UNIVERSITY OF EDUCATION, WINNEBA

COLLEGE OF AGRICULTURE EDUCATION

DEPARTMENT OF ANIMAL SCIENCE EDUCATION

MAMPONG -ASHANTI

INNOVATION FOR SEXING GUINEA FOWLS (*Numida meleagris*) AT DAY ONE OF HATCHING USING BIOMETRIC AND MORPHOMETRIC TRAITS

BY

ARHIN ERIC

(B.Ed AGRICULTURE)

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INNOVATION FOR SEXING GUINEA FOWLS (*Numida meleagris*) AT DAY ONE OF HATCHING USING BIOMETRIC AND MORPHOMETRIC TRAITS IN GHANA

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DECLARATION

I, Arhin, Eric, declare that with the exception of quotations and references contained in published works which have all been identified and acknowledged, this dissertation is entirely my own original work and it has not been submitted either in part or whole for another degree elsewhere.

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(CO- SUPERVISOR)

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DEDICATION

I dedicate this thesis to my parents, Mr. and Mrs. Gyasi and my family and Gyamfuah Ellen Afia.

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
DNA	Deoxyribonucleic Acid
EL	Egg Length
ESI	Egg Shape Index
EW	Egg Width
EWI	Egg Weight
GSS	Ghana Statistical Service
mtDNA	Mitochondrial DNA
NAERLS	National Agricultural Extension and Research Liaison Services
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat

ABSTRACT

All over the world it is almost impossible to determine the sex of Guinea keets at day one of hatching. The aim of this study was to determine the sex of Guinea fowls at day-old. A total of 1176 eggs were set in an incubator. The study was conducted at the Poultry Unit of the Department of Animal Science, University of Education, Winneba, Mampong Campus, (Ghana) from April, 2015 to March, 2016. Hatchability of fertile eggs (728) was 44.6%. Keets were reared from day-old to four months. Differences between males and females were observed (confirmed) using morphometric and biometric traits at four months old. Data obtained from both traits were analyzed using the General Linear Model Procedure of the Statistical Analysis System (SAS) and Chi square test. Males and females did not differ ($P > 0.05$) in all morphometric traits measured at day one of hatching, indicating that sexual dimorphism had not taken place at that age. However, at four months, neck, body, shank and wattles lengths in males were longer ($P < 0.01$) than in female counterparts. The helmet in males was also thicker than in females. However, females had wider ($P < 0.01$) pelvic inlet than males. At day-old, swollen leg technique indicated 52% and 48% expected males and females, and at four months there were 52.8% and 47.2% observed males and females, respectively ($P > 0.05$). Results from stretched leg technique showed 56% and 44% expected males and females at day old, and 52.8% and 47.2% males and females, respectively, were observed at four months ($P > 0.05$). In conclusion, the swollen and stretched leg techniques are effective in sexing Guinea fowls at day-old. Farmers should use both swollen or non-swollen and stretched and non-stretched leg techniques to reduce cost of feeding males till four months before sexing and maximize profit.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Domestic Guinea fowl (*Numida meleagris*) is a common farm animal which originated from Africa. It can also be located in Europe, specifically France, Hungary, Poland, Belgium and Russia (Darre, 2007). It is one of the common poultry species found in Northern Ghana that has different strains which includes pearl, white, black and the lavender (Dei *et al.*, 2007). According to Annor *et al.* (2013), the pearl is the commonest strain found in the Northern region. In the Northern savanna it can be found in the Upper East, Upper West and Northern regions of Ghana, where they live in groups, but can also be found in the southern parts of Ghana (Embury, 2001; Apiiga, 2004). They are mostly reared on free- range and normally found around the dwelling places where they sleep on trees and feed in farm fields and around houses (Moreki, 2009).

They are used as a source of income and protein because of low cholesterol level. The yield of edible meat is higher than that in chicken (Apiiga, 2007). The eggs are very popular and common in the market from May to July. Their thicker shells give them an advantage for longer storage and handling with less breakage (Dei *et al.*, 2007). Moreover, the birds are used for cultural purposes which includes funeral celebrations, sacrifices and as a token for settling disputes (Annor *et al.*, 2013).

Despite these importance derived from Guinea fowls; it is very difficult to determine the gender at the early age and even the first two months of age by using the external features (Apiiga, 2007; Naazie *et al.*, 2007).

However, it is easier to differentiate *Gallus domesticus* using the comb. Therefore people would expect that Guinea fowls have external features that could be used to distinguish the sexes of this species at the young age.

1.2 Problem Statement

The basic information about each individual includes its sex. However, telling the difference between the sexes in various taxonomic groups is not always as easy as in mammals. In birds that are sexually dimorphic, such as the house sparrow, mallard and collared flycatcher, it is very easy to distinguish between males and females (Apiiga, 2004).

As with the *Gallus domesticus,* the comb is important for sex differentiation. Therefore, one would expect Guinea fowl to have an external feature that could be used to distinguish between the sexes of this species.

However, males and females of many species have very similar phenotypic traits (sexual monomorphism), which can make sex identification challenging even for experienced ornithologists. At least, 60% of all passer-species are sexually monomorphic in colour (Iddriss *et al*., 2015).

Sexing is a major problem militating against efficient selection and breeding in Guinea keets. Farmers have difficulties in distinguishing between males and females at the early stages. To the untrained eye, it is not always an easy task to determine the gender of Guinea fowl.

This is because the exposed structures of the head, that is, the helmetic process of the frontal bone is found in both sexes and tend to have similar appearance (Teye and Gyawu, 2001). Idahor and Akinola, (2015) conducted a research using egg colour, weight and shape to predict the sex of Guinea keets. The results showed that both conical and oval eggs yielded both males and females. Teye and Gyawu, (2001) distinguished between males and females birds earlier at four weeks of age. They reported that males had vestigial phalli in their cloaca while females had none or at best possessed a labia-like structure at four weeks of age. Similarly, Iddriss *et al.* (2015) concluded that vent sexing was only possible from 8 weeks old. They observed that males from 4 weeks old and beyond had bigger wattles than females while the opposite was true for the width of pelvic inlet.

In spite of the various methods of sexing Guinea fowls made by some researchers, it is shown that none was able to sex Guinea fowls at day one. Evidence gathered from farmers in the Sekyere South District of Ashanti Region indicates that Guinea fowls can be sexed at day-old using swollen and non-swollen legs as well as males show stretched whilst females show non-stretched legs (Serekye Yaw Annor).

1.3 Research Objectives

The main objective of the study was to find a farmer friendly innovation for sexing Guinea fowls (*Numida meleagris*) at day one of hatching.

The specific objectives were to:

a. use the shape of the egg to pre-determine the sex of a guinea fowl.

b. use stretched and non- stretched legs to separate males from females at day-old.

c. distinguish between the sexes using swollen and non- swollen leg technique at day old.

d. use sound to differentiate between the sexes at four months old.

e. use morphometric and biometric traits to sex guinea fowls at day-old and four months old.

1.4 Significance of the Study

It has been observed that pullets precociously mate the females and farmers do not understand the behaviour of birds uncharacteristic to a peculiar sex. This increases the cost of feeding the pullets with layer ration. The aim of this study was to determine the sex of Guinea fowls at day-old using biometric and morphometric traits. This will reduce cost of ration and maximize profit. In large scale poultry production, it is important that the birds be sexed at a very early age so that resources are not unduly wasted feeding the males on expensive layer ration. The justification of this research work is to help farmers in early separation of males from females to provide enough space for the growth of pullets to prevent precocious mating.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Poultry Production in Ghana

Poultry meat and eggs have been a conventional protein source and serve as income in Ghana for many years (Teye and Gyawu, 2001), but the local domestic poultry in Ghana has been neglected as far as planned management practices are concerned, yet the indigenous fowl is reared all over the country in small backyard units and is well adjusted to its environment. Again birds such as Guinea fowls are used culturally for different purposes such as funeral celebrations, sacrifices, and courtships and as a token for settling disputes (Annor *et al*., 2013).

Ghana has estimated about 25 million poultry (Ghana Statistical Service, 2010). The birds are raised extensively with little financial input into production. Birds forage around compounds or farms with occasional feeding of grains or household wastes during the day and return to their owner's yard to roost at night. Birds are provided with housing (wooden coops or mud pens) only at night (Dankwa *et al*., 2000; Ikani and Dafwang, 2004).

Apart from being left on free range to scavenge for feed, birds are fed with a variety of feed supplements, which include grains (maize, millet and sorghum) termites and left-over food around the household (Annor *et al*., 2013). Inbreeding is a major concern about poultry in rural communities. Fowls that are hatched together from the same parents live together as adults and interbreed among themselves. However, commercial poultry farms are emerging in both southern and northern Ghana. In the south, Asamoah and Yamoah and Akate Farms are examples whilst Alhassan Farms and Atibire Farms are examples in the north (Moreki, 2009).

2.2 Guinea Fowls Production in Ghana

The Guinea fowl is a promising genetic resource for evolving a low-input poultry enterprise mostly in developing countries, and has the potential for reducing poverty (Teye and Gyawu, 2001). It is an integral part of the rural family system providing a sustainable family income for small, marginal and landless farmers. According to Annor *et al*. (2013), Guinea fowl is a poultry species suitable for use in meat production to expand and diversify the local poultry industry due to its consumer acceptance, resistance to common poultry disease and tolerance to poor management conditions.

Guinea fowls are commonly raised in the backyard system where they may be fed by their owners, but are mainly allowed to search for food during the day and return to their owner's yard to roost at night (Ikani and Dafwang, 2004; Teye and Gyawu, 2001). In Ghana, Guinea fowls are found mainly in the Northern sector (Naazie *et al*., 2007), particularly in the Northern, Upper East and Upper West Regions, where they have assumed socio-cultural, economic and nutritional significance (Dei and Karbo, 2004). The Guinea fowl is mostly found in every household in the rural areas in the three Northern Regions of Ghana. The three Regions together form about 40% of the total landmass of Ghana (Teye and Gyawu, 2001). Apiiga (2004) states that the Guinea fowl is an important bird in the Upper East Region and two out of five households own the bird. The number in each household ranges from 5 to 200 birds with an average of about 20. In almost all households, males and females as well as children rear these birds (Naazie *et al*., 2007).

Generally Guinea fowls are seasonal breeders. This has brought their laying periods to vary within the year and posed drawbacks in large commercial Guinea fowl production (Apiiga, 2007; Farrell, 2010). In the wild, production starts at 28-42 weeks with 15-20 eggs being laid each season whilst in captivity production starts at 28-32 weeks with 50-100 eggs laid per year (Annor *et al*., 2013). The eggs are normally hatched by chicken, ducks and turkeys. Guinea fowl eggs are slightly protected in hard shells and this helps to extend the shelf life by reducing spoilage. The hard shells also facilitate transportation over long distances and therefore reduce production losses (Farrell, 2010; Moreki, 2009).

Hatching Guinea fowl eggs takes a period of 26 to 28 days with average keets weight of 24 to 25 g (Farrell, 2010). The keets are raised till the domestic hen is ready to lay and brood over new eggs. Although the Guinea hens are not good brooders, the eggs are hatched artificially using incubators or other good brooding poultry species including chicken (Lislev *et al*., 2005).

Konlan *et al*. (2011), in a study which involved increasing Guinea fowl egg hatchability, reported a 69% rate with artificial incubation. Guinea fowls are reared traditionally in Ghana under the extensive system just like the local chicken. The farmer does little to influence what kinds of feed they eat. They are left to scavenge around farmsteads, open fields and compounds for food scraps, worms, insects, seeds, leaves, fruits etc. In the dry season, vitamin deficiency can develop since birds may not have access to green leaves (Annor *et al*., 2013). Consequently, their productivity is low as compared to that of the intensive and semi-intensive systems. However, when put under confinement, with good feeding and watering, improved housing and good medical care, the bird can lay between 150- 220 eggs per year (Apiiga, 2004) and weigh about 1.1125 kg at 18 weeks (Teye *et al.*, 2001).

Guinea fowls are hardy and are tolerant to mycotoxin and aflatoxin (Moreki, 2009). They are also resistant to most of the common poultry diseases, including coccidiosis, Newcastle disease, fowlpox and gumboro. Furthermore, they also easily adapt to harsh weather conditions as experienced in the tropics (Moreki, 2009; Singh *et al*., 2010).

At six months of maturity, Guinea fowls reach a slaughter weight of 1.5 kg to 2 kg but this relies greatly on the geographical location and management system (Ikani and Dafwang, 2004;Koney, 2004). Guinea fowl meat is a delicacy and can be used as a substitute for wild birds. Moreover, both the meat and eggs are healthier (lower cholesterol) compared to other poultry and are rich in minerals (magnesium, calcium and iron), vitamins (E, B1 and B2) and high in essential fatty acids (Moreki, 2009).

2.3 Guinea Fowl Types

Guinea fowl has been classified into four genera (*Agelastes, Numida, Guttera, and Acryllium*) (Ayorinde, 2004). The *Agelastes* consists of the White-breasted Guinea fowl (*meleagrides)* and the Black Guinea fowl (*niger*), whilst the *Numida* consists of the Helmeted type (*meleagris*). On the other hand, the *Guttera* consists of the Plumed (*plumifera)* and the Crested *(pucherani*) types, with the Vulturine type (*vulturinum*) being found under the last genera *Acryllium* (Ayorinde, 2004; Wikipedia, 2008).

Many varieties of Guinea fowls are found in West, South and Central Africa (Annor *et al*., 2013). Some are plain headed, plumed, crested, grey-breasted, helmeted and white-breasted. The grey-breasted and helmeted Guinea fowls (*Numida meleagris galeata pallas*) are the commonest types found in West Africa (Jacob and Pescatore, 2011) and has several subspecies distinguished by the size, shape, and colour of the wattles at the corner of the beak, and by the size and shape of the bony "helmet" on the crown (Jacob and Pescatore, 2011). According to the Annor *et al*. (2013) and Darre (2007), there are three principal varieties of domesticated Guinea fowl namely; Pearl, White and Lavender.

2.3.1 The Agelastes

Plate 1. ***The White-Breasted Guinea Fowl***

The white-breasted Guinea fowl (*Agelastes meleagrides*), black with a broad white collar, is considered one of the most endangered species of Africa because of habitat destruction and hunting pressure. The white-breasted Guinea fowl is one of the members of the two species of the genus *Agelastes*. However, Ayorinde (2004) believes that the white-breasted Guinea fowl is actually not a variety *per se* since it does not breed true and segregates to other colours when mated *inter se*. The white-breasted Guinea fowl is a medium-sized, terrestrial bird with a small head. It has a bare red head and upper neck, with a pure white lower neck, breast and upper back.

The female is similar to the male but slightly smaller. They have a low deep *kok-kok*, loud, ringing, melodious call, rather vocal, uttering dry ticking calls. They occur singly, in pairs or in small groups, but more commonly in groups of 15-24 birds, constantly moving in search of food and occupying large territories.

Plate 2.The Black Guinea Fowl

Source: FeatherSite.com, 2007

The black Guinea fowl (*Agelastes niger*) sometimes (*Phasidus niger*) is rare and is one of the two members of the genus *Agelastes*. The black Guinea fowl inhabit primarily, unspoiled, rainforests. They live in groups of 15- 20 and roost in trees at night. Their advertising calls are very different from other species of Guinea fowls. The calls consist of short, soft, low- pitched whistling sounds reminiscent of the cooing of doves (Martinez, 1994)

2.3.2 The Numida

Plate 3. The Pearl Guinea Fowl

The helmeted Guinea fowl, (*Numida meleagris*), is naturally distributed in West Africa along with the other species except the vulturine type (Ikani and Dafwang, 2004;

Kayang *et al*., 2010). In Northern Ghana, helmeted Guinea fowls have a high cultural value and make up about 25% of the poultry population in the zone (Kayang *et al*., 2010).

However, the Bawku, Zebilla and Binaba areas have bigger types than other areas. Those from the Upper East are the meat type, the egg type from the Upper West and the dual-purpose type from the Northern Region (Annor *et al*., 2013). It is able to thrive in various climatic conditions and is reared commercially in Europe, America and Asia (Dei and Karbo, 2004; Moreki, 2009). In Nigeria, 25% of the entire poultry population is made up helmeted Guinea fowl (Ikani and Dafwang, 2004). There are three well known types: the pearl (Plate 3), white (Plate 4) and lavender (Plate 5).

The nine subspecies of helmeted Guinea fowl has a characteristic helmet shape. The local breed of Guinea fowl (*Numida meleagris*) has a pointed comb or helmet and lateral wattles, and the bare skin of the neck, chin and throat. Like other Guinea fowls, this species has unfeathered head, in this case decorated with a dull yellow or reddish bony knob, and red and blue patches of skin. The wings are short and rounded, and the tail is also short. The pearl Guinea fowl is a large bird with a round body and small head. The pearl is the most numerous and has the purplish-grey plumage dotted with white (Darre, 2007; Wikipedia, 2008).

Helmeted Guinea fowls are 53 cm to 63 cm in length and are characterized by a bony helmet, naked grey neck and wattles on either side of the beak. They usually mature between 16 and 18weeks of age if fed well; weighing 1.25 to 1.50 kg. The hen may produce between 40 and 80 eggs per year; each egg weighing 30 to 40g (Kumssa and

Bekele, 2013). Male pearl Guinea fowl celebrate the beginning of breeding season with a parade, a single file of birds chasing each other with their heads lowered and their wings raised over humped backs. This ritual is a display of both aggression and courtship. The male capable of keeping up the chase for the longest distance emerges dominant. Its strength becomes evident to female observers evaluating their potential mates. Pearl Guinea fowl (*Numida meleagris)*, form stable pair bonds following a two to three-week "dating" period of temporary pairings (Leach, 2009).

Plate 4. The white Guinea fowl

Source: Annor *et al*., 2013

The white birds are not albino and are the only solid white bird that hatches solid white and not yellow offspring or keets. Its skin is lighter in color than that of the pearl variety and characterised by pure white plumage (Darre, 2007).

Plate 5. Lavender Guinea Fowl

Source: Annor, 2013

The lavender Guinea fowls are one of the three variety or subspecies of the helmeted Guinea fowl (Ayorinde, 2004; Jacob and Pescatore, 2011). The lavender Guinea fowls are similar to the Pearl, but with plumage that is light gray or lavender dotted with white (Darre, 2007; Bönner *et al*., 2004).

2.3.3 The Guttera

Plate 6. The Plumed Guinea Fowl
Source: FeatherSite.com, 2007

The Plumed Guinea fowl (*Guttera plumifera*) is a member of the Guinea fowl bird family. It is found in humid primary forest in Central Africa. It resembles some subspecies of the Crested Guinea fowl, but has a straighter (not curled) and higher crest, and a relatively long wattle on either side of the bill (Bird Life International, 2013). The bare skin on the face and neck is entirely dull grey-blue (Wikipedia, 2010). There are five subspecies of crested Guinea fowl, but only two subspecies of plumed Guinea fowl (*Guttera plumifera*), that vary mainly in the colour of the facial skin on the head and the form of the feathered crest (FeatherSite.com, 2007).

Plate 7. The Crested Guinea Fowl

Source: FeatherSite.com, 2007

The Crested Guinea fowl (*Guttera pucherani*) is found in open forest, woodland and forest-savanna mosaics in sub-Saharan Africa (Wikipedia, 2010). They have slightly curled feathers (FeatherSite.com, 2007) and the plumage is overall blackish with dense white spots. It has a unique black crest on the top of its head (Wikipedia, 2010; Clements, 2010). They occur in flocks averaging fewer than 20 birds. During early mornings after descending from their nightly roosts, crested Guinea fowl flocks move into forest glades to preen and socialize in the warmth of the morning sun. Crested Guinea fowl fly up into trees to feed on fruit rather than eat maize scattered on the ground (FeatherSite.com, 2007). Although deeper than the call of the helmetedGuinea fowl, the crested Guinea fowl alarm call is also a staccato "chuk- chuk- chukchukerr."

The lower pitch may be due, in part, to its windpipe being housed in the hollowed-out blade of its wishbone. Crested Guinea fowl are noisy, sometimes calling well into the night and during the quiet pre-dawn hours. Flock members keep in contact by emitting a low- pitched "chuk" call. Male crested Guinea fowl apparently do not have a hump-backed display as in the male helmeted Guinea fowl, but rather cock their tails like bantams when frightened (Martinez, 1994).

Plate 8. The Vulturine Guinea Fowl

Source: Bird Life International (2009).

The Vulturine Guinea fowl (*Acryllium vulturinum*) is the largest (61-71 cm in length) and most ornate, with a long, glossy-blue cape, white hackles extending from the neck and cobalt blue breast. It has a helmetless head and resembles the look of a vulture

(Jacob and Pescatore, 2011). This species may be readily seen in the non-breeding season in flocks of up to 30 individuals.

The head of the vulturine Guinea fowl is mostly featherless except for a chestnut- brown patch of short feathers on the sides and back of the head. It has black body plumage finely spangled white spots, with short rounded wings, and a tail longer than those of other members in the family Numididae (Bird Life International, 2009). The breast is blue, the edges of the wings are violet, and the rest of the plumage, except for the black ventral feathers, shows the usual spotted design.

This Guinea fowl is slender and has longer legs than the helmeted Guinea fowl. Their advertising call is similar to that of the crested and the plumed Guinea fowl, but is a much higher- pitched "keek-keek-keek," also given in series. Vulturine Guinea fowls also feed on a range of plant and animal items and will sometimes perch in bushes to feed on fruits. Unlike all other Guinea fowls, this species has the ability to survive for longer periods without water and is mostly found in East Africa (Martinez, 1994).

2.4 Sexual Dimorphism in Birds

Dimorphism means two forms. "Sexual dimorphism" means that the two sexes of a species differ in external appearance or other features (Butler and Losos, 2002). Males and females may differ in size, color, shape, the development of appendages (such as horns, teeth, feathers, or fins), and also in scent or sound production. Species in which male and females are identical in appearance or other features are said to be "monomorphic." The quality of the information available on sexual dimorphism varies widely across marine mammal species (Ayorinde and Olusegun, 2014).

Sexual dimorphism of Guinea fowls is not very clear (Agbolosu *et al*., 2012), even though the cry of the cock is distinct from that of the hen at nine weeks (Teye and Gyawu, 2001).

Other methods of sex identification including vent sexing, laparoscopy and polymerase chain reaction (PCR) have been used to differentiate between the sexes (Butler and Losos, 2002 ; Annor *et al*., 2013).

Sexual dimorphism may also only appear during mating season; some species of birds only show dimorphic traits such as Guinea fowls in seasonal variation. The males of these species will molt into a less bright or less exaggerated color during the off breeding season. Muscovy ducks are waterfowls characterized with visible sexual size dimorphism but scientific study on the relative contribution of the underlying morphological variables are scarce and empirical reports on its sexual size dimorphisms are scanty. Apart from sexual differences in total body size, the variation of relative size and shape of different body parts is of special interest in sexual dimorphism studies, because it could reveal differences in selection act on body parts of each sex (Butler and Losos, 2002).

2.5 Morphometric Trait and Biometric Trait

Morphometric refers to the quantitative analysis of form, a concept that encompasses size and shape. It is commonly performed on organisms and useful in analysing their fossil record, the impact of mutation on shape, developmental changes in form, covariance between ecological factors and shape as well for estimating quantitative-genetic parameters of shape (Singleton *et al*., 2011). Morphometric analyses of landmark data use a mathematical definition of shape. Shape encompasses all features of landmark configurations except for overall size, position, and orientation. Morphometric methods have been developed for analysing the outline of a part of the bird such as head length, head width, shank length, body length helmet thickness and wattle length (Currie *et al*., 2000). Biometric trait comprises of the physiological make-up of the animal. For instance, Guinea fowl has a unique feature that makes it different from other birds, such as the sound (Annor *et al*., 2013).

2.6 Mating Ratio of Guinea Fowl

Guinea fowls are naturally seasonal breeders because of their monogamous characteristic. Artificial insemination is important for a commercial breeding scheme (Annor *et al*., 2013; Ikani and Dafwang, 2004). However, for the small scale farmer who keeps the birds on the range, the practice of keeping fewer number cocks in a flock is better. This is because the cock often prepares the nests for a group of Guinea hens that flock with him. This is why it is common to find 20 to 30 eggs in a single nest during the egg producing season in the wild (Annor *et al*., 2013). According to National Agricultural Extension and Research Liaison Services (NAERLS) (2004), the nests are usually located in well hidden places making it difficult for the farmer to locate the nests when many males are kept. Such eggs also may be of poor fertility due to the monogamous tendency of the males (Leach, 2009).

Once the egg nests are located, farmers are advised to leave at least three newly marked or dummy eggs in the nests during each collection to encourage the Guinea fowl hen to continue using the same nest during the next breeding season. In the wild reproductive pairs are established during the rains, the pairs and their offsprings merge together with others to form larger groups at the end of the breeding season (NAERLS, 2004). Under domestic conditions, it is not necessary to mate the birds in pairs to obtain fertile eggs. High ratios of females to males result in poor fertility (Moreki and Mack, 2013). Higher fertility rates are obtained in flocks where male to female ratio is 4-5 or of six females (Avornyo *et al*., 2007; Lourens, 2001). According to Bell and Smith (2003), when guineas are kept closely confined, one male may be mated with six to eight females and several hens will use the same nest. However, a ratio of 1 male to 5 females appears to give optimal fertility.

2.7 Incubation

There are two main methods used to incubate Guinea fowl eggs depending on the scale of production; these are natural and artificial incubations. Guinea fowls are not particularly good mothers and the eggs are best hatched under broody chicken hens naturally or by artificial incubation using incubators (Anonymous, 2001).

2.7.1 Natural Incubation

The incubation period of Guinea fowl egg is between 26 to 28 days (Apiiga, 2004; Bell and Smith, 2003). However, according to Avornyo *et al*. (2007), Guinea fowl eggs take 24- 28 days to hatch. The normal incubation period for Guinea fowl eggs is 26 to 28 days and 24 to 25 days for crossbreds (Bell and Smith, 2003).

The natural incubation method is commonly used by farmers with small flocks. The chicken hens are usually used because they are more adaptable than Guinea fowl hens which are too wild to be set anywhere except in the nests where they have become broody. From 12 to 15 eggs may be set under a Guinea hen, while 20 to 28 eggs can be set under a large chicken hen. (Embury, 2001; Ikani and Dafwang, 2004).

2.7.2 Artificial Incubation

The artificial method of incubation is by the use of machines called incubators (Annor *et al*., 2013; Ikani and Dafwang, 2004). The incubators which are of different types have in-built devices for the production of controlled heating at recommended temperature, controlled humidity level and egg turning ability. Temperature level of 38ºC with 58% humidity for the first 3 weeks is recommended. But the temperature should be lowered to 36ºC while the humidity is raised to 75% for the last week in the incubator. A constant incubation temperature of 37.8ºC is the thermal homeostasis in the chick embryo and gives the best embryo development and hatchability (Lourens *et al*., 2007). Each egg should be turned at least 4 to 5 times daily for the first 24 days of incubation (Ikani and Dafwang, 2004). The eggs will be hatched within 26-28 days after incubation (Anonymous, 2001; Embury, 2001; Smith, 2000).

2.8 Fertility and Hatchability

Fertility and hatchability are major constraints in Guinea fowl production. Feed is regulated in the management of poultry to prevent excessive weight gain which is main cause of poor quality of ejaculate and ovulation (Brillard, 2007). Low relative humidity, low rainfall and high temperature result in a reduction of semen production. This is also associated with low spermatozoa concentration, a high percentage of sperm abnormality and a high dead to live spermatozoa ratio (Brillard, 2003). The fertility of local Guinea fowl eggs ranges from 40 to 58% in naturally mated stock, while using artificial insemination results in egg fertility ranging from 70 to 88% (Annor *et al*., 2013). The low hatchability in local Guinea fowls results from the monogamous nature of birds. Again, under natural mating conditions the mating ratio is about 2 females to 1 male. If there is higher proportion of females to smaller number of males most females will go unmated. This will result in low fertilization of eggs and hence poor hatchability (Leach, 2009).

Similarly, roosters in natural mating breeding system are known to reduce mating activity and impaired libido due to heat stress, presumably through dehydration and alteration in secretion of sex hormones (Ayo *et al*., 2010; Penfold *et al*., 2000). On the other hand, handling of eggs before incubation and period of storage greatly affect the hatchability of Guinea fowl eggs. For every day of storage, hatchability deteriorated by 4% on average. Hatchability rates of 67% and 70-88% have been achieved under artificial incubation (Mao *et al*., 2006). Tiwari and Maeda (2005) reported that eggs stored with the small end up had higher hatchability as compared to the large end up.

2.9 Sexing in Poultry

There are different ways of determining the sex of poultry prior and after incubation; these are; the egg, feather colour sexing, the cloacal or vent sexing, feather sexing and ultrasonography methods (Kaleta and Redmann, 2008). In addition to these methods, detection of oestrogen versus androgen in incubated eggs, temperature variation during incubation and microscopic cytogenetic analysis of chromosomes (Kaleta and Redmann, 2008) are also used to determine the sex of the chick in the egg. But these methods need more intense labor and neither suitable nor practical for commercial use

2.9.1 Egg Characteristics

2.9.1.1 Egg Shape Index (ESI)

The easiest way to determine the outer form of avian eggs is the calculation of the shape index. This is done by dividing the width by the length of an egg and multiplying the result by 100 (Kaleta and Redmann, 2008). Completely round eggs have a shape index of 100 and elongated eggs with values between 50 and 80 will house a female keet. Most shape indices of the eggs of chickens are close to 74, e.g. 44 mm wide and 59 mm long. These values may vary between different breeds and can change with increasing age of chickens (Keith, 2000; Mao *et al*., 2006).

Variations exist also among one species of birds' subspecies or race of free living birds (Kilner, 2006; Mao *et al*., 2006). Narushin and Romanov (2002) also reported that long and slant eggs were less satisfactory in hatching. According to Karls (2012), both conical and oval eggs yield both male and female keets. That is elongated eggs will yield male chicks and round eggs result in female chicks. It is important to remember that the relative size and shape of eggs produced are, either actively or passively, under maternal control and may provide a mechanism of sex detection. This method can also be used to separate male eggs from female ones and sold or consumed as table eggs (Bönner *et al*., 2004; Pike and Petrie, 2003).

2.9.1.2 Egg Width (EW) and Egg Length (EL)

The pair-wise analysis of male and female eggs within the same clutch indicated that the sex of the embryo explained 7% of the variance for relative egg length (Kudu *et al*., 2010). According to Yilmaz and Dikmen (2013), hatching a female chick was increased with the increase of the width of the hatching eggs with rounded shape. However, pointed and narrow shape eggs were more likely to produce male chicks. On the other hand hatching a female chick was decreased with the increase of the length of the hatching egg. However, Kudu *et al.* (2010) found that Peregrine Falcon egg length and width were poor predictors of chick sex.

2.9.1.3 Egg Weight (EWI)

Larger eggs might be considered to contain absolutely more nutrients and produce more vigorous and larger chicks than smaller eggs. Intra-clutch variation exists in egg size and shape and in some species egg size seemingly correlates with sex (Alabi *et al.*, 2012). Under such conditions, (Islam *et al.* 2002; Kudu *et al.*, 2010) predicted that most of the larger eggs would be male. That large eggs tend to produce more male chicks and small eggs tend to produce more female chicks was the observation of Kudu *et al.* (2010).

The effect of egg size on sex determination was also found in the white crowned sparrow (*Zonotrichia leucophrys oriantha*) (Magrath *et al.*, 2003), American kestrel (*Falco sparverius*) and the house sparrow (*Passer domesticus*) (Kudu *et al.*, 2010; Ottwall and Gunnarsson, 2007).

2.9.2 Feather Sexing by the Colour of the Plumage

Some hybrid chickens transmit sex-linked variances in the colour of the plumage at the day of hatching. Commercial brown egg layers can be distinguished into females and males by the colour of their down feathers on the neck and back (Flock, 1999). This is possible when using what is called sex-linked color traits (Kelata and Redmann, 2008).

Mating barred hens (black and white striped feathers) with non-barred males results in barred males and non-barred female chicks. This can also be accomplished using birds carrying specific genes for silver and gold color patterns in the roosters and hens (silver males bred with gold females results in silver pullets and gold cockerels). From a genetic standpoint (excluding mutations), this method is always accurate (Kelata and Redmann, 2008).

2.9.3 Cloacal (Vent) Sexing

The sex is determined by visualisation of the interior of the cloaca (Annor *et al*., 2013). Male chicks display a rudimentary phallus in form of a cone-like elevation on the ventral part of the vent just below the opening of the intestine. Female chicks have at the same location only a small hemisphere-like structure. This method needs a great deal of experience and is sometimes considered an art, due to the skill required.

Vent sexing appears to be stressful for the chicks, causing up to 1.0 % increases in early chick mortality (Phelps *et al*., 2003). Also, microbial cross contamination from chick to chick may occur if the meconium in the cloaca contains egg-transmitted pathogenic bacteria or viruses. After being taught the basics of this technique from nonprofessionals, most people would be doing well to obtain 60-70% accuracy at best (Keith, 2000). Although cloacal sexing has a number of disadvantages, many chicks are sexed by this method (Ellendorff and Klein, 2003).

2.9.4 Feather Sexing of Slow and Rapid Feathering Chicks

Day old chicks can be determined by comparing the lengths of their feathers being long or short. That is some breeds of chicken have rapid or slow growth of feathers. For instance, leghorn chickens have rapid growth of feathers whilst other heavy breeds slow feathery growth (Kelata and Redmann, 2008). Female chicks are rapid feathering and male chicks are slow feathering because the genes that control feather growth are linked to the sex gene, and slow are dominant over rapid feathering (Kelata and Redmann, 2008; Preisinger and Kühne, 1999). It is quiet significant to observe the feather length at one day after hatching. Hence feather sexing gained popularity in some major hybrid lines of chickens (Preisinger, 2003).

2.9.5 Ultrasonography of Day-Old Chicks

In an attempt to see in your mind's eye the gonads of newly hatched chicks by transcutaneous ultrasound, neither the dorso-ventral nor latero-lateral position of the chicks allowed recognition (Kaleta *et al*., 1994). This is because the subtle, small structure of the testes and the ovaries, combined with the almost complete absence of differences in tissue density or reflection of the ultrasonic beam prevent picturing of the gonads. Trans-intestinal ultrasonography was also applied to commercial poultry. However, this laborious method is not suitable for mass application and did not gain wide application due to the risk of cross-contamination (Kelata and Redmann, 2008).

2.9.6 Detection of Oestrogen versus Androgen in Incubated Eggs

The yolk of poultry eggs contains maternal oestrogen during formation in the ovary (Badyaev *et al*., 2006; Bennowitz-Fredericks *et al*., 2005; Eising *et al*., 2006; Engelhardt *et al*., 2005; Pilz *et al*., 2005). Differentiation of the sex of growing embryos on the basis of female hormones has been reported by Phelps *et al*. (2003). Oestrogen radio-immune assays conducted on allantoic fluids (20 µl) of embryos allow the sex discrimination from 15-17 days after incubation starts. After incubation for 7 to 14 days, the hormones of both sexes are present in the allantoic fluid and can be measured (Phelps *et al*., 2003). Oestradiol levels in the allantoic fluid of male embryos are either not detectable or less than 42 pg/ml. However, the oestradiol levels of female embryos are in the range of 113 and 830 pg/ ml. Technical equipment is available to facilitate the removal of small amounts (< 40μl) of allantoic fluid from eggs during the later stages of development (Phelps *et al*., 2003).

The results of oestrogen measurements are available within a few hours, and mass screening of several thousands of eggs per hour is possible (Phelps *et al*., 2003). Furthermore, embryonic development and hatchability of punctured eggs remain almost entirely unaffected.

2.9.7 Temperature Variation during Incubation

In some species of reptiles the proportion of male and female offspring can be altered by the ambient temperature during incubation (Booth, 2006). A number of studies on avian eggs demonstrate unequivocally that a slightly increased incubation temperature (more than 38.0°C) resulted in a large proportion of embryos displaying severe malformations of the head, extremities and internal organs, whilst high temperatures (above 39°C) resulted in death of the embryos (Baarendse *et al*., 2007; Nichelmann and Tzschenske, 2002). Thus, in contrast to reptiles, deviations from the optimal incubation temperature cannot be employed for alterations of the ratio between both sexes of aquatic and terrestrial birds including chickens. However, it is known that the Australian brush-turkey (*Alectura lathami*) a mound-building megapode, uses environmental heat sources such as decaying plant material for incubation. The usual 1:1 ratio between both genders changes in favour of males at low temperature and creates more females at high temperatures (Goth and Booth, 2005).

2.9.8 Microscopic Cytogenetic Analysis of Chromosomes

In avian, males have one paired sex chromosome (homogenetic, ZZ) whereas females have sex chromosomes that consist of a larger and smaller unit (heterogametic, ZW) (Klein *et al*., 2003). In order to analyse the sex chromosome, cells were grown in tissue culture media and the cell division stopped during the metaphase with colchicin. Rupture of the cells was obtained with hypotonic saline solution and cells were stained and microscopically monitored for sex chromosomes (Kelata and Redmann, 2008).

Chromosome counting and measurements used to be a common method of sexing birds at any age, until polymerase chain reactions (PCR) (Hoffmann, 2005) and endoscopy (Rühle, 2006) were established. Successful sexing is unlikely if chromosomal aberrations are present (Saefudin *et al*., 2005).

Cytogenetic sexing requires fresh sample material, free of bacterial or fungal contamination, and established cell culture techniques; and it is time-consuming and costly. This method can be performed independently from age and size of birds but blood sampling from embryos may be cumbersome (Cerit and Avanus, 2007).

2.10 Sexing Guinea Fowls

A male Guinea fowl is termed a cock, while a female is called a hen. Like any species, there are some signs to look for when determining gender. The ability to determine sex aids the local breeder, farmer and fancy bird show-person to appropriately house and feed both genders (eHow, 2008).

According to Bell and Smith (2003), Guinea fowl UK (2009), Teye and Gyawu (2001), there are no outstanding external features that can be easily used in the first two months of age to identify the sex of the bird but the present study indicates that Guinea keets can be sexed at day one of hatch. That is at one day one of hatch, males have swollen legs whilst females do not and males also stretch their legs when raised above the ground but females do not. According to Umosen *et al*. (2008), when keets are gently handled from about the fourth week of age, it is possible to identify a rudimentary phallus in the males, which distinguishes them from the females.

The phallus becomes fully developed and protrudes when a slight pressure is applied on the cloaca when the bird is about three months of age. At this age, the female exhibits a labia-like structure in the cloaca or there may be no structure at all (Teye and Gyawu, 2001). Although Guinea keets are naturally loud from hatch, the distinctive call that most easily determines the gender is not fully developed until two months.

At two months of age and older, a Guinea fowl will emit either a one or two syllable call. The female emits a two syllable call, which, according to folklore sounds like "buck-wheat, buck-wheat". A male emits a one syllable call that sounds like "chit chit chit chit." (Bell and Smith, 2003; eHow, 2008).

Differences in the size and shape of the head can also be observed. Males exhibit a more elongated and larger head, especially in the rear section of their heads. Females' skulls appear much more rounded. The bird's wattles (the ornamentation on the sides of their heads) vary in size and males exhibit much larger and more erect wattles (eHow, 2008). Studies at the Animal Science Department (University of Ghana) have shown that the length and width of head, width of abdomen, length of ceres (the leathery patch of skin just above the beak where their nostrils are located and length of tail feathers of young Guinea fowls are related to body weight rather than to the sex of the bird and cannot therefore be used as sexing indicators.

Studies however, showed that young female Guinea fowls tend to have a wider pelvic inlet than the males and this difference in pelvic inlet width is evident as early as 2 weeks of age. At this age the pelvic inlet width of the male averaged 9.0 mm (6.5–11.0 mm) while the average for the females was 12.8 mm (8.6–20.9 mm) (Iddriss *et al.*, 2015). The male has slightly larger head appendages (Annor *et al.*, 2013).

According to, Guinea fowl UK (2009) and Teye and Gyawu (2001) the surest way to determine sex differences in Guinea fowls is to wait until they start calling at about 9 weeks as only the females have a two note sound. The male birds produce a single note. Yet, females can also produce a single call (Guinea fowl UK, 2009; Teye and Gyawu, 2001). The male is generally slightly larger, has larger wattles, its voice is a more shrill shriek and it has a peculiar habit of strutting on tiptoe and arching his back. Perhaps the female is easier to recognize, since, the hen alone uses the call note 'come back, come back,' accenting the second syllable strongly, from which they are often called 'come backs' (Annor *et al.*, 2013).

2.10.1 Genetic Markers

Genetic markers are Deoxyribonucleic acid (DNA) sequences linked to specific locations on chromosomes and related to specific traits (Moore and Hansen, 2003). According to Lenstra *et al.* (2012), the most advanced and current techniques are molecular genetic markers, which include mitochondrial DNA (mtDNA) sequences (which are maternally inherited), Y-chromosomal haplotype (which are paternal linked) and autosomal DNA which are related more to phenotype.

2.10.1.1 Mitochondrial DNA and Y-chromosomal haplotype

The mtDNA are maternally inherited, circular DNA molecules located outside the nucleus, and are capable of evolving 5 to 10 times more rapidly than nuclear (autosomal) DNA, especially the displacement-loop (D-loop) region which is the control region of mtDNA located in the non-coding region. Most studies, however, emphasise on the highly polymorphic D-loop, but whole genome sequences have been reported as informative (Achilli *et al.*, 2008). mtDNA can easily be isolated but rely on the recognition of nuclear mtDNA insertions (Hassanin *et al.*, 2010), especially when diverse species-specific primers are used (Den Tex *et al.*, 2010). However, Y-chromosome is paternally inherited and is a large linear molecule located in the nucleus. The Y-chromosomal haplotypes have slow mutation rates and are powerful tools used to trace gene flow by male introgression (Petit *et al.*, 2002).

2.10.1.2 Autosomal DNA

Initial genetic diversity studies relied on blood groups and protein polymorphisms. Recently, autosomal DNA (Whittaker *et al.*, 2003) is the most used markers (Bruford *et al.*, 2003; Soller et al., 2006). Autosomal markers include Amplified Fragment Length Polymorphism (AFLP), Random Amplification of Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Single nucleotide polymorphism (SNP) and microsatellite markers (FAO, 2007). Microsatellite markers or simple sequence repeats (SSR) and SNPs are the most recent autosomal DNA markers, however microsatellite markers have been identified as the most powerful markers (Tóth *et al*., 2000; FAO, 2007; Tenva, 2009).

2.10.1.2.1 Amplified Fragment Length Polymorphism (AFLP)

According to FAO (2007), this technique involves the digestion of DNA with restriction enzymes and the selective amplification of the digested fragments using a Polymerase chain reaction (PCR). The output is a significant number of informative markers which can be located reliably in the genome allowing a quick scan of the entire genome. These markers are biallelic, easily reproducible and are capable of estimating relationships between breeds and related species (Buntjer *et al*., 2002).

2.10.1.2.2 Random Amplification of Polymorphic DNA (RAPD)

RAPD markers are detected using random PCR primers. They are the most popular molecular tools capable of recognizing polymorphisms in large portions of the genome based on minute quantities of DNA. RAPD analysis is quick and simple, because a single RAPD primer can anneal to various locations in a genome (multiple loci). Although RAPD are dominant markers, they however have the tendency to underestimate genetic variability and are not easily reproducible (SanCristobal *et al*., 2006). Negrini *et al*. (2006) estimated the intra and inter varietal genetic variation in three varieties of guinea fowl (Lavender, Pearl and White) with RAPD markers. The results showed a very low level of intra and inter varietal genetic variation in the three guinea fowl varieties, implying low genetic variation between the populations. The genetic homogeneity found in the study was attributed to the fact that the guinea fowl populations were diluted, closed, reproduced from small number of sires and subjected to similar type of selection programmes. The ability of RAPD markers to underestimate genetic variability as reported by Buntjer *et al*. (2002) was also indicated as a reason for the low genetic diversity realized in the above study.

2.10.1.2.3 Restriction Fragment Length Polymorphism (RFLP)

RFLP markers also rely on the use of restriction enzymes and occur as variations in the length of DNA fragments, after restriction enzyme digestion at precise restriction sites (FAO, 2007). The difference between RFLP and AFLP markers is that the PCR is done prior to restriction enzyme digestion in RFLPs (FAO, 2007). The advantages of this technique include its ability to discriminate between homozygotes and heterozygotes (co-dominant markers). They are also stable markers and therefore produce reproducible results. However, the methodology is long, labourious and demands the use of DNA of both high quality and quantity. They are also non-informative and therefore are unable to identify whole genome variation especially when inbreeding is high (Tenva, 2009).

2.10.1.2.4 Single Nucleotide Polymorphism (SNP)

SNPs are single base or nucleotide (A, T, G or C) variations or alterations that occur in DNA sequence (Vignal *et al*., 2002) and do not directly affect the phenotype of organisms (FAO, 2007). They are abundant in genomes (mostly in non-coding regions) and this makes them easy to find. Li *et al*. (2004) reported one SNP per 1,000 bp in the human genome. Vignal *et al*. (2002) has also reported that in most genomes SNPs occur as one SNP per 1,000 bp in both coding and non-coding regions. Although SNPs are biallelic and stable with low mutation rates, they are highly non-informative, compared to microsatellites (Tenva, 2009). Studies involving SNPs are costly and require high numbers of the markers to provide little information (FAO, 2007).

2.10.2 Microsatellite Markers

Microsatellite markers are defined as a class of highly informative, repetitive DNA sequences, based on nucleotide repeats (Gurdebecke and Maelfait, 2002). According to Tenva (2009) microsatellites markers are also referred to as short tandem repeats (STR), simple sequence tandem repeats (SSTR), variable number tandem repeats (VNTR), simple sequence length polymorphisms (SSLP), simple sequence repeats (SSR) and sequence tagged microsatellites (STM). Microsatellites can range from between two to six base pairs in length (Wang *et al*., 2010). The most popular class of microsatellites are dinucleotides (Adenyo *et al*., 2012), followed by tri-, tetra-, penta and hexanucleotide repeats. Dinucleotide microsatellites have been reported as the most polymorphic and are known to be characterised by higher repeat numbers (Li *et al*., 2004) with low repeat numbers being observed in trinucleotide repeats (Tóth *et al*., 2000; Thiel *et al*., 2003). According to Tong *et al*. (2009), dinucleotides occur more frequently in vertebrates whilst in plants the commonest classes of repeats are trinucleotides.

Table 1 shows some indicators that may be used to tell the sex of a Guinea fowl from the egg stage through the various stages of development.

Table 1 Sex indicators at different stages of life

Stage of development	Indicators	Sex
Egg	Eggs with the narrow end more pointed	Male
	Eggs with narrow end slightly rounded	Female
4-weeks old keet	Longer necks	
	Bigger keets in the clutch	Male
	Shorter necks	
	Smaller keets in the clutch	Female
10 -12 weeks old	Bigger body frame	
	Pronounced and more concave wattles	
	More protruding horn	
	Monosyllabic sound like “kir ke ke ke ke ke”	Male
	Smaller body frame	
	Less pronounced and flatter wattles	
	Less protruding horns	
	Disyllabic sound like “chekwen chekwen”	Female
Above 12 weeks of age	Presence of phallus	
	Much bigger helmet	
	Shortly attached wattle with much to the with much bigger, and thicker dangling lobe	Male
	Absence of phallus	
	Shortly attached wattle with less dangling lobe	Female

Source: Annor *et al*. (2013).

2.11 Importance of Sexing Guinea Fowls

Sexing and the separation of male and female animals are performed, based on differences in the nutrient requirements, growth rate, marketing age, management, and nutrition of the two sexes. The sexing of day-old chicks is performed by companies with expertise in animal improvement and grandparent breeding stocks for the purpose of marketing the males of the father line and the females of the mother line, while commercial egg companies perform sexing for the selection of female chicks. Furthermore, in broiler production, sexing is required when males and females are raised separately (Kaleta and Redmann, 2008; Saatci *et al*., 2011; Sari *et al*., 2013; Shafey *et al*., 2013; Tran *et al*., 2010).

Sexing allows for the identification and removal of male embryos from the incubator, saving space and energy and contributing to the alleviation of welfare issues (Phelps *et al*., 2003). This is because the relationship with morphological characteristics of the egg and sex of the chick might be helpful for poultry industry in several different ways (Lislev *et al*., 2005). When more female chicks are incubated, incubation capacity increases and less male layer chicks are killed (Magrath *et al*., 2003). It has been observed that pullets precociously mate the females and farmers do not understand the behaviour of birds uncharacteristic to a peculiar sex. This increases the cost of feeding the pullets with layer ration.

Sexing Guinea keets at early stage will reduce cost of ration and maximize profit. In large scale poultry production, it is important that the birds be sexed at a very early age so that resources are not unduly wasted feeding the males on expensive layer ration.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site and Period of the Study

The study was conducted at the Poultry Unit of the Department of Animal Science, University of Education, Winneba, Mampong Campus, (Ghana) from April, 2015 to March, 2016. Mampong is located about 40km North-East of Kumasi and lies on latitude 7° 4'0"N and longitude 1° 24'0" W. It has an average annual rainfall of 1300mm with major rainy season in March and peaks in May/June and long dry season extending from December to February. Temperatures are generally high with minimum and maximum values of 21°C and 30°C recorded in March and December, respectively with average temperature of 23.7°C (GSS, 2010).

3.2 Animals and Management

3.2.1 Egg Collection and Incubation

A total of 1176 eggs were obtained from Mampong College of Agriculture Guinea Fowl Unit. Eggs were collected in the morning in containers which were cushioned to avoid breaking or shaking (Moreki and Mack, 2013). Dirty eggs were discarded during egg collection. Each egg was individually marked with a pencil before incubation and thereafter weighed using an electronic weighing scale. Eggs were incubated at 37.5-37.8°C and 60% relative humidity for 28 days as noted by (Harun *et al*.2001; Tebesi *et al*.2012). Candling was done at 14 days after egg set to determine fertile and unfertile eggs. Hatched keets were sexed as described by Teye and Gyawu (2001). During egg transfer from setter to the hatcher (i.e., 3 days prior to hatching) hatching egg trays were partitioned using wire mesh to separate males from females that resulted from the use of egg shape to separate the sexes.

Hatchability of fertile eggs (728) was 44.6%. Keets were identified by wing tagging soon after hatching, and were reared from day-old to four months.

3.3 Housing of Experimental Birds

Birds were brooded for 6 weeks (Teye and Gyawu, 2002) in a brooder house, and then transferred to a deep litter floored house of size 49.9m x 8.17m x 2.48m. Males and females were housed in separate rooms. Birds were individually caged at the age of three to four months. Each cage measured 0.68m x 0.60m x 0.44m. At four months the sound, wattles and phallus, and other morphological traits were used to distinguish between males from females. Feed and water were provided *ad libitum*.

3.4 Management of Experimental Birds

3.4.1 Health and Medication

The Guinea fowl keets were given glucose as an anti-stress on day-old, dewormed later and appropriate medication was given thereafter throughout the experimental period. Birds were observed for signs of ill-health and the right steps taken to restore health. Dead birds were examined by the Veterinarian of the Department of Animal Science for post-mortem analysis. Reports from the post-mortem examinations determined the appropriate management practices to check and the medication to use to curtail the problem.

Table 2 Health management guide (Vaccination and de-worming schedule)

Age (days)	Medication
1-2	Glucose in water
6	Antibiotics plus vitamin premix*
10	Coccidiostat
16	Newcastle (HB1)
23	Gumboro
25	Antibiotics plus vitamin premix
30	Coccidiostat
35	Dewormer
38	Fowl pox vaccination
44	Coccidiostat
49	Newcastle (Lasota) vaccination
52	Antibiotics plus vitamin premix
56	Dewormer
60	Coccidiostat
84	Fowl pox vaccination
98	Dewormer
112	Newcastle (Lasota) vaccination

*- Vitamin premix: (A, C,D,E,K, Riboflavine, Thiamine, B6, B12, Panthenate)

Source: (Annor *et al*., 2013)

3.4.2 Formulation of Experimental Diet

Maize was bought from Mampong market in the Ashanti Region and milled at the Feed Formulation Unit at the University of Education, Winneba, Mampong campus. The other feed ingredients were purchased from commercial supplies at Kumasi. Experimental diets were formulated with the required feed ingredients (Table 3).

Table 3 Composition of feed ingredients for starter, grower and finisher Guinea fowl

Ingredients (Kg)	Starter	Grower	Finisher
Maize	57.5	58	53
Wheat bran	11	21	20
Soyabean	8.5	5	8
Tuna fish	11	6	7
Russia fish	9	7	3
Oyster shell	1.5	1.5	7.5
Calcium	0.5	0.5	0.5
Premix	0.5	0.5	0.5
Salt	0.5	0.5	0.5
Total	**100**	**100**	**100**

3.4.3 Feeding

Birds were then maintained at ambient temperatures between 21°C and 30°C until the end of the experiment. Feed and water were supplied *ad libitum*. Day old keets were fed ground maize in flat feeders followed by a starter ration from day 2 until 6 weeks of age. This was followed by a grower ration from 6 weeks of age until 21 weeks of age, and then a finisher feed until the end of the experiment. The starter (24% crude protein and 3100 Kcal ME/kg diet), grower (22% crude protein and 3150 Kcal ME/kg diet), and finisher (18% crude protein and 3,200 Kcal ME/kg diet) rations were formulated at the farm for optimum growth and performance in Guinea fowl breeders (Table 4).

Table 4 **Nutrient requirements of Guinea fowl**

Requirement	**Starter (0-4 weeks)**	**Grower (4-8 weeks)**	**Finisher (8-12 weeks)**
Metabolizable Energy(Kcal/kg)	3100	3150	3200
%Crude protein	24	22	18-20
%Methionine	0.60	0.57	0.50
%Methionine+Cystine	1.00	0.94	0.85
% Lysine	1.35	1.15	0.95
% Calcium	1.20	1.00	0.90
% Phosphorous	0.50	0.45	0.40
% Sodium	0.17	0.17	0.17

Source: (Annor *et al*., 2013)

3.4.4 Watering

The water troughs were washed thoroughly with soap and water every morning and fresh, clean water provided *ad libitum* daily. Troughs were disinfected fortnightly.

3.5 Experimental Procedure

All procedures used followed approved guidelines for the ethical treatment of animals. Birds were sexed using the following methods:

3.5.1 The shape of the egg

The shape of the eggs was used as a feature to pre-determine the sex of Guinea fowls. The egg shape was indicated by the shape-index, which is the ratio between egg width and egg length multiplied by 100. That is, $SI = \frac{W}{L} \times 100$. An optimum shape-index is 74 i.e. an egg which is 4.2 cm wide and 5.7 cm long. An egg with a shape index of 72 is too long. An egg with a shape index of 76 is too round. In using egg shape to pre-determine the sex of Guinea fowls, eggs with narrow and pointed end indicated males whereas those with the narrow end slightly rounded were females (Plate 9) (Annor *et al*., 2013).

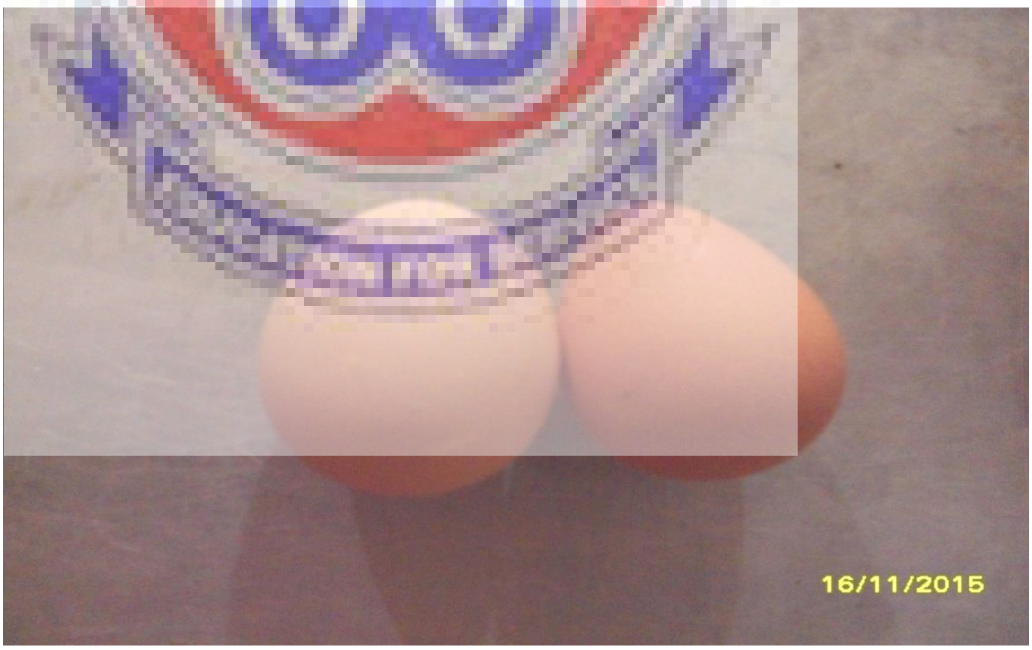

Plate 9 Female (left) egg oval at the end and male (right) egg pointed at the end

3.5.2 Swollen and Stretched Leg Techniques

At day old of hatch, keets were sexed using swollen and stretched leg technique. Swollen leg indicated males (Plate 10) whilst non swollen leg depicted females (Plate 11). Day old keets were held and raised above the ground. Those with stretched legs showed males (Plate 12) whilst non-stretched legs indicated females (Plate 13). They were individually identified using tags placed through their inner wings. The procedure was repeated three times to ensure accuracy and consistency.

***Plate 10** Swollen Guinea keet leg*   ***Plate 11** Non swollen Guinea keet leg*

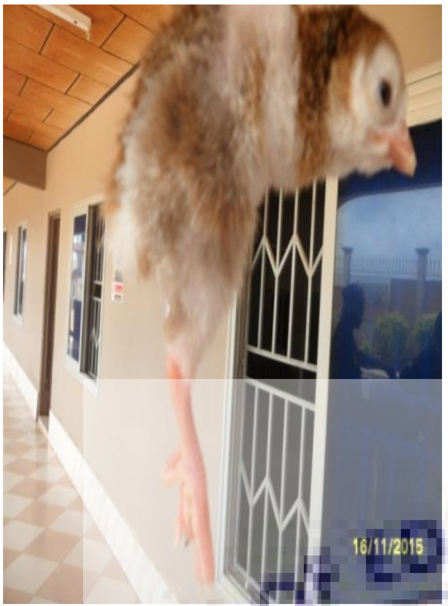

Plate 12 Stretched leg Guinea keet ***Plate 13 Non-Stretched leg Guinea keet***

3.5.3 Sound and Wattles

At three months old the birds were transferred into individual cages of size 0.68m x 0.595m x 0.44m and observed their sounds for a period of one week. Males gave monosyllabic sound like "kirkekekekeke" but their female counterparts produced disyllabic sound such as "chekwenchekwen". Observations from the wattles showed that males (Plate 14) had their wattles shortly attached to the jaws with a much bigger, coarse and thicker dangling lobe whilst females (Plate 15) indicated less pronounced and flatter wattles.

Plate 14. Male Guinea fowl (cupped wattles)

Plate 15.Female Guinea fowl (flat wattles)

3.5.4 Phallus

The birds were individually observed closely through the vent to finally confirm the sex. Vents were inspected at four months old for the appearance of a rudimentary phallus as a feature to differentiate between both sexes. This examination was made easier if two people were available. The bird was tipped upside down with one hand and the tail pushed towards the head. Concurrently, the first finger and thumb were placed on the opposite sides of the vent and slowly separate with a gentle but firm pressing motion, stretching and turning inside out the cloaca to expose the phallus, if present. Males (Plate 16) show the phallus when they are 4 weeks old but females (Plate 17) do not at that age (Idahor and Akinola, 2015; Teye and Gyawu, 2001).

***Plate 16** Presence of phallus*

***Plate 17** Absence of phallus*

3.6 Morphometric Parameters Measured

The following morphometric traits (head length, head width, neck length, body length, shank length, tail length, thigh length, wattle length, helmet thickness, pelvic inlet, wing span, and leg length) were measured at day-old and four months old. The anatomical measurements followed process described by Teguia *et al*. (2008) and Yakubu (2009).

The length and circumference measurements were effected using a measuring tape calibrated and a pair of calipers in centimetres (cm). All measurements were taken by the same individual early in the morning before the birds were fed. To ensure accuracy, each measurement was taken twice, the same person throughout took all measurements and weighing, thus eliminating error due to personal difference. The following morphological traits were measured at four months

i. Head width: Measured from one side of the face to the other between calipers.
ii. Head length: This was taken with a pair of calipers and measured from the tip of the beak to the back of the occipital bone.
iii. Neck length: Measured with a flexible tape, it spanned from the occipital condyle to the point of attachment of the neck to the rest of the body.
iv. Body length: This was measured from the tip of the beak to the tip of the pygostyle using a flexible tape.
v. Tail length: This was measured from the tip of the pygostyle to the tip of the tail feather using a flexible tape.
vi. Shank length: This was measured with a ruler the distance between the footpad and the hock joint when the tibia-tarsus and tarsometa-tarsus were held at right angles to each other.
vii. Thigh length: This was measured with a ruler from the tip of pubic bone to the trabecular bone (knee joint).
viii. Wattle length: This was measured with a ruler and spanned from the point of attachment to the tip of the wattle.

ix. Helmet thickness: This was measured with the helmet trapped in between the calipers from left to right at the base (not front to back, which may be considered as helmet width).

x. Width of pelvic inlet: measured with a pair of caliper as the distance between the tips of the 2 pubic bones.

xi. Leg length: This was measured with a ruler as the distance between the pubic bone and the tip of the metatarsal bone (drumstick leg).

xii. Wing length: Measured from the glenohumeral joint to the farthest feather on the phalanges on each side (i.e. left and right wings).

3.7 Data Collection and Statistical Analysis

Data obtained from both the morphometric and biometric traits were analysed using Statistical Analysis System (General Linear Model procedure) (2010) and Chi square test (Steel and Torrie, 1980).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 RESULTS

4.1.1 Summary of techniques used for sexing Guinea fowl

Different techniques for sexing Guinea fowls have been presented in Table 5.

Table 5 Different methods of sexing Guinea fowl

Technique	Sex	%Expected	%Observed	X^2	P-level
	Male	53.6	52.8		
Egg	Female	46.4	47.2	0.03	3.84
	Total	100	100		
	Male	52	52.8		
Swollen	Female	48	47.2	0.03	3.73
	Total	100	100		
Stretched	Male	56	52.8		
	Female	44	47.2	0.03	2.85
	Total	100	100		
	Male	52.8	52.8		
Sound	Female	47.2	47.2	0.00	1.84
	Total	100	100		
	Male	52.8	52.8		
Wattles	Female	47.2	47.2	0.00	2.61
	Total	100	100		
	Male	52.8	52.8		
Phallus	Female	47.2	47.2	0.00	3.66
	Total	100	100		

4.1.2 *Morphometric Traits*

Morphometric parameters measured in the study for day-old keets and four months old Guinea fowls have been presented in Table 6 and Table 7, respectively.

Table 6 Means and standard errors for day-old body measurement

Parameter (cm)	Male	Female	Probability value
Head width	1.80±0.02	1.74±0.01	0.0878
Head length	3.1±0.04	3.0±0.03	0.0736
Body length	11.08±0.04	11.01±0.02	0.2109
Neck length	3.1±0.02	3.1±0.02	1.0000
Shank length	1.2±0.03	1.16±0.02	0.2952
Leg length	6.12±0.03	6.07±0.03	0.3284
Pelvic inlet	2.08±0.03	2.04±0.02	0.3098
Wing length	3.05±0.03	3.05±0.02	0.9151

Means with different letters in the same row are significantly different (P < 0.05)

Means and standard errors for four months old body measurement are presented in Table 7.

Table 7 Means and standard errors for 4-month old body measurement

Parameter (cm)	Female	Male	Probability Value
Head width	2.49±0.03	2.54±0.03	0.2771
Head length	6.91 ±0.04	6.97 ±0.04	0.3552
Neck length	12.59 ±0.14[b]	13.42 ±0.14 [a]	0.0001
Body length	52.91 ±0.28[b]	53.99 ±0.26[a]	0.0055
Tail length	16.07 ±0.13	15.85 ±0.12	0.2132
Shank length	5.34 ±0.03[b]	5.42 ±0.03[a]	0.0389
Thigh length	12.93 ±0.08	12.80 ±0.07	0.2261
Wattle length	2.30 ±0.06[b]	3.21 ±0.06 [a]	0.0001
Helmet thickness	0.84±0.02[b]	1.06±0.02[a]	0.0001
Pelvic inlet	6.03 ±0.08[a]	3.49 ±0.08 [b]	0.0001
Leg length	24.43 ±0.15	24.40 ±0.14	0.7447
Wing length	19.18 ±0.13	18.89 ±0.13	0.0866

Means with different letters in the same row are significantly different (P < 0.05)

4.2 DISCUSSION

4.2.1 The Shape of the Eggs

The present study revealed that egg shape was found to be related to the probability of the sex of the hatching chick. Using the shape of the egg to differentiate between the sexes, the expected proportion of males and females were similar ($p > 0.05$) to the observed proportions (Table 5). Pointed shaped eggs were more likely to produce male chicks and the rounded shaped eggs were more likely to produce female chicks ($p > 0.05$). Similarly, Mao *et al*. (2006) reported that rounded (oval) shaped eggs were most likely to produce pullets (female) and pointed end (pointy) shaped eggs were most likely to produce cockerels (male). Again, Idahor and Akinola (2015) conducted a research using egg colour, weight and shape to predict the sex of Guinea keets.

The results showed that both conical and oval eggs yielded both males and females, indicating that the use of egg shape in pre-determining sex was effective in that study. Furthermore, Yilmaz-Dikmen and Dikmen (2013) conducted a study on the use of shape index, width, length, weight, volume and replicate and concluded that only the effect of shape index on the hatching chick sex was the most prominent factor for determining the sex of the egg in white layers.

4.2.2 Swollen/Non-Swollen and Stretched/Non Stretched Legs

At day-old, swollen leg indicated males (Plate 10) whilst non-swollen leg depicted females (Plate 11). Males did not differ ($p > 0.05$) from their female counterparts (Table 5). Stretched legs showed males whereas non-stretched leg depicted females.

The observations for males were higher than that of females for both swollen/non-swollen and stretched/non-stretched legs techniques. Many scientists have concluded that it is difficult to determine the sex of Guinea keets at day one of age, unless they are about 8 weeks old (Iddriss *et al.*, 2015; Teye and Gyawu, 2001). However, these two methods have worked successfully in the determination of Guinea keets at day old. Hence the present study has proven otherwise.

4.2.3 Sound

It was observed that males gave monosyllabic sound like "kir ke ke ke ke ke" but their female counterparts produced disyllabic sound when they were four months old such as "chekwen chekwen" as reported by Annor *et al.* (2013). Similar observations were made by Iddriss *et al.* (2015) and Teye and Gyawu (2001). The present study therefore agrees with the works of the aforementioned scientists.

4.2.4 Wattles and Phallus

Again, it was observed that at 4 months old the wattles of a male Guinea fowl was cupped and inclined at an angle of 90^o to the side of the upper jaw (plate 14) whereas their female counterparts had a flat wattle attached to upper jaw (plate 15). This observation agrees with the report of Umosen *et al.* (2008).

Moreover, it was observed that males had phallus present while females exhibited a labia like structure. This confirms the work of Teye and Gyawu, (2001), who distinguished between males and female birds earlier at four weeks of age by observing vestigial phalli in the cloaca of males while females had none or at best possessed a labia-like structure at four weeks of age. Similar observation was made by Iddriss *et al.* (2015) in 8 weeks old Guinea fowls.

4.2.5 Morphometric Traits Measured for Day-Old Guinea Keets

Results obtained from morphometric traits measured at day one of hatching indicated that males did not differ ($p > 0.05$) from females in head width, head, body, neck, shank, leg and wing lengths, and pelvic inlet (Table 6). These indicate that sexual dimorphism had not taken place at that age. In consonance with Moreki and Mack (2013), it was very difficult to sex Guinea fowls at day-old since males and females all look exactly the same morphologically. Similarly, Ikani and Dafwang (2004) reported that it is difficult to differentiate between the sexes at day-old since there is little difference in their appearance.

4.1.6 *Morphometric Traits Measured for Guinea Fowls at four months*

At 4 months old, males did not differ ($P > 0.05$) from females in head width, head, tail, thigh, leg and wing lengths, but neck, body, shank and wattles lengths in males were longer ($P < 0.01$) than in female counterparts (Table 7). Nsoso *et al.* (2006) reported that body length, neck length for males were higher than females. In another study by Nsoso *et al.* (2008), Guinea fowl body length and shank length were higher in males than in females. The helmet in males was also thicker than in females.

However, females had wider ($P < 0.01$) pelvic inlet than males. These observations give an indication that it is possible to differentiate between the sexes in four months old Guinea fowls using neck, body, shank and wattles lengths, and helmet thickness. Iddriss *et al.* (2015) concluded that males from 4 weeks old and beyond had bigger wattles than females while the opposite was true for the width of pelvic inlet.

The present study indicates that at four months, neck, body, shank and wattles lengths in males were longer ($P < 0.01$) than in female counterparts. The helmet in males was also thicker than in females. However, females had wider ($P < 0.01$) pelvic inlet than males (Table 7).

This is in consonance with Annor *et al.* (2013) who reported that between four to twelve weeks, male Guinea fowls have longer necks, bigger body frame, pronounced and more concave wattles and much bigger helmets.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The shape of eggs was successfully used to sex Guinea fowls prior to hatching. This method can be used to separate male eggs from female ones and sold or consumed as table eggs. Guinea fowls were sexed using swollen and stretch leg techniques at day-old.

At four months, it was easier to use sound, phallus and morphormetric traits (neck, body, shank and wattles lengths, helmet thickness and pelvic inlet) to distinguish between the sexes.

5.2 RECOMMENDATION

Farmers should use both swollen or non-swollen and stretched and non-stretched leg techniques to reduce cost of feeding males till four months before sexing and maximize profit.

REFERENCES

Achilli, A., Olivieri, A., Pellecchia, M., Uboldi, C., Colli, L., Al-Zahery, N., Accetturo, M., Pala, M., Kashani, ,B .H., Perego, U.A., Battaglia, V., Fornarino, S., Kalamati, J.,Houshmand, M., Negrini, R., Semino, O., Richards, M., Macaulay, V., Ferretti, L., Bandelt, H. J., Ajmone-Marsan, P. and Torroni, A. (2008). Mitochondrial Genomes of Extinct Aurochs Survive in Domestic Cattle. *Journal of Current Biology* 18: 157–158.

Adenyo, C., Hayano, A., Inoue, E., Kayang, B. B. and Inoue-Murayama M. (2012). Development of microsatellite markers for grasscutter (*Thryonomis swinderianus, Rodentia*) using next-generation sequencing technology. *Journal of Conservation Genetic Resources* 4: 1011-1014.

Agbolosu, A . A., Teye, G.A., Adjetey, A.N.A., Addah, W. and Naandam, J. (2012). Performance characteristics of growing indigenous Guinea fowls from Upper East, Upper West and Northern regions of Ghana. *Agriculture and Biology Journal of North America* 10: 336-339.

Alabi, O.J., Ng`ambi, J.W. and Norris, D. (2012). Effect of egg weight on physical egg parameters and hatchability of indigenous Venda chickens. *Asian Journal of Animal Veerinary Advances*, 7: 166-172.

Annor, S.Y., Ahiaba, J. and Apiiga, S.Y. (2013). *Guinea fowl Production: A Handbook*. Qualitype Limited, Accra, Ghana, pp.1-2.

Anonymous, (2001). *Guinea fowl*. Nova Scotia Department of Agriculture and Fisheries Archives. Available: http://www.gov.ns.ca/nsaf/elibrary/archive/lives/feather/ guineas1.htm.

Apiiga, S. Y. (2004). *Guinea Fowl Production and Poverty Alleviation in the Upper East Region: A Call for Presidential Special Initiative*. Ministry of Food and Agriculture, P. O. Box 3, Bolgatanga.

Apiiga, S.Y. (2007). *Improving Guinea Fowl Production in the Upper East Region.* Ministry of Food and Agriculture, Bolgatanga.

Avornyo, F. K., Munkaila, L., Karbo, N., Mbii, P., Abukari, A. and Allegye-Cudjoe, E. (2007). Towards Reducing Guinea Fowl Mortality in Northern Ghana: *Research and Development Experiences*, Vol.8 No- 2.

Ayo, J. O., Obidi J.A. and Rekwot, P. I. (2010). Seasonal variation in feed consumption, hen-day, mortality and culls of Bovans Black chickens. *Proceedings of the 13th annual conference of the Nigerian society of Animal Production*. 14-17th March, pp. 415-418.

Ayorinde, K. L. (2004). The Spice of Life. *71st Inaugural Lecture, University of Ilorin, Nigeria.*

Ayorinde K. L. and Olusegun, A. O. (2014). Sexual size dimorphism and sex determination by morphometric measurements in locally adapted muscovy duck (*Cairina moschata*) in Nigeria.

Baarendse, P. J. J., Debonne, M., Decuypere, E., Kemp, B. and Van Den Brand, H. (2007) Ontogeny of avian thermoregulation from a neural point of view. *Journal of World's Poultry Science* 63: 267- 276.

Badyaev, A.V., Acevedo Deaman, D., Navara, K.J., Hill, G.E. and Mendoca, M.T. (2006) Evolution of sex-biased maternal effects in birds: III. Adjustment of ovulation order can enable sex-specific allocation of hormones, carotenoids, and vitamins. *Journal of Evolutionary Biology* 19: 1044-1057.

Bell, M. and Smith, K. (2003). *Guinea Fowl Production.* Department of Primary industries and Fisheries, State of Queensland.

Bennovitz- Fredericks, Z. M., Kitaysky, A. S. and Wingfield, J. C. (2005). Steroids in allantoic waste: an integrated measure of steroid exposure in ovo. *Annales of the New York Academy of Sciences* 1046: 204-213.

BirdLife International (2009). Globally Threatened Birds; White- Breasted Guinea Fowl. The Official Red List Authority for Birds for IUCN.

BirdLife International (2013). IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 11 July, 2016.

Booth, D.T. (2006). Influence of incubation temperature on hatching phenotype in reptiles. *Physiology and Biochemistry in Zoology* 79: 274-281.

Bönner, B. M., Lutz, W., Redmann, T., Jäger, S., Reinhardt, B., Wissing, J. and Booth, D.T. (2004). Morphometric and allometric studies on eggshells and embryos of free-living Canada geese (Branta canadensis Linnaeus, 1758). *European Journal of Wildlife Research,* 50: 179-186.

Brillard, J. P. (2003). Practical aspects of fertility in poultry. *World's Poultry Science Journal* 59: 441-446.

Brillard, J. P. (2007). Control of fertility in turkeys: the impact of environment, nutrition and artificial insemination technology. *Poultry Industry Technical articles.*

Bruford, M. W., Bradley, D. G. and Luikart, G. (2003). DNA markers reveal the complexity of livestock domestication. *Journal of Nature Reviews Genetics* 4: 900–910.

Buntjer, J. B., Otsen, M., Nijman, I. J., Kuiper, M.T. and Lenstra, J. A. (2002). Phylogeny of bovine species based on AFLP fingerprinting. *Journal of Heredity* 88: 46–51.

Butler, M. A. and Losos J. B. (2002). Multivariate sexual dimorphism, sexual selection, and adaptation in Greater Antillean Anolis lizards. Ecological Monographs, 72, 4: 541–559, doi:10.1890/0012-9615(2002)072[0541:MSDSSA]2.0.CO;2

Cerit, H. and Avanus, K. (2007). Sex identification in avian species using DNA typing methods. *World's Poultry Science Journal*, 63: 91-99.

Clements, J. F. (2010). *The Clements checklist of birds of the world*. Sixth Edition. Cornell University Press, Ithaca. ISBN 978-0801445019.

Currie, A. J., Ganeshanandam, S., Noiton, D. A., Garrick, D., Shelbourne, C. J. A. and Oraguzie, N. (2000). Quantitative evaluation of apple (*Malus domestica*) fruit shape by principal component analysis of Fourier descriptors. *Euphytica* 111, 219–227.

Dankwa, D., Nelson, F. S., Mzama, K. B. and Oddoye, E. O. K. (2000). A Survey of Rural Poultry Management in the West Mamprusi District and the Ga Rural District of Ghana. *Animal Research Institute, Council for Scientific and Industrial Research.*

Darre, M. J. (2007). *Guinea Fowl Management Extension Poultry Specialist*. Department of Animal Science, University of Connecticut. Storrs CT 06269-4040.

Dei, H. K. and Karbo, N. (2004). Improving smallholder guinea fowl production in Ghana: *A training Manual.* Cyber Systems, Tamale, Ghana. 27pp.

Dei, H. K., Tetteh, E. and Otchere, E. O**.** (2007). Nutritive Value of a Processed Product from Cassava and Blood in the Diets of Layer Chickens. *Ghana Journal of Animal Science*, Vol. 2 and 3. No. 1, Pp 45- 51.

Den Tex, R.J., Maldonado, J. E., Thorington, R. and Leonard, J.A. (2010). Nuclear Copies of Mitochondrial Genes: Another Problem for Ancient DNA. *Journal of Genetics* 138: 979-84.

eHow Article (2008). Copyright © 1999- 2008. eHow- Inc. *How to Determine the Sex of a Guinea Fowl*. www.ehow.com

Eising, C.M., Muller, W. and Groothuis, T.G. (2006). Avian mothers create different phenotypes by hormone deposition in their eggs. *Biological Letters*, 2: 20-22.

Ellendorff, F. and Klein, S. (2003) Current knowledge on sex determination and sex diagnosis: potential solutions. *World's Poultry Science Journal*, 59: 7.

Embury, I. (2001). Raising Guinea fowl. Agfact. A5.0.8. *New South Wales AgriculturePublications*, New South Wales, USA, pp 4.

Engelhardt, A., Von, N. and Groothuis, T.G. (2005). Measuring steroid hormones in avian eggs. *Annales of the New York Academy of Sciences*, 1046: 181-192.

FAO, (2007). The State of the World's Animal Genetic Resources for Food and Agriculture. FAO, Rome.

Farrell, D. (2010). *Guinea Fowl*. PoultryHub.org.Australia. Friday, 20th August, 2010.

Feathersite.com(2007).GuineaFowl-*Numidameleagris*. www.feathersite.com/Poultry/Guineas/BRKGuineas.htm

Flock, D.K. (1999). Entwicklung der reziproken rekurrenten Selektion (RRS) in der LTZ-Legehennenzucht (1969-1999). *In: Anonym, 40 years of layer breeding in Cuxhaven.* Jubiläumstagung, Bremen, pp. 83-99.

GSS (2010). *Ghana Statistical Service*. www.statsghana.gov.gh.

Goth, A. and Booth, D. T. (2005). Temperature-dependent sex ratio in a bird. *Biological Letters*, 1: 31-33.

Guinea Fowl UK (2009). *About Guinea Fowl*. www.guinea.fowl.co.uk

Gurdebecke, S. and Maelfait, J. (2002). RAMS technique fails in developing microsatellite markers. Proceedings of 20th European Colloquium of Arachnology, Szombathely, Europe, 22-26 July, pp 49 -56.

Harun, M. A. S., Veeneklaas, R. J., Visser, G. H. and Vankampen M. (2001). Artificial incubation of Muscovy duck eggs: Why some eggs hatch and others do not. *Poultry Science*, 80(2): 219-224.

Hassanin, A., Bonillo, C., Nguyen, B. X. and Cruaud, C. (2010). Comparisons between mitochondrial genomes of domestic goat (*Capra hircus*) reveal the presence of numts and multiple sequencing errors. *Mitochondrial DNA* 21: 68–76.

Hoffmann, B. (2005). *Geschlechtsdiagnose bei Vögeln mit Hilfe der Polymerase-Ketten-reaktion (PCR).* Veterinary Medicine Dissertation, University of Giessen.

Idahor, K. O. and Akinola, L. A. F. (2015). Keets Sex Predetermination Using Egg Colour, Weight and Shape. *Journal of Recent Advances in Agriculture*, 3(1): 345-350.

Iddriss, I. A., Bawa, A., Ian, A.J., Robinson, J. E. and Obese, F.Y. (2015). Sexing in Guinea fowls (*Numida meleagris*). *Poultry Science*, 94:311-318.

Ikani, E. L. and Dafwang, I. I. (2004). National Agricultural Extension and Research Liaison Services, Poultry Series No. 8, Extension Bulletin No. 207, Zaria, Nigeria

Islam, M.S., Howlider, M.A.R., Kabir, F. and Alam, J. (2002). Comparative assessment of fertility and hatchability of Barred Plymouth Rock, White Leghorn, Rhode Island Red and White Rock Hen. *International Journal of Poultry Science*, 1(4): 85- 90.

Jacob, J. and Pescatore, T. (2011). *Keeping Guinea Fowls*. Cooporative Extension Service. University of Kentucky. College of Agriculture. Lexington, KY, 40546

Kaleta, E.F. and Redmann T. (2008). Approaches to determine the sex prior to and after incubation of chicken eggs and of day-old chicks. *World's Poultry Science*, 64:391-399.

Kaleta, E.F., Schildger, B., Enders, F., Herzberger, S. and Roschinsky, M. (1994). Möglichkeiten zur Schätzung des Alters von Hühnerküken innerhalb der ersten Lebenswoche. *Archiv für Geflügelkunde*, 58: 261-267.

Karls, R.O. (2012). Factors influencing the egg shape of Guinea fowls. *Journal of Animal Science*, 7: 21.

Kayang, B. B., Youssao, I., Inoue, E., Naazie, A., Abe, H., Ito, S. and Inoue-Murayama M. (2010). Genetic diversity of Helmeted Guinea fowl (*Numida meleagris*) based on microsatellite analysis. *Journal Poultry Science, 47*: 120-124.

Keith, B. (2000). *Sexing chicks in the backyard flock.* Cooperative extension service. College of Agricultural and Environmental Sciences / Athens, Georgia 30602-4356.

Kilner, R. M. (2006). The evolution of egg colour and patterning in birds. *Biological Reviews of the Cambridge Philosophical Society*, 81: 383-406.

Klein, S., Baulain, U., Rottika, M., Marx, G., Thielenbein, J. and Ellendorff, F. (2003). Sexing the freshly laid egg – development of embryos after manipulation; analytical approach and localization of the blastoderm in the intact egg. *World's Poultry Science Journal*, 59: 39-45.

Koney, E.B.M. (2004). *Poultry Health and Production*. Second edition. Advent press, Accra, Ghana. pp. 91- 94.

Konlan, S.P., Avornyo, E.K., Karbo, N. and Sulleyman, A. (2011). Increasing Guinea fowl eggs availability and hatchability in the dry season. *Journal of World's Poultry Research* 1(1): 1-3.

Kudu, Y.S., Ayanwale, B.A., Shiawoya, S.L., Aremu, A., Jiya, E.Z. and Masodami, V. (2010). Growth performance of wild indigenous Guinea fowls fed graded levels of roasted Negro Coffee meal. *Proceeding of the 35th Conference NSAP*, Ibadan Nigeria. pp. 402-404.

Kumssa, T. and Bekele, A. (2013). Population status, feeding ecology and activity pattern of helmeted Guinea fowl (*Numidia meleagris*) in Abijata-Shalla Lakes National Park *African Journal of Environmental Science and Technology* 7 (1):49-55.

Leach, T. (2009). Helmeted Guinea Fowl. *Oxford Scientific Films/ BBC Natural History Sound Library.*

Li, Y. C., Korol, A. B., Fahima, T. and Nevo, E. (2004). Microsatellites within genes: structure, function, and evolution. *Journal of Molecular Biology Evolution* 21: 991-1007.

Lislev, T., Byrkjedal, I., Borge, T. and Saetre G.P. (2005). Egg size in relation to sex of embryo, brood sex ratios and laying sequence in northern lapwings (Vanellus vanellus). *Journal Zoological Society of London*, 267:81-87.

Lourens, A., (2001). The importance of air meteorology in animal production. *International Journal of Biometry*.

Lourens, A., H. Van den Brand, M.J.W. Heetkamp,R .Meijerhof and Kemp, B. (2007). Effects of eggshell temperature and oxygen concentration on embryo growth and metabolism during incubation. *Poultry Science*, 86: 2194-2199.

Magrath, M. J. L., Brouwer, L. and Komdeur J. (2003). Egg size and laying order in relation to offspring sex in the extreme sexually size dimorphic brown songlark (*Cinclorhamphus cruralis*). *Behavioral Ecology Sociobiology*, 54:240–248.

Mao, K. M., Sultana, F., Howlider, M. A., Iwasawa, A. and Yoshizaki, N. (2006). The magnum-isthmus junction of the fowl oviduct participates in the formation of the avian-type shell membrane. *Zoological Sciences*, 23: 41-47.

Martinez, I. (1994). Family Numididae (Guinea Fowl). *Handbook of the Birds of the World, Vol. 2. New World Vultures to Guinea Fowl*. Lynx Editions, Barcelona, ISBN 8487334156. Pp. 554- 570 .

Moore, S. S. and Hansen, C. (2003). Genomics: delivering added value to the beef industry? *Outlook on Agriculture* 32 (4): 247-252.

Moreki, J.C. (2009). *Guinea Fowl Production*. Reach Publishers, Wandsbeck, South Africa, pp. 7-31.

Moreki, J. C. and Mack, N. (2013). Effect of storage time and egg position on hatchability of Guinea fowl eggs. *Journal of Animal Science Advances*, 2(5):.259-261.

Naazie, A., Canacoo, E. A. and Mwinbong, C. (2007). Guinea Fowl Production Practices and Marketing in Northern Ghana. Agricultural Research Centre-Legon, College of Agriculture and Consumer Sciences. University of Ghana, Legon, Ghana. *Ghana Journal of Animal Science*, 2 and 3(1):35-44.

Narushin, V.G and Romanov M.N. (2002). Egg physical characteristics and hatchability. *World Poultry Science Journal*, 58: 297-303.

National Agricultural Extension and Research Liaison Services (NAERLS) (2004). The production of Guinea fowl in Nigeria. Ahmadu Bello University, Zaria, Nigeria. Extension Bulletin No. 207, *Poultry Series* No. 8.

Negrini, R., Milanesi, E., Bozzi, R., Pellecchia, M. and Ajmone-Marsan, P. (2006). Tuscany autochthonous cattle breeds: an original genetic resource investigated by AFLP markers. *Journal of Animal Breeding and Genetics* 123: 10–16.

Nichelmann, M. and Tzschenske, B. (2002). Ontogeny of thermoregulation in precocial birds. *Comparative Biochemistry and Physiology*, 131: 751-763.

Nsoso, S. J., Mareko, M.H.D., and Molelekwa, C. (2006). Comparison of growth and morphological parameters of Guinea fowl (*Numida meleagris*) raised on concrete and earth floor finishes in Botwana. *Livestock Reproductive and Rural Development*, 18: 101.

Nsoso, S. J., Mareko, M.H.D.,Manyanda, S., and Legodimo, P.P. (2008). The effect of housing type on body parameters, feed intake and fedd conversion ratio of Guinea fowl (*Numida meleagris)* keets and chemical composition of their meat during growth and development in Botswana. *Reprouctive Journal of Animal Science*, 2(2), 36-40.

Ottwall, R., and Gunnarsson, G. (2007). Morphological and molecular sex identification of Redshanks (*Tringa totanus*). *Bird Study*, 54:127–129.

Penfold, L.M., Wildt, D.E., Herzog, T.L., Lynch, W., Ware L., Derrickson, S.E and Monfort, S.I. (2000). Seasonal patterns of luteinizing hormone, testosterone and semen quality in Northern Pintail duck. *Reproductive Fertility Development*, 12: 229-235

Petit, E., Balloux, F. and Excoffier, L. (2002). Mammalian Population Genetics: Why Not Y? *Journal of Trends in Ecology and Evolution* 17: 28-33.

Phelps, P., Bhutada, A., Bryan, S., Chalker, A., Ferrell, B., Neuman, S., Ricks, C., Tran, H. and Butt, T. (2003). Automated identification of male layer chicks prior to hatch. *World's Poultry Science Journal*, 59: 33-38.

Pike T.W. and Petrie M. (2003). Potential mechanisms of avian sex manipulation. *Biological Review*, 78:553-574.

Pilz, K.M., Adkins-Regan, E. and Schwabl, H. (2005). No sex difference in yolk steroid concentrations of avian eggs at laying. *Biological Letters*, 1: 318-321.

Preisinger, R. (2003). Sex determination in poultry – a primary breeder's view. *World's Poultry Science Journal*, 59: 54-58.

Preisinger, R. and Kühne, W. (1999). Legehennenzucht van der Schwelle zum nächsten Jahrtausend. 40 Jahre Legehennenzucht in Cuxhaven. *Vorträge der Jubiläumstagung Cuxhaven, S.* 116-123.

Rühle, D.M. (2006). Untersuchungen zur Endoskopie juveniler Psittaziden unter besonderer Berücksichtigung der Geschlechtsorgane und ausgewählter biometrischer Befunde. Veterinary Medicine Dissertation University of Giessen.

Saefudin, A., Saar, W., Schmutz, M., Preisinger, R. And Schüler, L. (2005). Chromosomal aberrations and early embryonic mortality in laying hens. *Archiv für Geflügelkunde*, 69: 146-150.

Saatci, M., Arslan, C., Unal, Y., Tilki, M. and Aksoy, A.R. (2011). Effect of fattening length, sex and feather color on growth and fattening performance in native Turkish geese. *Eurasian Journal of Veterinary Science*, 27(3):183-189.

SanCristobal, M., Chevalet, C., Peleman, J., Heuven, H., Brugmans, B., van Schriek, M., Joosten, R., Rattink, A. P., Harlizius, B., Groenen, M. A., Amigues, Y., Boscher, M.Y., Russell, G., Law, A., Davoli, R., Russo, V., Desautes, C., Alderson, L., Fimland, E., Bagga, M., Delgado, J. V., Vega-Pla, J. L., Martinez, A. M., Ramos, M., Glodek, P., Meyer, J. N., Gandini, G., Matassino, D., Siggens, K., Laval, G., Archibald, A., Milan, D., Hammond, K., Cardellino, R., Haley, C. and Plastow, G. (2006). Genetic diversity in European pigs utilizing amplified fragment length polymorphism markers. *Journal of Animal Genetics* 37: 232–238.

Sari, M., Sari, T., Onk, K. and Isik, S. (2013). Effects of production system and gender on liveweight and body measurements of pekin ducks. Atatürk University. *Journal of Veterinary Science* 8(2):112-121.

SAS Institute (2010). Version 10 Series Statistical Analysis System Software. SAS Institute, Inc. *Anatomical record*, 294(2): 322-334. North Carolina, USA.

Shafey, T.M., Alodan, M.A., Hussein, E.O.S. and Al-Batshan, H.A. (2013). The effect of sex on the accuracy of predicting carcass composition of roos broiler chickens. *Journal of Animal and Plant Science*, 23(4):975-980.

Singh, S.K., Mehra, S., Kumar, V., Shukla, S.K., Tiwari, A., Mehra, M., Goyal, G., Mathew, J. and Sharma, D. (2010). Sequence variability in the BLB2 region among guinea fowl and other poultry species. *International Journal of Genetics and Molecular Biology*, 2 (3): 48-51.

Singleton, M., Rosenberger, A. L., Robinson, C., O'Neill, R. (2011). "Allometric and metameric shape variation in pan mandibular molars: A digital morphometric analysis".

Smith, J. 2000. *Guinea fowl*. Diversification Data Base. Scottish Agricultural College. Available:http://www.sac.ac.uk/management/external/diversification/tableofcontents. htm, Date accessed: 24 July 2016.

Soller, M., Weigend, S., Romanov, M. N., Dekkers, J. C. M. and Lamont, S. J. (2006). Strategies to assess structural variation in the chicken genome and its associations with biodiversity and biological performance. *Journal of Poultry Science* 85: 2061–2078.

Steel, R.G.D. and Torrie, J.H. 1980. *Principles and Procedures of Statistics: A Biometrical Approach*. 2nd Edition, McGraw-Hill International Book Company, Singapore, pp. 633

Tebesi, T., Madibela, O. R. and Moreki, J. C. (2012). Effect of storage time on internal and external characteristics of Guinea fowl (*Numida meleagris*) eggs. *Journal of Animal Science Advances*, 2(6): 534-542.

Teguia, A., Ngandjou, H.M., Defang, H. and Tchoumboue J. (2008). Study of the live body weight and body characteristics of the African Muscovy Duck (*Cairina moschata*). *Tropical Animal Health Production,* 40, 5-10. 18.

Tenva, A. (2009). Molecular markers in animal genome analysis. *Journal of Biotechnology in Animal Husbandry* 25 (5-6): 1267-1284.

Thiel, T., Michalek, W., Varshney, R. K. and Graner, A. (2003). Exploiting EST databases for the development and characterization of gene-derived SSRmarkers in barley *(Hordeum vulgare L.). Journal of Theoretical and Applied Genetics* 106: 411-422.

Teye, G.A. and Gyawu, P. (2001). The benefits of intensive indigenous guinea fowl production in Ghana. *World Poultry- Elsevier* 17 (9): 53-54.

Teye, G. A., Gyawu, P. and Agbolosu, A. A. (2001). *Growth Potential and Carcass Yields of Exotic and Indigenous Guinea Fowls in Ghana*. Department of Animal Science, University for Development Studies, Tamale.

Tiwari, A.K.R. and Maeda, T. (2005). Effects of egg storage position and injection of solutions in stored egg on hatchability in chickens (*Gallus domesticus*) research note. *Journal of Poultry Science*, 42: 356-362.

Tran, H.T., Ferrell, W. and Butt, T.R. (2010). An estrogen sensor for poultry sex sorting. *Journal of Animal Science*, 88:1358-1364.

Tong, J., Wang, D. and Cheng, L. (2009). *Development of Microsatellite Markers by Data Mining from DNA sequences data mining and knowledge discovery in* *real life applications*. Ed. Julio Ponce and Adem Karahoca. ISBN 978-3-902613-53-0, pp. 438, I-Tech, Vienna, Austria

Tóth, G., Gáspári, Z. and Jurka J. (2000). Microsatellites in different eukaryotic genomes: survey and analysis. *Journal of Genome Research* 10: 967-981.

Umosen, A.D., Onyeanusi, B.I., Salami, S. O., Nzalak, J.O. , Imam J. and Ibe, C.S. (2008). Observations on the Wattles of Adult Helmeted Guinea Fowls (*Numida meleagris galeata*) *International Journal of Poultry Science*, 7 (12): 1204-1206.

Vignal, A., Milan, D., SanCristobal, M. and Eggen, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. *Journal of Genetic Selection Evolution* 34: 275-305

Wang, Y., Wang, A. and Guo, G. (2010). Development and characterization of polymorphic microsatellite markers for the Northern Quahog (Mercenaria mercenaria). *Journal of Shellfish Research* 29 (1): 77-82.

Whittaker, J. C., Harbord, R. H., Boxall, N., Mackay, I., Dawson, G. and Sibly, R. M. (2003). Likelihood-based estimation of microsatellite mutation rates. *Journal of Genetics* 164: 781–787.

Wikipedia, (2008). *Guinea fowl*. The free encyclopedia. Wikimedia, Foundation, Inc., U.S. registered 501.

Wikipedia, (2010). *Crested Guinea Fowl*. The free encyclopedia. Wikimedia, Foundation, Inc., U.S. registered.

Yakubu, A. (2009). An assessment of sexual dimorphism in African Muscovy ducks using morphological measurements and discriminant analysis. *Proceedings of the 4th Waterfowl Conference*, November 11- 13, 2009, Kerala, India. Pp. 69-75.

Yilmaz-Dikmen, B. and Dikmen, S. (2013). A Morphometric Method of Sexing White Layer Eggs. *Brazilian Journal of Poultry Science*. ISSN 1516-635X v.15 / n.3 / 169-286.