 2	Week 3	Week 4	Average Docility
Sex						
Male	49	2.54±0.040	2.19±0.036	1.95±0.033	1.94±0.031	2.16±0.025
Female	30	2.51±0.045	2.13±0.041	1.90±0.037	1.86±0.035	2.10±0.028
Mean Doc.		2.53±0.043	2.16±0.039	1.93±0.035	1.90±0.033	2.13±0.027
P Value		*0.569*	*0.217*	*0.294*	*0.047*	*0.097*
Variety						
Pearl	57	2.56±0.031	2.21±0.028	1.92±0.025	1.92±0.023	2.15±0.019
White	10	2.45±0.070	2.08±0.064	1.90±0.058	1.90±0.055	2.08±0.044
Lavendar	12	2.56±0.065	2.19±0.059	1.96±0.053	1.88±0.050	2.15±0.040
Mean Doc.		2.52±0.055	2.16±0.050	1.93±0.045	1.90±0.043	2.13±0.034
P Value		*0.338*	*0.192*	*0.705*	*0.797*	*0.334*

Method						
HPT	79	1.90±0.055^{c}	1.49±0.050^{b}	1.24±0.045^{c}	1.23±0.04^{c}	1.47±0.034^{d}
NOT	79	2.44±0.055^{b}	2.22±0.050^{a}	1.95±0.045^{b}	1.92±0.042^{b}	2.14±0.034^{c}
CT	79	2.64±0.055^{b}	2.42±0.050^{a}	2.12±0.045^{b}	2.11±0.042^{b}	2.33±0.034^{b}
HT	79	3.11±0.055^{a}	2.49±0.050^{a}	2.39±0.045^{a}	2.33±0.042^{a}	2.58±0.034^{a}
Mean Doc.		2.52±0.055	2.16±0.050	1.93±0.045	1.89±0.042	2.13±0.034
GAD		**2.52±0.051**	**2.16±0.046**	**1.93±0.042**	**1.89±0.039**	**2.13±0.032**
P Value		*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
St Dev.		*0.62*	*0.56*	*0.56*	*0.53*	*0.49*

No. = number, St Dev. = Standard deviation, P Value = Probability Value, HPT = Human Presence Test, NOT = Novel Object Test, CT = Contact Test, HT = Handling Test; GAD = Grand Average Docility Score; Doc = Docility
NB: Means between/among weeks with different superscripts are significantly different (p<0.05).

4.2 Effects of Fixed Factors on Docility

4.2.1 Effect of Sex on Docility

The sex of the birds did not significantly influence the behavioural pattern of the birds. Male or female birds did not change in their behaviour throughout the period when the test was performed. Both males and females were observed to be flighty (2.16 and 2.10 respectively).

It must be noted that, the effect of sex on temperament is debatable. This result agrees with Annor *et al*. (2011) who did not find any effect of sex on docility of grasscutter. Similarly, Burdick *et al*. (2009) and Burdick *et al*. (2011) did not also find the effect of sex on docility in cattle in their studies. Moreover, Pajor *et al*. (2008), and Pajor (2011) reported no difference (P<0.05) between temperament scores and sex in sheep.

The results of this study is contrary to that of Voisinet *et al*. (1997) who indicated in their study in different breeds of cattle, that females had greater temperament scores than males, meaning male cattle were more docile than female cattle. It also agrees with the study of Burrow (1997) in *Bos indicus* crossbreds.

4.2.2 Effect of Variety on Docility

Among the three varieties, there were no significant differences in behavioural response to docility tests. Among the three varieties, Pearl and Lavendar had some resemblance in their docility scores (Table 4.2). Within each week the docility scores were barely same for the Pearl and the Lavendar varieties and reduced across the weeks with high scores at the beginning. The docility scores remained constant from the third week to the fourth week for all the breeds. The pattern was observed with both the sex and the variety. Comparatively the White scored 0.1 docility score value below the Pearl and Lavendar in weeks one and two. But this was not enough to make the White a better variety because there was no significant ($P> 0.05$) difference between them.

The deductions from the results on the variety factor effects on docility indicated, variety has no significant ($P<0.05$) influence on docility of Guinea fowls. However, there have been some reports concerning differences among breeds in terms of temperament scores (Morris *et. al.* 1994). One of such reports is also by Paranhos da Costa *et. al.*, (2002), who indicated that there was significant differences in temperament between Caracu breed (*Bos taurus taurus*) and Nelore, Gir and Guzerá (*Bos Taurus indicus*). That, the *Bos Taurus taurus* were found to be less reactive than *Bos taurus indicus* while among the Zebu breeds, Nelore were less reactive than Gir and Guzerá (Paranhos da Costa *et al.*, 2002). Burrow and Corbet (2000) also indicated that there were significant differences in temperament scores between sire breeds used in the crossbreeding experiment of zebu and zebu-derived beef cattle.

4.3 Effects of Test Methodology on Docility

The method used in determining docility of the birds significantly influence the behaviour and response of the birds.

Table 4.2 indicates that docility scores due to the methods had similar patterns within and across the methods. For instance, there was a consistent significant increasing trend in the docility scores for each method across the weeks up to the week three. From the third week onwards there were decreasing trends in docility scores within all the methods (i.e. across the weeks). The visible trend is that, from the third to the fourth week of assessment, the docility scores recorded remained relatively constant (Table 4.2) within each method (i.e. across the weeks).

Since a significant effect was found between the test method and docility ($P< 0.001$), it means in the assessment of docility trait, the test methodology is very important as a non-genetic factor.

The individual methods were also found effective in determining the docility status of the birds because each of them had a significant ($P< 0.001$) effect on the weekly docility scores. The significant differences were observed between the individual docility scores for all the methods from week one to week three signified the effectiveness of each method and that was confirmed by the average docility scores for all the methods (i.e. 1.5±0.03, 2.1±0.04, 2.3±0.03 and 2.6±0.04 respectively for HPT, NOT, CT and HT). However, the use of HT showed that the birds were rather somehow restless (2.6) in nature whiles the others (i.e. NOT and CT) indicated that the birds were flighty. Also HPT average docility score (1.5) indicated that the birds were either docile or flighty in nature. These differences indicate how the birds perceived the various methods. Some of the methods could have been very much stressful and caused a lot of fear and anxiety in the birds.

The birds showed restlessness with the use of HT which agrees with the reports by Fordyce *et al.* (1988); Grandin (1997); Curley Jr *et al.* (2006); and Burrow and Dillon (1997), who stated that during handling, fear is a major determinant of an animal's behaviour and the degree of the effects of handling felt by animals depends on the temperament of the animal. Grandin (1997) specifically proposed that, handling may be more stressful to temperamental animals than to those animals that are calmer.

Despite this, there were some similarities and dissimilarities between the methods in terms of their effect on docility. Those that were resilient in effect (i.e. those that show consistency in docility score) throughout in this research are adjudged reliable (i.e. HPT, NOT and CT). But even though HT produced inconsistent results it was adjudged the most effective because it rather recorded wider docility score differences in successive times. The NOT and CT were similar in effect on docility since there were no significant differences (P> 0.001) between their respective docility scores.

The decreasing pattern in docility scores among the methods with some level of uniformity across the weeks (i.e. 1.9 to 1.2, 2.4 to 1.9, 2.6 to 2.1 and 3.1 to 2.3) is in conformity with the findings of Hearnshaw and Morris (1984); Grandin (1993); Burrow and Dillon (1997); Gibbons *et al*. (2009) and Turner *et al*. (2011) who say animal responses to each of these measures of temperament have been shown to be repeatable overtime.

4.4. Effect of Bird's Temperament on Feed Intake

There was no significant effect (P> 0.05) of docility on the feed intake of Guinea fowls (Table 4.3). The feed intake between the docile and the aggressive numerically was 5g.

Table 4.3: Effects of Docility on production traits

	Production trait		
Docility	**Feed Intake (g/day)**	**Weight Gain (g/day)**	**H/L Ratio**
Docile	83.22±2.51	- 1.24±0.76	2.01±0.07
Flighty	83.85±1.18	- 1.43±0.35	2.04±0.03
Restless	85.00±2.12	- 0.95±0.64	2.02±0.06
Aggressive	78.18±8.43	- 2.00±2.53	2.38±0.23
Mean	83.92±16.87	-1.32±5.07	2.037±0.47
P Value	*0.8480*	*0.9162*	*0.5093*

NB: H/L ratio = Heterophil/Lymphocyte ratio; P Value = Probability value

Docility did not influence feed intake (P > 0.05). Even though this research did not find any statistical significant influence of docility on this phenotypic trait (feed intake) but was able to record some marginal increment in the level of feed intake from the docile to the aggressive.

In this research the flighty and restless birds turned to consume a little more feed (83.85±1.18 g/day, 85.00±2.12 g/day respectively) as compared to the docile birds (83.22±2.51 g/day), (Table 4.3). The aggressive type on the other hand even though consumed slightly lesser than that of the docile, flighty and the restless they equally lost more weight (Table 4.3). This finding agrees with the works of Café *et al*. (2011) and Fordyce *et al.* (1985), that the docility character negatively influences production traits. This result also agrees with the finding of Burrow and Dillon (1997) that, the animals (cattle) with good temperament scores may grow faster in a feedlot than animals with poorer temperament scores.

4.5 Effects of Bird's Temperament on Weight Gain

There was no significant (P > 0.05) effect of docility on weight gain in the Guinea fowls. All the birds lost weight numerically, though not statistically sufficient to make comparisons. Again the non-docile birds (i.e. the flighty, restless and aggressive) even though ate more relatively than the docile birds they did lose more weight than the docile except the restless which did quite well which could been due to certain reasons.

Docility did not influence weight gain of the birds since there was no significant influence (P > 0.05) of docility on the weight gain but was able to record some marginal decrement in the level of resilience in weight loss from the docile to the aggressive. That is, all the docility categories of the birds lost some weight also in the same direction (i.e. from docile to aggressive).

The reasons scientists like Agarwal *et al.* (2009), Echternkamp (1984) and Post *et al.* (2003) among others assigned to the phenomenon of the loss in weight is that, birds with calm temperament (docile) have relatively reduced corticosterone compared to the temperamental ones (the non-docile). A high corticosterone level has been shown to retard growth. Agarwal *et al.* (2009) in particular explained that, glucocorticoids (e.g. corticosterone) negatively affect growth by increasing the production of leptin which reduce feed intake while Pugh *et al.* (2011) explained that, corticosterone break down protein, glycogen and fat to increase the amount of circulating glucose.

4.6 Effects of Bird's Temperament on Heterophil-Lymphocyte Ratio

There was no significant influence ($P > 0.05$) of docility on heterophil-lymphocyte ratio of the Guinea fowls. The maximum difference between docile and the non-docile bird (aggressive type) was 0.37 which is 37% change in heterophil to lymphocyte levels in the blood. This change indicates the degree to which a bird could easily be stressed. As a biomarker of stress, H/L ratio is said to be a very reliable indicator of stress levels and so in this experiment, the aim was to establish its effect and relationship with docility character in the Guinea fowls.

This observation corresponds with Krams *et al.* (2012) who postulated that the less stress individuals, that is birds with low H/L ratios will be less aggressive and respond to behavioural temperamental test better than birds with high H/L ratios. This prediction reflects the extent of docility of the birds. The finding is in conformity with Ots *et al*. (1998), who also showed that birds that recorded reduced body mass also had the highest increase in H/L ratios. It is confirmed by Krams *et al*. (2011), who said there was a negative correlation between H/L ratio and body mass.

However, the research did not notice any significant influence ($P > 0.05$) of docility on the heterophil-lymphocyte ratio of the Guinea fowls. The reason for the phenomenon could be that docility is a stress response trait while H/L ratio is a biomarker for stress. Stress in general is governed by many factors including hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system and one of the products of these systems is the circulating corticosterone concentration that tends to decrease the number of lymphoctyes and cytokines (Jain *et al*., 1991) relative to the number of heterophils.

Other factors which were beyond the control of the experiment and which govern the stress levels could have influence on the birds at the time of taking the blood samples, since heterophils proliferate in circulation in response to infections, inflammation and stress (Jain, 1993; Campbell, 1995; Rupley, 1997; Harmon, 1998; Thrall, 2004).

4.7 Effects of Sex and Variety on Blood Parameters (Biomarker of Docility)

4.7.1 Effects of Sex on Heterophils, Lymphocytes and H/L Ratios

As part of the objective of establishing the relationship between blood parameters and docility of birds, the sex and breed effect on some blood parameters were computed. The results showed that sex had no significant effect (P>0.05) on H/L ratios. The males had slightly higher (0.108±0.002) H/L ratios than the females (0.106±0.002). In terms of heterophils and lymphocytes, males recorded slightly high heterophils (7.42±0.13) and little low lymphocytes (68.88±0.28) than the females. On the other hand, the females recorded slightly low heterophils (7.33± 0.14) and slightly high lymphocytes (69.39±0.31).

These results agree with Burdick *et al.* (2011) and Vleck *et al.* (2000), that there is no significant effect of sex on H/L ratio. Burdick (2011) specifically mentioned that there was no significant (P>0.05) difference between males and females (chicken) on the basis of their H/L ratios. The figures for the H/L ratios recorded in this study were far lower than the figures recorded by Burdick *et al.* (2011) in chicken where he studied the association between heterophil/lymphocyte ratio as a marker of 'resistance' to stress and some production and fitness traits in chickens. The mean H/L ratios for males and females, respectively were 0·87±0·03 and 0·83±0·03 (*P*>0·05).

The interpretation of the results of H/L ratio is that, heterophils increase with stress leading to a relative decrease in the number of lymphocytes (Gross and Siegel, 1983). The H/L ratios supposed to be very stable (i.e. less variable) (Gross and Siegel, 1983), but fluctuate according to the ability of the bird to resist stress.

According to the concept of H/L ratios as biomarker of resistance to stress (Burdick *et al.*, 2011), the birds with low H/L ratios are less aggressive and supposed to respond well to behavioural/temperamental tests better than birds with high H/L ratios (Krams *et al*., 2012).

From this study, it therefore means the females were less aggressive since they recorded slightly lower H/L ratio.

Table 4.4: Effect of Sex and Variety on Heterophils, Lymphocytes counts, and H/L Ratios of Guinea fowls

	Heterophil (%)	**Lymphocyte (%)**	**H/L Ratios**
Sex			
Male	7.42±0.13	68.88±0.28	0.108±0.002
Female	7.33± 0.14	69.39±0.31	0.106±0.002
P Value	*0.5899*	*0.1666*	*0.4398*
Variety			
Lavendar	6.76±0.20^{b}	69.60±0.45^{a}	0.09±0.003^{b}
Pearl	7.74±0.09^{a}	68.45±0.21^{b}	0.11±0.002^{a}
White	7.61±0.22^{a}	69.35±0.49^{ab}	0.11±0.004^{a}
P Value	*0.0002*	*0.0345*	*0.0002*
Grand mean	7.59±0.71	68.67±1.57	0.11±0.011
St Dev.	0.78	1.63	0.013

NB: St Dev = Standard Deviation, P Value = Probability value,

Means having common superscript in the same column are not significantly different (p > 0.05)

4.7.2 Effects of Variety on Heterophils, Lymphocytes and H/L Ratios

The variety factor had significant effect on H/L ratios (P = 0.0002). The variety factor again had effect on heterophil (P = 0.0002) and lymphocytes counts (P = *0.0345*) of the Guinea fowls. There was a significant (P <0.05) difference between Lavender and Pearl varieties and between Lavendar and White but no difference between Pearl and White (P = 0.5822) in heterophilia (i.e. increase in number of heterophils in stress condition). There was a significant (P = 0.0236) difference between Lavendar and Pearl and no difference between Lavendar and

White (P = 0.7097) and between Pearl and White (P = 0.0995) in times of lymphopaenia (i.e. reduction in the number of lymphocytes during stress).

The Lavendar recorded the lowest H/L ratio (0.09±0.003) among the three strains. This was significantly different (P <0.05) from the Pearl and White. There was no statistical difference (P = 0.3871) between the Lavendar and Pearl Guinea fowls. The above results means the Lavendar had the greatest capacity to resist stress and by extension were less aggressive in nature. The Pearl and White Guinea fowls recorded similar results meaning they were similar in the ability to withstand stress with regards to the heterophil counts. The strain which recorded the lowest counts was the Lavender followed by the White and then the Pearl. However there was no significant difference (P >0.05) between the pearl and the White in terms of the bird’s heterophil increasing in response to stress. Additionally in terms of the lymphocytes, the strain that recorded the lowest was the Pearl followed by the White and then the Lavendar. In the presence of stress, the Pearl would easily be stressed followed by the White and then the Lavendar.

Statistically there was no difference (P >0.05) between Lavender and White in terms of lymphocytes counts in times of stress. With regards to the heterophils there was a significant difference (P <0.05) between the Lavender and the White. That is why there was a statistical difference between the H/L ratios of the two strains. Thus, the Lavender is the preferred strain followed by the White and then the Pearl by the use of the H/L stress/docility indicator index. As at the time of this analysis there was no available literature to support or deny this result.

4.8 Phenotypic Correlations between Docility with Blood Parameters

Phenotypic correlations between docility and blood parameters are presented in Table 4.5. The phenotypic correlations between docility and heterophil as well as docility and lymphocytes were low (i.e. 0.042 and 0.072 respectively). Correlation between H/L ratios and docility was equally low (0.023). The mean counts of the heterophils (7.59) showed comparatively low variability as compared to the mean counts of the lymphocytes (68.68) as indicated by their standard deviations (Table 4.5). The mean H/L ratios (0.11) rather showed less variability among the birds as indicated by its standard deviation (0.01). In addition, none of the parameters showed any significant relationship with docility. However, the direction of the relationships for any of the parameters and docility was positive, meaning should any of them increase or decrease may lead to increment or reduction in the docility.

The above observation is in conformity with the findings of Gross *et al.* (1983) who stated that, the ratio of heterophils to lymphocytes is less variable than the number of heterophil or lymphocyte cells.

Table 4.5 Phenotypic correlations between docility and Heterophils, Lymphocytes and H/L Ratios

Trait	Mean	Standard deviation	Docility	*P Value*
Heterophil	7.59	0.78	0.042	*0.72*
Lymphocytes	68.68	1.63	0.072	*0.53*
H/L Ratios	0.11	0.01	0.023	*0.84*

The above results (Table 4.5) showed that there is a relationship existing between the docility of a bird and H/L ratio of that same bird but only that it was a relatively weak one. Scientists such as Banbura *et al*. (2013), rather found the ratio as a reliable indicator of inherent stress condition and hence docility. In other words the H/L ratio was a biomarker of the capacity of the bird to withstand stress. The association between docility and H/L ratios was a positive one. That is an increment in the H/L ratio level might lead to higher score for stress and the relationship was such that, the more stressed a bird was, the more aggressive it would become. The relationship meant that less stressed individuals were less aggressive. Thus, the higher levels of H/L ratio also meant relatively high levels of docility score.

4.9. The Most Suitable Test Methodology in Measuring Docility

4.9.1 Consistency of the Test Methodology

The first criterion employed to establish the most suitable measures of docility was based on finding the test methodology that was consistent in producing results that were repeatable since research showed that docility character of an animal was repeatable irrespective of the method used or circumstance (Hearnshaw and Morris, 1984; Grandin, 1993; Burrow and Dillon, 1997; Gibbons *et. al.*, 2009; Turner *et. al.*, 2011). From Table 4.2, it was observed that all the methods HPT, NOT, CT and HT produced docility scores across the weeks in a pattern of decreasing order that was consistent throughout the experiment. All the methods used performed exceptionally well in producing consistent significant differences between the weeks' docility scores from the start of the experiment up to the third week.

Those that produced repeated minimal score differences at different times (across the weeks) or situations were seen with NOT, CT and HPT methods since their average docility score differences were only 0.2 score points.

In the NOT, a docility score difference of 0.2, 0.3, 0.0 were produced respectively for week one and two, week two and three and three and four. This was the same for the CT method. On the other hand, the use of the HPT method produced docility score differences of 0.4, 0.3 and 0.0 respectively for every two successive weeks. Finally the HT also produced docility score differences of 0.6, 0.1 and 0.1 respectively for every two successive weeks.

The above figures indicated HPT, NOT and CT as being consistent and similar in effect. Among the four methods the one which produces the greatest impact on the bird was one with highest correlations coefficient with docility.

4.9.2 Correlation between Docility and Behavioural Measures of Docility

Another strategy was to measure the strength of the method in assessing the underlying trait. The correlations of docility with each of the methods were conducted and these are shown in Table 4.6.

Table 4.6 Strength of relationship between behavioural measures and docility scores

Method	Mean	Standard deviation	Docility	P Value
Handling Test	2.61	0.16	0.672**	0.000
Human Presence Test	1.50	0.28	0.619**	0.000

Novel Object Test	2.16	0.24	0.569**	0.000
Contact Test	2.33	0.24	0.425**	0.000

*** = Correlation is significant at the 0.01 level (2-tailed)*

In terms of the method that produced the greatest effect on docility of the birds, the order is from the handling test to contact as showed in Table 4.6.

From the above deduction, handling test seemed to have the greatest effect on docility score of the birds. Despite this, it also provided wider docility score difference between the first week and the second week signifying the greater effect it leaves on the birds and how that method was perceived by the birds. The human presence test, the novel object test and the contact test on the other hand were very much similar in effect; they showed resilience in producing consistent docility scores throughout the experiment. The consistency of producing repeated minimal score differences between any two successive times or situations judges the reliability of the method used. In terms of reliability therefore, the HPT, NOT and CT can be counted on. Most importantly they each produced significant effect in assessing the underlying trait since they each yielded above average correlation coefficient.

4.9.3 Assessing the Underlying Trait

The most suitable test methodology in this study was taken to be one which actually assesses the underlying trait (docility). In order to ascertain this, the various tests were correlated with each other to yield a pair of tests which recorded the highest correlation coefficient. The result in Table 4.7 shows the relationship among the behavioural docility measures. According to

Grandin (1993) for the sake of accuracy, it is appropriate to adopt multiple methods to assess behavioural docility. However, it is of interest to understand whether these different tests measure the same underlying trait through correlation analysis. On the overall, this would suggest that these tests are assessing similar if not identical underlying traits (Burdick *et al.*, 2011).

A number of studies have found a significant relationship among the measures. For example, Fell *et al.* (1999); Olmos and Turner (2008); Hoppe *et al.* (2010) and Café *et al.* (2011) recorded a significantly moderately correlated result for flight speed and chute test score beef cattle. A similar strategy was adopted here in this study, by finding the strength of the relationship that existed between the measures to represent the level of suitability of the pair in assessing the behavioural docility of the birds. They were arranged in order of importance from highly suitable to the least suitable. It shows that the human present test with the novel object test was the most suitable among the six available pairs. The least suitable was using contact test alongside the human presence test.

Table 4.7 Correlational Relationships among the Behavioural Measures

Pair of test method	Level of suitability
Human presence – Novel Object test	0.409**
Contact test – Handling test	0.177
Human Presence – Handling test	0.148

Novel Object – Handling test	0.043
Novel Object – Contact test	0.058
Human Presence – Contact test	- 0.052

*** = Correlation is significant at the 0.01 level (2-tailed)*

The contact with the human presence test from the analysis would yield negative results (effect) whenever used together in assessing the behavioural docility. The results are in agreement with Turner *et al*. (2011) who found a positive correlation between chute score and flight speed.

The other pairs such as Human presence – Novel Object test, Contact test – Handling test, Human Presence – Handling test, Novel Object – Handling test, and Novel Object – Contact test produced positive relationships which were in agreement with the findings of other researchers like Curley *et al*. (2006b) who found a moderate relationship between chute scores and response to confinement in a pen. However there have been mixed reports concerning these relationships since others have reported weaker correlations. An example of those who reported weak relationship was the Burrow and Corbet (2000).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Sex as a fixed factor had no influence on docility of the Guinea fowls. The novel object and handling techniques for measuring behavioural docility have proven efficient and reliable enhancing the possibility to accurately measure docility. The docility trait for Guinea fowls was that of flighty. The Lavendar variety had the greatest capacity to withstand stress and by extension was less aggressive. The H/L ratio was less variable hence making it very suitable to be used in determining docility of the birds. It has been confirmed that an increment in the H/L ratio level might lead to higher score for stress. The research showed that docility scores of the birds showed marginal variations for the first two weeks but reduced across the weeks and the scores of all the three breeds became unity in the third week onwards.

The methods, HPT, NOT and CT produced consistent docility scores across the weeks. The HPT, NOT and CT were similar in effect. The method which produced the greatest impact on the birds was HT. The research revealed that the test criterion has significant effect on docility.

5.2 Recommendations

1. The docility trait should be included in the breeding objectives of the Guinea fowls in order to achieve more acceptable docility scores.

2. The economic value of docility in Guinea fowls should be assessed (in terms of mortality, performance, carcass quality, growth rates, feed conversion, treatment cost, net returns per head)
3. Breeders, while testing the docility character of birds in order to classify them into their docility classes should extend the testing to the third and fourth week of the assessment to ensure reliable results.
4. Similar experiment should be conducted to verify these results, comparing the Lavendar, Pearl and White as to which one is the best variety in terms of acceptable docility score.

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APPENDIX

Appendix A: The weekly trend of behavioural docility scores, effects of method, sex and variety

```
                    The SAS System        1

                  The MEANS Procedure

Variable   N      Mean        Std Dev      Minimum      Maximum

VARIETY   316   1.4303797   0.7420870   1.0000000   3.0000000

METHOD    316   2.4968354   1.1169642   1.0000000   4.0000000

SEX       316   1.3797468   0.4860935   1.0000000   2.0000000

WEEK 1    316   2.5503165   0.6210110   1.2500000   4.0000000

WEEK 2    316   2.1971519   0.5697673   1.0000000   3.7500000

WEEK 3    316   1.9272943   0.5629541   1.0000000   3.5000000

WEEK 4    316   1.9194620   0.5384326   1.0000000   3.3750000


                    The SAS System        2

                    The GLM Procedure

                    Class Level Information


              Class      Levels     Values

              VARIETY    3          1 2 3

              METHOD     4          1 2 3 4

              SEX        2          1 2

          Number of Observations Read       316

          Number of Observations Used       316
```

NB: breed:– 1=pearl, 2 = white, 3 = Lavendar

Method:– 1 = HPT, 2 = NOT, 3 = CT, 4 = HT

Sex:– 1= male, 2 = female

```
The SAS System        3

The GLM Procedure

Dependent Variable: WEEK 1

                                     Sum of
Source                  DF          Squares         Mean Square    F Value    Pr > F
Model                    6        59.3382941        9.8897157      49.18      <.0001

Error                   309       62.1429243       0.2011098

Corrected Total         315      121.4812184

                 R-Square     Coeff Var      Root MSE    WEEK 1 Mean

                 0.488457      17.58420      0.448453       2.550316

Source                  DF      Type III SS     Mean Square    F Value    Pr > F

VARIETY                  2      0.43782994      0.21891497      1.09      0.3380

METHOD                   3     58.82121093     19.60707031     97.49      <.0001

SEX                      1      0.06509266      0.06509266      0.32      0.5698
```

```
The SAS System        4

The GLM Procedure

Dependent Variable: WEEK 2

                                     Sum of
Source                  DF          Squares     Mean Square    F Value    Pr > F
Model                    6       50.2677742      8.3779624      49.79    <.0001

Error                  309       51.9921625      0.1682594

Corrected Total            315    102.2599367

                 R-Square     Coeff Var      Root MSE    WEEK 2 Mean

                 0.491569      18.66937      0.410194       2.197152

Source                  DF      Type III SS      Mean Square    F Value    Pr > F
```

```
VARIETY            2    0.55770019     0.27885009     1.66      0.1924

METHOD             3   49.37185465    16.45728488    97.81     <.0001

SEX                1    0.25722653     0.25722653     1.53      0.2172
```

```
The SAS System        5

The GLM Procedure

Dependent Variable: WEEK 3

                                  Sum of
Source                DF         Squares      Mean Square    F Value   Pr > F
Model                  6     57.32985170       9.55497528      69.47   <.0001

Error                309     42.49910993       0.13753757

Corrected Total      315     99.82896163

              R-Square     Coeff Var     Root MSE    WEEK 3 Mean

              0.574281      19.24255     0.370861       1.927294

Source                  DF     Type III SS    Mean Square    F Value   Pr > F

VARIETY                  2      0.09641551     0.04820776       0.35   0.7046

METHOD                   3     57.08663643    19.02887881     138.35   <.0001

SEX                      1      0.15223406     0.15223406       1.11   0.2936
```

```
The SAS System        6

The GLM Procedure

Dependent Variable: WEEK 4

                                   Sum of
Source                  DF         Squares          Mean Square   F Value    Pr > F
Model                    6         53.84439275      8.97406546      73.99    <.0001

Error                  309         37.47716580      0.12128533

Corrected Total        315         91.32155854
```

```
                R-Square     Coeff Var      Root MSE    WEEK 4 Mean

                0.589613      18.14365      0.348260      1.919462

         Source                DF    Type III SS     Mean Square    F Value    Pr > F

         VARIETY                2     0.05518844      0.02759422     0.23        0.7966

         METHOD                 3     53.29457747    17.76485916   146.47      <.0001

         SEX                    1     0.48363023      0.48363023      3.99       0.0467


      The SAS System        7

      The GLM Procedure

      Dependent Variable: AV. DOC

                                           Sum of
         Source                DF         Squares      Mean Square    F Value    Pr > F
         Model                  6     53.69573354      8.94928892     113.46    <.0001

         Error                309     24.37224114      0.07887457

         Corrected Total          315     78.06797468

                R-Square     Coeff Var      Root MSE    AV. DOC Mean

                0.687807      13.04725      0.280846      2.152532

         Source                DF  Type III SS     Mean Square    F Value    Pr > F

         VARIETY               2    0.17346560      0.08673280      1.10      0.3343

         METHOD                3    53.27723403    17.75907801     225.16    <.0001

         SEX                   1    0.21838219      0.21838219      2.77      0.0971


      The SAS System        8

      The GLM Procedure

       Least Squares Means

                                                       Standard
                VARIETY    WEEK 1 LSMEAN     Error                Pr > |t|
```

1	2.56292488	0.03070661	<.0001
2	2.45000000	0.07090659	<.0001
3	2.56002163	0.06487499	<.0001

VARIETY	WEEK 2 LSMEAN	Standard Error	Pr > |t|
1	2.20989501	0.02808697	<.0001
2	2.08125000	0.06485743	<.0001
3	2.18778162	0.05934039	<.0001

VARIETY	WEEK 3 LSMEAN	Standards Error	Pr > |t|
1	1.91774109	0.02539370	<.0001
2	1.90312500	0.05863821	<.0001
3	1.96235569	0.05365020	<.0001

VARIETY	WEEK 4 LSMEAN	Standard Error	Pr > |t|
1	1.91782672	0.02384621	<.0001
2	1.90312500	0.05506481	<.0001
3	1.88126535	0.05038077	<.0001

VARIETY	AV. DOCILITY LSMEAN	Standard Error	Pr > |t|
1	2.15615704	0.01923020	<.0001
2	2.08500000	0.04440568	<.0001
3	2.15379383	0.04062835	<.0001

NB: 1 = Pearl, 2 = White; 3 = Lavendar

METHOD	WEEK 1 LSMEAN	Standard Error	Pr > |t|
1	1.90103055	0.05520757	<.0001
2	2.44248624	0.05520757	<.0001
3	2.64784917	0.05501421	<.0001

```
4             3.10589606    0.05540688     <.0001
```

NB: 1= Human Presence Test, 2 = Novel Object Test, 3 = Contact Test, 4 = Handling Test.

```
The SAS System        9

The GLM Procedure

Least Squares Means

              WEEK 2        Standard
METHOD        LSMEAN        Error          Pr > |t|
1             1.49614170    0.05049771     <.0001

2             2.22525562    0.05049771     <.0001

3             2.42550476    0.05032085     <.0001

4             2.49166676    0.05068003     <.0001


                WEEK 3      Standard
METHOD          LSMEAN      Error            Pr > |t|
1              1.24169203    0.04565546     <.0001

2               1.95340089   0.04565546     <.0001

3              2.12476161    0.04549555     <.0001

4              2.39110784    0.04582029     <.0001

              WEEK 4        Standard
METHOD        LSMEAN        Error          Pr > |t|
1             1.23500888    0.04287322     <.0001

2             1.92076837    0.04287322     <.0001

3             2.11322601    0.04272307     <.0001

4             2.33395284    0.04302801     <.0001

                                           Standard
METHOD        AV. DOCILITY LSMEAN          Error           Pr > |t|
1               1.47451130                 0.03457407     <.0001

2               2.13780244                 0.03457407     <.0001

3               2.32918311                 0.03445298     <.0001
```

```
4              2.58510431                  0.03469889     <.0001


                              Standard
SEX    WEEK 1 LSMEAN          Error          Pr > |t|
1       2.53918570            0.04012553     <.0001

2       2.50944531            0.04509411     <.0001

 NB: 1 = Male; 2 = Female

                              Standard
SEX    WEEK 2 LSMEAN          Error          Pr > |t|
1       2.18920249            0.03670235     <.0001

2       2.13008193            0.04124705     <.0001

 NB: 1 = Male; 2 = Female

The SAS System        10

The GLM Procedure

 Least Squares Means

                                     Standard
SEX        WEEK 3 LSMEAN             Error          Pr > |t|
1           1.95048144               0.03318294     <.0001

2           1.90499975               0.03729184     <.0001

 NB: 1 = Male; 2 = Female

                              Standard
SEX    WEEK 4 LSMEAN          Error          Pr > |t|
1       1.94127190            0.03116078     <.0001

2       1.86020615            0.03501929     <.0001

                                     Standard
SEX    AV. DOCILITY LSMEAN           Error          Pr > |t|
1      2.15888729                    0.02512886     <.0001

2      2.10441329                    0.02824045     <.0001

The SAS System        11

The GLM Procedure
```

Least Squares Means

VARIETY	WEEK 1 LSMEAN	LSMEAN Number
1	2.56292488	1
2	2.45000000	2
3	2.56002163	3

NB: 1 = Pearl; 2 = White; 3 = Lavendar

Least Squares Means for effect VARIETY

Pr > |t| for H0: LSMean (i) = LSMean (j)

Dependent Variable: WEEK 1 Docility Score

i/j	1	2	3
1	-	0.1449	0.9675
2	0.1449	-	0.2532
3	0.9675	0.2532	-

VARIETY	WEEK 2 LSMEAN	LSMEAN Number
1	2.20989501	1
2	2.08125000	2
3	2.18778162	3

NB: 1 = Pearl; 2 = White; 3 = Lavendar

Least Squares Means for effect VARIETY

Pr > |t| for H0: LSMean (i) = LSMean (j)

```
                Dependent Variable: WEEK 2 Docility Score

            i/j        1         2         3

             1         -       0.0697    0.7348

             2       0.0697      -       0.2265

             3       0.7348    0.2265      -


                                 WEEK 3      LSMEAN
                VARIETY          LSMEAN      Number
                1                1.91774109     1

                2                1.90312500     2

                3                1.96235569     3

             NB: 1 = Pearl; 2 = White; 3 = Lavendar


The SAS System       12

The GLM Procedure

Least Squares Means

                Least Squares Means for effect VARIETY

                Pr > |t| for H0: LSMean (i) = LSMean (j)

                Dependent Variable: WEEK 3 Docility Score

            i/j        1         2         3

             1         -       0.8192    0.4498

             2       0.8192      -       0.4567

             3       0.4498    0.4567      -


                          WEEK 4     LSMEAN
                VARIETY  LSMEAN      Number
                1        1.91782672     1
```

```
        2          1.90312500        2

        3          1.88126535        3

       NB: 1 = Pearl; 2 = White; 3 = Lavendar


       Least Squares Means for effect VARIETY

       Pr > |t| for H0: LSMean (i) = LSMean (j)

          Dependent Variable: WEEK 4 Docility Score

   i/j          1          2          3

     1          -       0.8066     0.5096

     2      0.8066         -       0.7698

     3      0.5096     0.7698        -


                                     LSMEAN
        VARIETY    AV. DOC LSMEAN    Number
        1               2.15615704   1

        2               2.08500000   2

        3               2.15379383   3

     NB: 1 = Pearl; 2 = White; 3 = Lavendar


The SAS System        13

The GLM Procedure

Least Squares Means

Least Squares Means for effect VARIETY

       Pr > |t| for H0: LSMean (i) = LSMean (j)


          Dependent Variable: Average Docility Score

   i/j          1          2          3
```

1	-	0.1425	0.9578
2	0.1425	-	0.2539
3	0.9578	0.2539	-

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

METHOD	WEEK 1 LSMEAN	LSMEAN Number
1	1.90103055	1
2	2.44248624	2
3	2.64784917	3
4	3.10589606	4

NB: 1 = HPT, 2 = NOT, 3 = CT, 4 = HT

Least Squares Means for effect METHOD

Pr > |t| for H0: LSMean (i) = LSMean (j)

Dependent Variable: WEEK 1 Docility Score

i/j	1	2	3	4
1	-	<.0001	<.0001	<.0001
2	<.0001	-	0.0042	<.0001
3	<.0001	0.0042	-	<.0001
4	<.0001	<.0001	<.0001	-

METHOD	WEEK 2 LSMEAN	LSMEAN Number
1	1.49614170	1
2	2.22525562	2
3	2.42550476	3
4	2.49166676	4

NB: 1 = HPT, 2 = NOT, 3 = CT, 4 = HT

```
 The SAS System        14

The GLM Procedure

Least Squares Means

                    Least Squares Means for effect METHOD

                    Pr > |t| for H0: LSMean (i) = LSMean (j)

                       Dependent Variable: WEEK 2

          i/j        1          2          3          4

            1        -       <.0001     <.0001     <.0001

            2     <.0001        -       0.0023     <.0001

            3     <.0001     0.0023       -        0.3116

            4     <.0001     <.0001     0.3116       -


                               WEEK 3      LSMEAN
                    METHOD     LSMEAN      Number
                    1          1.24169203     1

                    2          1.95340089     2

                    3          2.12476161     3

                    4          2.39110784     4

                    NB: 1 = HPT, 2 = NOT, 3 = CT, 4 = HT

                    Least Squares Means for effect METHOD

                    Pr > |t| for H0: LSMean (i) = LSMean (j)

                       Dependent Variable: WEEK 3

          i/j        1          2          3          4

            1        -       <.0001     <.0001     <.0001

            2     <.0001        -       0.0038     <.0001

            3     <.0001     0.0038       -        <.0001

            4     <.0001     <.0001     <.0001       -
```

METHOD	WEEK 4 LSMEAN	LSMEAN Number
1	1.23500888	1
2	1.92076837	2
3	2.11322601	3
4	2.33395284	4

NB: 1 = HPT, 2 = NOT, 3 = CT, 4 = HT

The SAS System 15

The GLM Procedure

Least Squares Means

Least Squares Means for effect of METHOD

Pr > |t| for H0: LSMean (i) = LSMean (j)

Dependent Variable: WEEK 4 Docility Score

i/j	1	2	3	4
1	-	<.0001	<.0001	<.0001
2	<.0001	-	0.0006	<.0001
3	<.0001	0.0006	-	<.0001
4	<.0001	<.0001	<.0001	-

METHOD	AV. DOC LSMEAN	LSMEAN Number
1	1.47451130	1
2	2.13780244	2
3	2.32918311	3
4	2.58510431	4

NB: 1 = HPT, 2 = NOT, 3 = CT, 4 = HT

Least Squares Means for effect METHOD

```
                 Pr > |t| for H0: LSMean (i) = LSMean (j)

                    Dependent Variable: AV. Docility Score

            i/j          1          2          3          4

              1          -       <.0001     <.0001     <.0001

              2       <.0001        -       <.0001     <.0001

              3       <.0001     <.0001        -       <.0001

              4       <.0001     <.0001     <.0001        -

NOTE: To ensure overall protection level, only probabilities associated with pre-planned

    comparisons should be used.

                                      H0:LSMean1= LSMean2

                        SEX    WEEK 1 LSMEAN       Pr > |t|

                        1      2.53918570           0.5698

                        2      2.50944531


The SAS System      16

The GLM Procedure

Least Squares Means

                                      H0:LSMean1= LSMean2

                        SEX    WEEK 2 LSMEAN       Pr > |t|

                        1      2.18920249           0.2172

                        2      2.13008193


                                      H0:LSMean1= LSMean2

                        SEX      WEEK 3   LSMEAN       Pr > |t|

                        1      1.95048144               0.2936

                        2      1.90499975
```

```
                    H0:LSMean1= LSMean2

        SEX    WEEK 4 LSMEAN       Pr > |t|

        1       1.94127190          0.0467

        2       1.86020615

    NB: 1 = male, 2 = female


                H0:LSMean1= LSMean2

        SEX    AV. DOC LSMEAN      Pr > |t|

        1       2.15888729          0.0971

        2       2.10441329

        NB: 1 = male, 2 = female
```

Appendix B: PHENOTYPIC CORRELATION BETWEEN DOCILITY AND HETETEROPHIL, LYMPHOCYTE, H/L RATIO 1

```
                  The CORR Procedure

    3 With Variables:   HET     LYMP     H/L RATIO

    1      Variables:       DOC

                  Simple Statistics

Variable      N     Mean        Std Dev      Sum           Minimum      Maximum

HET          79     7.58861     0.77922      599.50000     5.60000      8.80000

LYMP         79     68.67722    1.63252      5426          64.80000     72.60000

HLRAT        79     0.11068     0.01259      8.74400       0.08000      0.13500

DOC          79     2.15190     0.16082      170.00000     1.70000      2.50000


         Pearson Correlation Coefficients, N = 79

              Prob > |r| under H0: Rho=0

                                   DOC
```

```
                    HET                 0.04161

                                        0.7158


                    LYMP                0.07244

                              0.5258


                    H/L RATIO      0.02277

                                   0.8421
```

Appendix C: THE RELATIONS BETWEEN DOCILITY AND PERFORMANCE TRAITS (FEED INTAKE, WEIGHT GAIN) AND H/L RATIO

```
                         The SAS System      1

                    The MEANS Procedure

Variable              N       Mean            Std Dev        Minimum        Maximum

DOCILITY                  316      2.0822785      0.6219803      1.0000000       4.0000000

FEED INTAKE     316       83.9189873     16.8076481     47.0000000      120.0000000

WEIGHT GAIN     316      -1.3164557      5.0484492     -12.0000000      12.0000000

H/L RATIO       316      2.0368592       0.4691621      0.1103000       2.4843750


                        The SAS System       2

                     The GLM Procedure

                    Class Level Information

                 Class        Levels    Values

                 DOCILITY:    4         Docile (1) Flighty (2) Restless (3) Aggressive (4)

              Number of Observations Read        316


                         The SAS System       3
```

```
                        The GLM Procedure


Dependent Variable:   FEED INTAKE

                               Sum of
   Source              DF      Squares         Mean Square   F Value    Pr > F
   Model                3      229.31442       76.43814        0.27       0.8480

   Error              312      88757.25165   284.47837

   Corrected Total   315     88986.56608

              R-Square     Coeff Var     Root MSE      FEED INTAKE Mean
              0.002577      20.09853     16.86649     83.91899


 Source                DF     Type III SS          Mean Square    F Value    Pr > F

   DOCILITY              3      229.3144228     76.4381409       0.27        0.8480


                         The SAS System        4

                        The GLM Procedure

Dependent Variable:      WEIGHT GAIN

                               Sum of
   Source              DF      Squares              Mean Square    F Value    Pr > F
   Model               3      13.146961            4.382320         0.17       0.9162
   Error                312    8015.207470        25.689768
   Corrected Total   315    8028.354430

              R-Square     Coeff Var     Root MSE      WT Mean

              0.001638     -385.0116      5.068507     -1.316456

   Source                  DF     Type III SS          Mean Square    F Value    Pr > F

   DOCILITY                   3       13.14696073     4.38232024      0.17        0.9162


                          The SAS System        5

                          The GLM Procedure

Dependent Variable:      HL RATIO

                                    Sum of
   Source                  DF       Squares              Mean Square    F Value    Pr > F
```

```
Model              3     0.51215640     0.17071880     0.77      0.5093
Error              312   68.82346286    0.22058802
Corrected Total  315   69.33561926

           R-Square    Coeff Var      Root MSE      HL RATIO Mean

           0.007387     23.05844     0.469668      2.036859

Source              DF    Type III SS    Mean Square   F Value   Pr > F

DOCILITY             3     0.51215640   0.17071880     0.77       0.5093


                          The SAS System          6

                          The GLM Procedure

                         Least Squares Means

                                                Standard
           DOCILITY STATUS   Feed Intake      LSMEAN      Error          Pr > |t|
          Docile              83.2177778      2.5143074                    <.0001

           Flighty          83.8509804     1.1808903               <.0001

           Restless         85.0047619     2.1249776               <.0001

           Aggressive       78.1750000     8.4332433               <.0001


                                                Standard
           DOCILITY STATUS   Weight Gain    LSMEAN     Error             Pr > |t|
           Docile           -1.24444444    0.75556848              0.1006

           Flighty          -1.43137255    0.35486650                <.0001

           Restless         -0.95238095    0.63857191              0.1369

           Aggressive       -2.00000000    2.53425371              0.4306


                                                   Standard
           DOC           HL RATIO          LSMEAN   Error       Pr > |t|
           Docility      2.01583889     0.07001397                <.0001

           Flighty      2.04109589     0.03288334                <.0001

           Restless     2.01656151     0.05917260               <.0001

           Aggressive  2.37695312     0.23483400          <.0001
```

```
                  The SAS System        7

                  The GLM Procedure

                  Least Squares Means

                        LSMEAN

      DOCILITY STATUS    Feed Intake LSMEAN   Number

      Docile                    83.2177778            1

      Flighty                   83.8509804            2

      Restless            85.0047619            3

      Aggressive          78.1750000            4

  Least Squares Means for effect of DOCILITY

  Pr > |t| for H0: LSMean (i) =LSMean (j)

 Dependent Variable: FEED INTAKE

    i/j     1              2          3          4

    1       -               0.8198     0.5876     0.5670

    2       0.8198         -          0.6354     0.5056

    3              0.5876       0.6354       -         0.4329

     4   0.5670        0.5056     0.4329     -


                              LSMEAN

            DOCILITY STATUS      WEIGHT GAIN LSMEAN   Number

            Docile               -1.24444444            1

            Flighty              -1.43137255            2

            Restless                   -0.95238095            3

            Aggressive           -2.00000000            4



            Least Squares Means for effect DOCILITY
```

```
            Pr > |t| for H0: LSMean (i) =LSMean (j)

                Dependent Variable: WEIGHT GAIN

i/j       1         2         3         4

 1      -         0.8230    0.7680    0.7753

 2    0.8230    -           0.5125    0.8243

 3    0.7680    0.5125    -           0.6888

 4    0.7753    0.8243    0.6888    -


                         LSMEAN

             DOCILITY STATUS      HL RATIO LSMEAN     Number

             1                    2.01583889          1

             2                    2.04109589          2

             3                    2.01656151          3


                   The SAS System       8

                  The GLM Procedure

                 Least Squares Means

                  HLRATIO     LSMEAN

             DOC        LSMEAN      Number

             4      2.37695312        4

            Least Squares Means for effect DOC

            Pr > |t| for H0: LSMean (i) =LSMean (j)

              Dependent Variable: HLRATIO

i/j       1         2         3         4

 1        -       0.7442    0.9937    0.1416

 2    0.7442        -       0.7173    0.1577

 3    0.9937    0.7173        -       0.1377
```

```
4       0.1416      0.1577      0.1377      -
```

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Appendix D: EFFECTS OF SEX AND VARIETY ON H/L RATIOS

```
                 The SAS System          1

                      The MEANS Procedure

Variable    N        Mean        Std Dev       Minimum       Maximum

HET        79    7.5886076    0.7792151     5.6000000     8.8000000

LYMP       79   68.6772152    1.6325181    64.8000000    72.6000000

HLRAT      79    0.1106835     0.0125948    0.0800000     0.1350000
```

```
                       The SAS System        2

                        The GLM Procedure

                      Class Level Information

                  Class          Levels    Values

                  SEX              2               Female Male

                  VARIETY          3               Lavendar Pearl  White

                  Number of Observations Read          79

                  Number of Observations Used          79
```

```
                      The SAS System        3

                      The GLM Procedure

                      Dependent Variable: HETEROPHILS

                            Sum of
Source              DF      Squares          Mean Square    F Value    Pr > F
Model                3      9.89153269     3.29717756      6.60       0.0005
```

```
Error                  75     37.46821414    0.49957619
Corrected Total     78     47.35974684

               R-Square     Coeff Var      Root MSE      HET Mean

               0.208859      9.314054      0.706807      7.588608

Source          DF     Type III SS      Mean Square    F Value   Pr > F

SEX               1      0.14639989   0.14639989      0.29        0.5899

VARIETY        2      9.61747293   4.80873647      9.63         0.0002


                            The SAS System         4

                             The GLM Procedure

Dependent Variable: LYMPHOCYTES

                              Sum of
Source                 DF     Squares           Mean Square    F Value    Pr > F
Model                  3      23.8810247      7.9603416       3.24        0.0266

Error                  75    183.9979627    2.4533062

Corrected Total             78     207.8789873

               R-Square     Coeff Var      Root MSE     LYMP Mean

               0.114879      2.280674      1.566303      68.67722

Source        DF   Type III SS          Mean Square   F Value  Pr > F

SEX             1      4.78673033      4.78673033    1.95        0.1666

VARIETY      2     17.27915299    8.63957650     3.52         0.0345


                                 The SAS System         5

                                         The GLM Procedure

Dependent Variable: HLRATIO

                             Sum of
Source                DF    Squares            Mean Square    F Value    Pr > F
Model                 3     0.00268568     0.00089523      6.93         0.0004

Error                 75     0.00968740     0.00012917
```

```
Corrected Total   78      0.01237309

            R-Square     Coeff Var      Root MSE    HLRATIO  Mean

            0.217059      10.26810      0.011365     0.110684

Source                  DF     Type III SS     Mean Square    F Value    Pr > F

SEX                      1      0.00007792      0.00007792       0.60    0.4398

VARIETY                  2      0.00255397      0.00127699       9.89    0.0002


                              The SAS System         6

                                        The GLM Procedure

                              Least Squares Means

                                    Standard
             SEX      HET LSMEAN      Error                       Pr > |t|
             F        7.32667461        0.14214551        <.0001

             M       7.41587604         0.12648143        <.0001

                                          Standard
             SEX     LYMP LSMEAN           Error               Pr > |t|
             F        69.3885578              0.3149983      <.0001

             M         68.8784982             0.2802862      <.0001



                                      Standard
             SEX    H/LRATIO LSMEAN     Error              Pr > |t|
             F        0.10578848           0.00228563        <.0001

             M       0.10784640           0.00203376         <.0001

                                        Standard
            VARIETY HET LSMEAN          Error              Pr > |t|
            L          6.75923321           0.20449916      <.0001

            P          7.74459277           0.09678995      <.0001

            W          7.61000000         0.22351201       <.0001



                                      Standard
            VARIETY LYMP LSMEAN      Error                  Pr > |t|
            L          69.6008383          0.4531756            <.0001
```

```
P        68.4497457        0.2144891          <.0001

W        69.3500000       0.4953086          <.0001


                                        Standard
VARIETY    HLRATIO LSMEAN     Error              Pr > |t|
L              0.09724517           0.00328824      <.0001

P              0.11330715           0.00155633      <.0001

W              0.10990000           0.00359396      <.0001

              The SAS System        7

                        The GLM Procedure

              Least Squares Means

                   H0:LSMean1= LSMean2

       SEX       HET LSMEAN     Pr > |t|

       F        7.32667461        0.5899

       M        7.41587604

                        H0:LSMean1=LSMean2

       SEX      LYMP LSMEAN        Pr > |t|

       F        69.3885578              0.1666

       M        68.8784982


                        H0:LSMean1=LSMean2

       SEX    HLRATIO LSMEAN        Pr > |t|

       F        0.10578848                  0.4398

       M       0.10784640

                            LSMEAN

       VARIETY       HET LSMEAN       Number

       L                 6.75923321              1

       P                 7.74459277              2
```

```
        W           7.61000000          3


        Least Squares Means for effect VARIETY

        Pr > |t| for H0: LSMean (i) = LSMean (j)

            Dependent Variable: HETEROPHILS

    i/j         1          2          3

      1         -        <.0001     0.0063

      2       <.0001       -        0.5822

      3       0.0063     0.5822       -


                        LSMEAN

        VARIETY     LYMP LSMEAN Number

        L         69.6008383         1

        P         68.4497457         2

        W          69.3500000        3



              The SAS System          8

              The GLM Procedure

              Least Squares Means

        Least Squares Means for effect VARIETY

        Pr > |t| for H0: LSMean (i) =LSMean (j)

            Dependent Variable: LYMP

    i/j         1          2          3

      1         -        0.0236     0.7097

      2       0.0236       -        0.0995

      3       0.7097     0.0995       -
```

LSMEAN

VARIETY	HLRATIO LSMEAN	Number
L	0.09724517	1
P	0.11330715	2
W	0.10990000	3

Least Squares Means for effect VARIETY

Pr > |t| for H0: LSMean (i) =LSMean (j)

Dependent Variable: HL RATIO

i/j	1	2	3
1	-	<.0001	0.0113
2	<.0001	-	0.3871
3	0.0113	0.3871	-

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.