Behaviour, physiological responses, meat yield and gut morphology of free-range chickens raised in a hot environment

By

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Submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY IN ANIMAL SCIENCE

in the

Discipline of Animal and Poultry Science

College of Agriculture, Engineering and Science

School of Agricultural, Earth and Environmental Science

University of KwaZulu-Natal

November 2016

Pietermaritzburg, South Africa

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Declaration

I, **Tonderai Mutibvu,** vow that this Dissertation has not been submitted to any other University other than the University of KwaZulu-Natal and that it is my original work conducted under the supervision of Professors Michael Chimonyo and Tinyiko Edward Halimani. All assistance towards the production of this work and all the references contained herein have been duly accredited.

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November 2016

List of abbreviations

ACTH	Adrenocorticotropic Hormone
ALP	Alkaline Phosphatase
AOAC	Association of Analytical Chemists
ARC	Agricultural Research Council
AST	Aspartate Transaminase
aVSA	Apparent Villus Surface Area
bpm	Beats per minute
BR	Breathing Rate
BW	Body Weight
CK	Creatine Kinase
CP	Crude Protein
EC	European Commission
EDTA	Ethylene Diamine Tetra-acetic Acid
EE	Ether Extract
EU	European Union
GLM	General Linear Models
HR	Heart Rate
LDH	Lactate Dehydrogenase
MUFA	Monounsaturated Fatty Acids
Na	Naked Neck gene
NN	Naked Neck
OV	Ovambo
PABM	Provitamin A bio-fortified maize
PDIFF	Probability of Differences

PK	Potchefstroom Koekoek
PUFA	Polyunsaturated Fatty Acids
RH	Relative Humidity
RJF	Red Jungle Fowl
RT	Rectal Temperature
SA	South Africa
SAS	Statistical Analysis System
SD	Standard Deviation
SE	Standard Error
SEM	Standard Error of the Mean
SFA	Saturated Fatty Acid
SFRB	Scavenging Feed Resource Base
SSA	Sub-Saharan Africa
T_a	Ambient Temperature
THI	Temperature Humidity Index
TI	Tonic Immobility
TNZ	Thermoneutral Zone
VD	Villus Density
VH	Villus Height
VW	Villus Width
WM	White Maize

Abstract

Behaviour, physiological responses, meat yield and gut morphology of free-range chickens raised in a hot environment

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Tonderai Mutibvu

It is vital to minimise thermal stress and associated welfare problems for birds reared in hot environments and behaviour is a good indicator of thermal stress. The broad objective of the study was to investigate behavioural, physiological and gut morphological responses of free-range slow-growing chickens raised in a generally hot environement. A total of 488 Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) chickens were used in the study. The experiments conducted in this study explored effect of strain, sex, rearing system and provitamin A bio-fortification of maize on gut development in chickens in the context of thermal stress.

Two hundred and eighty-eight NN, OV and PK chickens were separated by sex and reared in either intensive or extensive rearing pens, with twelve birds from each strain per pen. Time budgets on free-ranging and related behavioural activities were determined at 3 different observation periods (0800 h, 1200 h and 1600 h) for 3 weeks. Body weight (BW), random environmental effects; ambient temperature (T_a) and relative humidity (RH) as well as bird stress indicators; rectal temperature (RT), heart rate (HR), breathing rate (BR), tonic immobility (TI), spleen and liver weights were determined for the free-ranging and confined flocks. On the last day of the trial, blood samples were collected from randomly selected birds via brachial venepuncture. Body weight (BW), carcass weight (CW), dressed weight (DW), portion and giblet yields were determined. Gut organs were recovered and weighed on a digital

scale within 10 min of slaughter. Intestinal length, weight, ileal villus parameters; villus height (VH), villus density (VD), villus width (VW) and muscularis externa (ME) thickness and apparent villus surface area (aVSA) were assessed. Ambient temperature (T_a) and RH were used to compute a temperature humidity index (THI) and data were subjected to ANOVA with strain, sex and rearing system as the main effects.

Time of day influenced ($P < 0.01$) free-ranging-related behaviours namely; foraging, drinking and preening. Females spent more time compared to males on the same activity and also appeared, generally, more stressed than males. Physiological responses of PK, OV and NN were generally comparable under similar rearing conditions and none of the factors studied had an effect ($P > 0.05$) on RT. Sex influenced ($P < 0.05$) VH, aVSA, VW and gizzard weight. Villi were taller, wider, hence greater aVSA in males than females on WM and PABM while ME thickness decreased ($P < 0.01$) between 18 and 21 weeks of age. Strain influenced ($P < 0.05$) VW, aVSA, ME thickness, intestine length, liver, gizzard, pancreas and heart weights. Sex of bird influenced ($P < 0.05$) carcass weight (CW), heart, proventriculus and abdominal fat pad (AFP) weight. The heart, liver and pancreas weights were significantly higher in OV than PK and NN chickens. Strain influenced ($P < 0.05$) BW, H/L ratio, spleen, relative liver weights, thigh, neck, pancreas, gizzard and crop weights but not TI ($P > 0.05$). Sex of bird affected ($P < 0.05$) BW, spleen, relative liver weights, H/L ratio, shank, drumstick and abdominal fat pad (AFP) and pancreas weight. Strain × sex interactions were observed ($P < 0.05$) on spleen and liver weights. There was negative correlation between time spent foraging and THI. Higher BW and heavier portions were obtained with OV than with NN and PK chickens.

Generally, males yielded heavier portions than females of the same strain. Free-range birds experienced crop and gizzard hypertrophy and pancreas atrophy. Free-range males yield

heavier cuts and females were fattier than males. It was concluded that rearing system, strain and sex of bird influence gut morphology, physiological responses, meat and fat yield in free-range slow-growing chickens. While free-ranging could minimise stress in birds, mechanisms should be devised to prevent predation in outdoor rearing of birds. Endo- and ecto-parasite infestation, behavioural studies using more elaborate techniques and evaluation of fatty acid profiles are possible areas of future research to help understand, hence improve bird welfare for slow-growing chickens in outdoor systems.

Key words: Behaviour; Chicken; Free-range; Strain; Sex; Temperature; Welfare.

Acknowledgements

I acknowledge the leading role, mentorhip, and dedication showed by Prof. M. Chimonyo in the conception, organisation and execution of the study. The success of this work is owed unto your guidance, advice, patience, expertise, experience and much more. Prof T. E. Halimani, the writing process was much easier with your assistance, thank you for the support. I am grateful to staff and students at the Animal and Poultry Science Discipline, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The following gentlemen set aside all their work to assist with constructing the poultry houses at Cedara College of Agriculture; Mthokozisi Kwazi Zuma (your energy and oneness are amazing, thank you), Cypril Ndumiso Ncobela, Luxolo (Runyararo) Qokweni, Zwelethu Mdletshe, Thando Conference Mpendulo, Vuyisa Andreas Hlatini, you are dearly appreciated, I know I owe you one and all. To Ms S. Ndlela, words fail me, from setting up office space to IDEX samples and much more. Brighton and Kudzai Chimonyo, thank you very much for all the support, Kudzi could not eat chicken, for a while, after witnessing and participating in the *exsanguination* of (innocent) chickens at the farm. If all mothers in the world could emulate Amai Chimonyo, no place would be too far from home. To the entire Chimonyo family for making this “our struggle together”. Titus J. Zindove, a comrade in the struggle, your guns were instrumental, somehow when I wielded them I shot not to miss. Dr V. E. Imbayarwo-Chikosi, thank you a million times for your patience, guidance and expertise. To Mabel, Shelton and Sekuru Manu; a simple reminder that someone is carrying a similar load lifts the burden off one’s shoulder. Dr D. T. Mbiriri, the ingenuity of your support is worthy the world’s carats put together. To Mr T. Chimwa, Mrs P. Chimwa and family, each trip from home was a visit to yet another warm home. To my sister-cum-mother, Mrs B. F. Tsunguro, you set me off on this path. Thank you always. My heartfelt gratitude also extends to Cedara College of Agriculture for facilities used during data collection, we are heavily indebted to Dr H. K. Swatson from Cedara College of Agriculture without whose support this study would not have succeeded. T-man was instrumental during the inception of the trial. To Feyisayo Odunitan-Wayas, thank you for the support and encouragement, you walked the long walk. Mr G. Ashley, Biological Sciences, University of Zimbabwe (UZ), Ms Musekiwa (Animal Science, UZ), it is a family affair! The list of individuals explicitly named here is by no means exhaustive, that some names do not appear here does not, in any way be-little nor render their contribution insignificant. To ALL of you I say, with a turgid yet sublime heart, you are gems only crafted for the glory of heaven and I pray that God richly bless you all.

In the beginning was the word, and the word was with God, and the word was God! And so it shall be in the end, and so it is to this end. When all else failed, when the funds were low, my faith, soul and will smitten and challenged, resolve questioned, you remained steadfast by my side…..to the Lord God Almighty I say, ebenezer and may your mighty name be glorified now and forever more.

Publications

1. Mutibvu T., Chimonyo M. and Halimani T. E. 2017. Effects of strain and sex on the behaviour of free-range slow-growing chickens raised in a hot environment. Journal of Applied Animal Research: DOI: 10.1080/09712119.2017.1287079.

2. Mutibvu T., Chimonyo M. and Halimani T. E. 2017. Tonic immobility, heterophil to lymphocyte ratio and organ weights in slow-growing chickens. Journal of Applied Poultry Research. DOI: 10.3382/japr/pfw066.

3. Mutibvu T., Chimonyo M. and Halimani T. E. Effect of strain and sex of bird on carcass and fat yield of slow-growing chickens. The Indian Journal of Animal Sciences. *In Press*

Dedication

To my wife Precious, daughters Lindsay N. and Louise and the Heir, Alex T. Jr.

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Chapter 1: General Introduction

1.1 Background

Poultry production is a vital activity in most developing countries contributing to poverty reduction and improving food and nutrition security. Common species kept by farmers include chickens, guinea fowls, turkey and pigeons. Indigenous chicken (*Gallus gallus domesticus*) strains are by far the most dominant, and arguably the most important in both communal and commercial establishments. In the communal setup, almost every rural household owns indigenous chickens (Sonaiya, 2004; Mtileni *et al*., 2013). These slow-growing chickens provide meat and eggs and a reliable source of income (Adomako *et al*., 2009). They are hardy and require low levels of input (Van Marle-Köster *et al*., 2008; Dyubele *et al*., 2010) being adaptable to a wide range of habitats and conditions. The chickens are thought to have originated from the red jungle fowl (RJF) of Southeast Asia (Lindqvist, 2008). Common examples in sub-Saharan Africa (SSA) include the Naked Neck, Ovambo, Potchefstroom Koeoek and Venda chickens. The Naked Neck and Ovambo strains are closely associated with rural livelihoods in Southern Africa where they are used to meet nutritional and economic needs of households (Mapiye *et al.,* 2008).

In sub-Saharan Africa (SSA), 70 % of the total chicken population is reared under the extensive system (Mapiye *et al*., 2007). Indigenous birds are preferred to exotic chickens, because of their pigmentation, taste, flavour and leanness (Moreda *et al*., 2013). The health and well-being of chickens depends upon the exogenous factors such as adequate nutrition, proper growth environment, reduced exposure to stress and appropriate management practices (Burgos *et al*., 2006). The large majority of chickens are raised in indoor production systems which differ dramatically from organic housing systems and especially outdoor runs and pastures

(Thamsborg and Roepstorff, 2003). The rearing system has serious implications on the welfare of birds.

Consumers have become increasingly conscious about the quality of meat products they choose, thereby increasing the demand for free-range and organic livestock production in recent years (Thamsborg and Roepstorff, 2003). Free-range chickens have a high potential to produce organic products. The shift in consumer preference is driven and argued from an animal welfare and consumer health awareness perspective (Sutherland *et al*., 2013). Free-range systems often entail allowing birds access to the outdoors, a change in housing conditions and abstaining from medical prevention in poultry production (Thamsborg and Roepstorff, 2003). Allowing outdoor access promotes the expression of normal behaviour, thus increasing bird comfort and welfare (Ponte *et al*., 2008; Wang *et al*., 2009). Several countries have crafted policies to regulate the rearing of poultry, with a view to increasing bird welfare. Brazil, countries in the European Union (EU), Canada and Australia, to name but a few, have come up with mechanisms to ensure humane poultry production and processing. In Brazil, policies on production through certification of bird quality were adopted in the year 1999 (Santos *et al*., 2005). In the EU, conventional cage systems for laying hens were banned in January 2012 (CEC, 1999). Certified products have a niche market and have recently continued to gain popularity.

Free-range chicken production systems, however, involve exposing chickens to various environmental stressors. Some of the most important stressors in outdoor systems are high ambient temperature and relative humidity. These, and other, meteorologic elements constitute a major variable for outdoor operations (Sossidou *et al*., 2011). They are inherently variable and change continuously (Lin *et al*., 2016; Ayo *et al*., 2011) and such cyclic exposure stresses chickens. Heat stress, as one of the major factors influencing feed intake and bird behaviour under free-range conditions, is likely to worsen given the effects of climate change. The global average surface temperature is anticipated to increase by between 1.88°C and 4.08°C in the next 60 years (Renaudeau *et al.,* 2011).

Slow-growing chickens are considered to be adaptable to harsh environmental conditions but their productivity still remains low. The contribution of environmental factors, interaction effects of the same with inherent bird traits on the observed productivity are not known. There is need to investigate the gut morphology, behavioural and physiological responses, tonic immobility (TI), heterophil to lymphocyte (H/L) ratio and organ weights as stress indicators in Naked Neck (NN), Ovambo (OV) and Potchefstroom Koeoek (PK) chickens raised under hot environmental conditions.

1.2 Justification

Village poultry are owned by almost all poor households in developing countries and are viewed as an excellent tool in poverty reduction due to their quick turnover and low capital requirements. Chickens present a potentially good entry point for developmental efforts aimed at improving food security among poor households. The international poultry meat market has taken a dramatic shift leading to an increase in the demand for free-range and organic products, hence opportunities for commercialization targeted at satisfying a niche market exist.

Slow-growing chicken strains are regarded to be adapted to adverse environmental and management conditions yet their productivity remains low. There is need to investigate the contribution of environmental factors and the interaction effects of the same with inherent bird traits on the observed productivity. An investigation on the performance of slow-growing chickens under outdoor conditions is, therefore, necessary. Such information help in the identification of appropriate strains for the emerging dominant rearing systems. To the best of our knowledge, there are few, if any, studies that have examined behaviour, physiology and gut morphology of free-range slow-growing chicken strains of Southern Africa.

1.3 Objectives

The broad objective of the study was to investigate the behavioural, physiological and gut morphological response parameters of slow-growing chicken strains reared in an outdoor high temperature environment. The specific objectives were to:

1. Determine the effect of strain, sex of bird and time of day on the behaviour of free-range NN, OV and PK chickens;
2. Assess physiological responses in NN, OV and PK chickens raised in a hot environment;
3. Determine the effect of provitamin A bio-fortified maize (PABM), rearing system, strain and sex of bird on gut and ileal villus morphology of NN, OV and PK chickens;
4. Determine the effect of strain, sex of bird and rearing system on the duration of tonic immobility (TI), heterophil to lymphocyte (H/L) ratio and organ weights of NN, OV and PK chickens;
5. Determine the effect of strain, sex of bird and rearing system on the meat and fat yield of NN, OV and PK chickens.

1.4 Hypotheses

The following hypotheses were tested;

1. Strain and sex of bird influence the behaviour of free-range NN, OV and PK chickens;
2. Strain, sex of bird and rearing system influence physiological responses of NN, OV and PK chickens;
3. Provitamin A bio-fortification, strain, sex and rearing system influence gut and ileal villus morphology in NN, OV and PK chickens;

4. Strain, sex and rearing system have an effect on duration of TI, heterophil to lymphocyte (H/L) ratio and organ weights of NN, OV and PK chickens;
5. Strain, sex and rearing system have an effect on the meat and fat yield of NN, OV and PK chickens.

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Chapter 2: Review of Literature

2.1 Introduction

Poultry production is a vital activity in most developing countries contributing to poverty reduction and improving food and nutrition security. Various species of poultry are kept and common species include chickens, guinea fowls, turkey and pigeons. Indigenous chicken (*Gallus gallus domesticus*) strains are the most dominant, and arguably the most important, poultry species in communal production systems. Almost every rural household owns indigenous chickens (Sonaiya, 2004; Mtileni *et al*., 2013). They are hardy, slow-growing and require low levels of input (Van Marle-Köster *et al*., 2008; Dyubele *et al*., 2010). The chickens make a significant contribution to household food security in the developing world (Besbes, 2009; Gondwe, 2004) providing a cheap, readily available source of high value protein (Jinga *et al*., 2012) in form of eggs and meat (Dyubele *et al.,* 2010). They have a direct impact on household nutrition and food security (Pedersen, 2002; Muchadeyi *et al*., 2004). Their low input (Abdelqader *et al.*, 2007) and space requirements suit the low intensity management found in rural households. Resource-limited households throughout Africa and Asia keep chickens to satisfy their protein requirements (Packard, 2014).

In sub-Saharan Africa (SSA), 70% of the total chicken population is reared under extensive systems (Mapiye *et al*., 2007). Slow-growing strains are preferred because of their pigmentation, taste, flavour and leanness (Moreda *et al*., 2013). *Gallus gallus domesticus* are thought to have originated from the red jungle fowl (RJF) of Southeast Asia (Lindqvist, 2008) and are adaptable to a wide range of habitats and conditions. The birds scavenge for most of their nutritional needs (Grobbelaar *et al*., 2010) and feed on a diverse feed resource base including leafy materials, insects, earthworms and kitchen waste (King'ori, 2004; Mwalusanya *et al*., 2002; Rashid *et al*., 2004), snails, slugs, leaves, flowers, sand and grits (Sonaiya, 2004; Goromela *et al*., 2007), berries and foliage (Sonaiya, 2004).

Chickens produced in extensive production systems have a high potential to produce organic products. The demand for organically-produced meat and eggs has increased recently (Thamsborg and Roepstorff, 2003). The shift in consumer preference is driven and argued along animal welfare and consumer health awareness dimensions. Organic and free-range rearing entail allowing access to the outdoors, a change in poultry housing conditions and abstinence from medical disease prevention (Thamsborg and Roepstorff, 2003). There is scope in exploiting the ability of local strains to adapt and survive under a wide range of challenging environmental and ecological conditions (Packard, 2014) in view of the increasing demand for free-range products across the world. This review assesses slow-growing chicken production, in view of the increasing popularity of organic meat and eggs, in the context of harsh environmental conditions prevalent in most parts of SSA.

2.2 Chicken production systems

Slow-growing chickens are kept under a wide range of conditions, which can be classified into one of four broad production systems; intensive, semi-intensive, foraging and extensive production systems.

2.2.1 Intensive systems

Intensive systems are used by medium to large-scale commercial enterprises, and are also used at the household level. Under such systems, birds are fully confined in houses or cages. Capital outlay is higher and the birds are totally dependent on their owners for all their requirements.

Productivity under such conditions is fairly high. Three deep-litter, slatted floor and battery cage systems are the common types of intensive systems for chicken production.

2.2.1.1 Deep litter system

The birds are fully confined with floor space allowance of 3 to 4 birds/m^2 within a house, but can move around freely. The floor is covered with a 5 to 10 cm deep layer of litter. Materials that can be used as litter include grain husks (maize or rice), straw, wood shavings among other absorbent and non-toxic materials. The fully enclosed system offers protection from thieves and predators. It is generally suitable for specially-selected commercial breeds of egg or meat producing poultry such as layers, breeder flocks and broilers. The deep litter system has not been widely explored with slow-growing strains.

2.2.1.2 Slatted floor system

Wire or wooden slatted floors are used instead of deep litter, which allows stocking rates to be increased to 5 birds/m^2 of floor space. Birds have reduced contact with soil and faeces and are allowed some freedom of movement.

2.2.1.3 Battery cage system

The battery cage system is usually used for laying birds, which are kept throughout their productive life in cages. Initial capital outlay is high as such the system has mostly been confined to large-scale commercial egg layer operations. Intensive systems of rearing slow-growing chickens commercially are uncommon. The battery cage system has become unpopular for animal welfare reasons and has been abolished in some parts of the world. Cage systems for laying hens were banned in the European Union as from January 2012 according to an EU Council Directive1999/74/EC on the welfare of laying hens (CEC, 1999).

2.2.2 Semi-intensive systems

These are a combination of the extensive and intensive systems where birds are confined to a certain area with access to shelter. They are quite common and can be found in urban and peri-urban as well as rural situations. The birds are confined in an enclosed area outside during the day and housed at night. Feed and water are available in the house to avoid spoilage and wastage by rain, wind and wild animals.

2.2.3 Foraging chicken production systems

There is limited data on the foraging behaviour of free-range chickens (Miao *et al*., 2005). Birds forage mainly within 30 to 40 m of a shelter. Birds would forage further out into the paddock only when attendants were present. Further research on foraging behaviour of free-range chickens is required as the outcomes of this type of research will assist free-range poultry producers to develop management strategies to improve foraging ability of chickens (Miao *et al*., 2005).

2.2.4 Extensive production systems

The village poultry production system, commonly known as traditional free-range system, is the most important poultry production system in rural communities of most developing countries in Africa and Asia (Goromela *et al*., 2008). In Africa, indigenous chicken production falls under either one of two traditional poultry production systems. Chickens are reared in small numbers in traditional extensive or semi-intensive low input-low output systems (Sonaiya, 2004). The birds scavenge for feed (Grobbelaar *et al*., 2010) during the day and may be confined at night (King'ori *et al*., 2010). Under the extensive production system, chickens have access to a wide range of feed materials including green grass, leafy materials, seeds and various grains, crawling and flying insects, earthworms, and for some strains, even small rodents (Grobbelaar *et al*., 2010). Feed quality and quantity, thus, varies with season. It is common that little or no shelter is provided (Grobbelaar *et al*., 2010) which exposes birds to predators and adverse environmental conditions. The birds sleep in trees to prevent predation. Where housing is provided, it is simple and rudimentary (King'ori *et al*., 2010). In spite of the challenges, the extensive production system is a suitable activity for rural women, youth and the marginalized farmers who derive income and food from these birds (King'ori *et al*., 2010). There is need to develop technologies to improve the productivity of slow-growing chickens especially in view of the damaging effects of climate change. Effects of variability in climate, escalating inconsistences in the seasonality of rainfall and other climate-related uncertainties, need to be thoroughly examined so that efforts to increase productivity are preceded by a thorough and systematic inventory. Physiological responses as stress indices and the interaction of the inherent traits of slow-growing birds with the environment, in particular, effects of thermal stress, need to be determined.

Exposing birds to environmental factors may negatively influence their health and welfare. One of the major challenges facing free-range chicken production is predation. To protect free-range flocks from nocturnal predators, birds must be secured indoors at night (The Human Society for the United States, 2010). A well-insulated house should protect birds from extreme weather conditions. Perimeter fences can be dug deep in the ground to prevent predators from digging underneath, and an overhang at the top to prevent animals from climbing over the fence (Thear, 2002).

Free-range scavenging chickens are in direct contact with and can contract diseases from parasite vectors, soil, faeces (Abdelqader *et al*., 2008) and wild birds (Lervik *et al*., 2007). Layer birds raised in a free-range system had a higher incidence of helminths than birds raised in cages (Lay *et al*., 2011). Many of the infectious diseases of layers are a result of contact with soil, litter and fomites (e.g., rodents, beetles and equipment) known to carry agents of those diseases (Lay *et al*., 2011). Low nutrient consumption, poor management, inclement weather, and the absence of biosecurity are among the key challenges.

Despite these ills, outdoor production systems, without any confinement on birds, could decrease stress and allow selection of strains that may increase comfort and bird welfare (Wang *et al*., 2009). Free-ranging offers the freedom for chickens to exercise in the paddock, which might reduce leg weakness problems and improve the development of the bone (Miao *et al*., 2005). It also allows the expression of normal behaviour including foraging, dustbathing and also flying.

The chicken production systems frequently overlap. For example, it is common practice for the free-range to be coupled with feed supplementation, backyard with night confinement but without feeding, and poultry cages in confined spaces.

2.3 Slow-growing chicken strains

Indigenous chickens are invaluable reservoirs of genes for adaptive and economic traits that provide a diversified genetic pool (Muchadeyi *et al*., 2007). Six major categories are recognized namely; normal feathered, Naked Neck, frizzle, silky, dwarf and the feathered feet (Mtileni *et al*., 2012). Most indigenous chicken populations arise from the uncontrolled crossbreeding of various local and imported strains. As a result, the chickens are viewed as indescript strains. Most of them, therefore, have no identified description and are generally unimproved (Pedersen, 2002).

Common strains of chickens in SSA, include the Naked Neck, Ovambo, Potchefstroom Koekoek and Venda chickens (Grobbelaar *et al*., 2010; Packard, 2014; Nthimo *et al*., 2004). Less popular strains, particularly in South Africa, are the Nguni, Natal Game and the Zulu (Grobbelaar *et al*., 2010). These birds show small genetic distances to justify population substructuring (Muchadeyi *et al*., 2007). Slow-growing strains are inseparable from the rural scenario due to their adaptability under harsh environmental conditions (Miao *et al*., 2005). Adaptability of indigenous chickens in the tropical environment has been through reduction in body sizes as a means of reducing maintenance feed requirement and increasing feed efficiency (Rashid *et al*., 2005). Their small body sizes reduce maintenance feed requirements and make survival on a low plane of nutrition possible. Apart from their low egg production potential and growth rates, there is a good market for both meat and eggs from slow-growing strains in both the European Union (EU) and Asia (Miao *et al*., 2005) presenting opportunities for commercialisation and export.

2.3.1 Naked Neck

The Naked neck (NN) chickens are thought to originate from Malaysia from where they spread to all parts of the world. The chickens are a widely distributed, multi-coloured, relatively light-weight strain kept for meat and eggs for household consumption. White, red and black feather combinations are common and the strain is adapted to hot environments. The NN is an adaptable strain and can be found in all diverse climates of Southern Africa. They have a more efficient heat dissipation mechanism due to the absence of feathers in the neck region resulting from reduced plumage cover which is an advantage when temperatures are high and birds have to dissipate excess heat (Deeb and Cahaner, 2001). The reduced feather cover aids in thermoregulation at high ambient temperature (Eberhart and Washburn, 1993) by increasing sensible heat loss. The Naked Neck gene (*Na*) is an autosomal, incompletely dominant gene

(Raju *et al*., 2004) which causes bare skin on the neck which becomes reddish towards sexual maturity. The *Na* allele is associated with increased tolerance to heat, which is probably due to the 30 % reduction in overall plumage for heterozygotes and 40 % for homozygotes (Raju *et al*., 2004; Rajkumar *et al*., 2010, Fathi *et al*., 2013).

Naked Neck chickens reach sexual maturity at 155 d of age, with males weighing about 1.95 kg and females 1.4 kg (Chikumba and Chimonyo, 2014). Naked Neck chickens possess better post weaning (>12 weeks of age) heat tolerance than OV and PK chickens owing to the presence of the major gene that causes reduced plumage cover (Cahaner *et al*., 1993; Fathi *et al*., 2013).

2.3.2 Ovambo (OV)

The OV are predominantly dark-coloured birds that are thought to have originated in the Ovamboland rural areas of Namibia (van Marle-Koster and Nel, 2000). The birds are capable of flying and roosting in trees to avoid predators (Grobbelaar *et al*., 2010; Nthimo, 2004). They are quite aggressive and can kill mice and small rats (Grobbelaar *et al*., 2010; Nthimo, 2004). They attain sexual maturity at average weights of 2.16 kg for males and 1.54 kg for females at 20 weeks of age (Nthimo *et al.*, 2004). Ovambo chickens are a dual-purpose strain (van Marle-Koster and Nel, 2000) that can be used for both egg and meat production.

2.3.3 Potchefstroom Koekoek

Named in relation to its colour pattern, this strain is a composite dual purpose strain that was developed in the 1950s'. It is characterised by a barred colour pattern that is similar between males and females though the females tend to be darker than the males. The feature is, thus, used for colour sexing (Grobbelaar *et al*., 2010). The strain was developed by the crossing of

Black Australorp cockerels with White Leghorn hens and the Plymouth Rock (Grobbelaar *et al*., 2010; Packard, 2014). The PK is popular among rural farmers in SSA for egg and meat production as well as their ability to hatch their own offspring (Grobbelaar, 2008). The meat of this strain is still popular among local communities and is preferred to that of commercial broiler hybrids (Grobbelaar *et al*., 2010).

2.3.4 Venda

The strain was first identified in Venda, Limpompo Province (Grobbelaar *et al*., 2010) and later discovered in other parts of South Africa. Venda chickens reach sexual maturity at the age of 143 days with an average body weight of 2.1 kg in males and 1.4 kg in females at 20 weeks old. The average egg weight for the strain is 52.7 g. These chickens have white and black or white and brown plumage with shades of dark green on the feather tips (Joubert, 1996). They are characterized by lower egg production, instinct to broodiness and adaptability for household production. A description of some common local strains of chickens is shown in Table 2.1.

Table 2.1. Description of the some of slow-growing chicken strains common to Southern Africa

Strain	Origin	Phenotypic appearance	Average weight (kg)		Source
			Male	Female	
Naked Neck	Introduced to Africa by traders from Malaysia	Very colourful, Naked Neck major gene	2.0	1.6	Van Marle Köster & Casey (2001)
Ovambo	Namibia, Ovambo land	Brown & black plumage, aggressive birds	2.2	1.9	Van Marle Köster & Casey (2001)
Potchefstroom Koekoek	Cross between Black Australorp and White Leghorn	Black & white speckles	3 - 4	2.1	Viljoen (1986); Van Marle Köster & Casey (2001)
Venda	First official recording in 1979 in Venda, Northern Province, SA	White & black /white & brown plumage, green on feather tips	2.0	1.9	Van Marle Köster & Casey (2001)

Source: *Van Marle Köster et al. (2008)*

2.4 Role and functions of indigenous chickens

In addition to providing meat and eggs to rural communities, indigenous chickens are a source of income (Abdelqader *et al*., 2008; Dana *et al*., 2010; Mtileni *et al*., 2012). They can be sold or bartered to meet household needs such as medical costs, school fees and village taxes thus they act as a ready source of cash for sustaining livelihoods. The birds are also used in various cultural/ religious applications (Dana *et al*., 2010). Their droppings are a rich source of manure (Jinga *et al*., 2012; Mungube *et al*., 2008; Mapiye *et al*., 2008). In addition to its use in improving soil fertility, hence crop production, manure can also be sold to earn income thus further diversifying sources of income. Chicken manure is a high value organic fertilizer owing to its rich nitrogen content.

Chickens are easily disposable hence act as a form of savings and insurance for households. The chickens occupy a special position in various other miscellaneous socio-economic roles in traditional, religious and other customs. Such roles include gift payments (McAinsh *et al*., 2004) to strengthen social relationships. The management of scavenging poultry is generally practised by women thus the chickens have social, cultural and symbolic roles that transcend their practical use as food or commodities (Chikumba and Chimonyo, 2014). This contributes to women's empowerment. It is the opinion of most researchers and of the leadership of poultry development networks and associations that, if properly managed, village poultry offer a viable means of alleviating poverty, generating income, achieving food security and empowering women in the poor rural regions of developing countries (Pym *et al*., 2006). The fowl have also provided a way of converting available feedstuffs around the rural setting into highly nutritious and well appreciated meat and eggs (Mtileni *et al*., 2011).

2.5 Trends in organic farming

Organic and free-range livestock production have increased dramatically, in recent years, in Europe and other parts of the world (Thamsborg and Roepstorff, 2003) owing to increased international demand for organic products (Hughner *et al.*, 2007). The increased demand suggests that the production of food and fibre with minimal or no chemical input is a desirable characteristic of organic farming systems (Sutherland *et al*., 2013). The resurgence in the interest in free-range poultry farming in developed countries is a result of welfare concerns associated with rearing of poultry under intensive conditions (Miao *et al*., 2005). Within the organic market, livestock products like milk, beef and eggs were amongst the five highest ranking products in 14 of 18 European countries (Michelsen *et al*., 1999). Although intensive indoor poultry management systems still exist, there has been a paradigm shift in the poultry industry across the world. Defined by Fanatico *et al*. (2007) as alternative production, free-range cage-free systems are a way of boosting farm income and adding fertility or diversity to a farm. This, in conjunction with a growing awareness on human health and nutritional concerns, has led to the development of specialty markets targeting poultry produced in alternative production systems such as free-range or organic systems (Fanatico *et al*., 2007).

There are several other facets to the popularity of organic and free-range products on the world market today. One is premised on the increasing consumer awareness on health and the need to control cholesterol intake. Consumers have the perception that free-range products are healthy and wholesome, low in calories and saturated fats, high in protein and vitamins and are, therefore, willing to pay a premium (Miao *et al*., 2005). On the other hand, free-range or organic systems allow birds access to an outside area promoting foraging, feed selection, and activity, thus theoretically improving the welfare of the birds (Ponte *et al*., 2008) compared to conventional systems which do not permit the expression of normal behaviour. These, in addition to other, views have led to extensive chicken rearing becoming increasingly popular. While extensive rearing systems are predominant in developing countries, more by default than otherwise, the systems are well developed with meat and other products of such production systems getting certified in the developed world.

In Brazil, policies concerning the criteria for production, supply, processing, distribution and certification of bird quality (DOI/DIPOA 007/99 of 05/19/1999) were enacted in 1999 (Santos *et al*., 2005). Conventional cage systems for laying hens were banned in the European Union since January 2012 in accordance with an EU Council Directive1999/74/EC on the welfare of laying hens (CEC, 1999). Furthermore, the organic market has grown by 20 % annually in the United States alone for the past decade (ERS, 2002) while in China, more than 30 % annual growth has been realised in the past five years (Jin, 2008). Free-range systems make use of slow-growing strains which are more adapted to these production systems (Castellini *et al*., 2002; Gordon and Charles, 2002). Outdoor production systems, without any confinement on birds, could decrease stress and allow selection of strains that may increase comfort and bird welfare (Wang *et al*., 2009).

2.6 Factors influencing productivity of free-range chickens

Slow-growing chicken productivity is influenced by several factors including strain of bird, feed and water quality, predation, diseases and environmental stressors such as extreme temperatures and humidity.

2.6.1 Strain and breeding for improved productivity

Village chicken strains are generally not genetically capable of high growth rates and feed conversion efficiencies. Attempts have been made to upgrade scavenging birds by the introduction of males of an imported high egg producing strain (Cumming, 1992). Literature suggest that crossbred pullets always showed a significant increase in egg size and production if evaluated in laying cages though in deep litter, this did not happen as the chickens would go broody after about a dozen of eggs (Cumming, 1992; Pym *et al*., 2006). The low rate of lay in slow-growing strains is also explained by the requirement for the hens to go broody after they have laid a clutch of eggs so as to hatch the eggs and rear the chicks (Pym *et al*., 2006). Strain-upgrading interventions have met with a lot of challenges in as far as implementation is concerned including failure of the high grade cockerels to cope with the harsh new environmental conditions. Though rather too bold, Cumming (1992) reported that no upgrading scheme of village or scavenging chickens has succeeded anywhere in the world.

2.6.2 Ambient temperature

The climatic environment is among the major factors limiting production efficiency (Renaudeau *et al*., 2011) and heat stress is one of the most important environmental stressors particularly in hot regions of the world (Altan *et al*., 2003). The challenges of raising slow-growing chickens are likely to be aggravated by the escalating uncertainties due to climate change. Climate change is expected to negatively impact free-ranging chicken production through altering the quantity and quality of feed, heat stress and changes in water availability (Thornton and Herrero, 2008). During hot weather, feed intake increases body temperature and chickens respond by reducing feed intake. The reduction in nutrient consumption compromises weight gain and egg production potential. The global average surface temperature is anticipated to increase by between 1.88°C and 4.08°C in the next 60 years (Renaudeau *et al*., 2011).

Under intensive management, birds are housed in temperature-controlled housing which prevents stress caused by cycling temperatures (Miao *et al., 2005*). Controlling room temperature enhances the achievement of optimum performance. Under free-range conditions, birds may be exposed to extremely high or low temperatures (Miao *et al., 2005*). This not only influences the performance of birds but also welfare. In winter, free-range chickens might need more protection from cold weather. Given the diversity of free-range systems, provisions can be made for free-range birds to behaviourally thermoregulate by remaining inside the poultry house to reduce heat losses during cold weather conditions.

2.6.3 Body temperature

The normal body temperature of an adult chicken is 40.6 to 41.7°C while the thermo-neutral zone (TNZ) ranges from 18 to 24°C (Fanatico *et al*., 2007). Temperatures above the TNZ trigger mechanisms of heat dissipation. Ambient temperature and diet influence the acid-base balance. Although birds are equipped to regulate body fluid pH during metabolism (Borges *et al*., 2004), chickens have no sweat glands for efficient external body temperature regulation. Strain differences in response to heat stress have been reported before and adaptation requires the functional integration of the endocrine, cardiorespiratory, digestive, excretory and immune systems (Altan *et al*., 2003).

High body temperatures can adversely affect the anatomy and physiology of the cells, impair protein synthesis, oxidative metabolism, membrane structure and function (Mager and de Kruijff, 1995; Iwagami, 1996). During long-term heat exposure, the effect of heat treatment may be attenuated by acclimation (Lin *et al*., 2005) or habituation, suggesting that chicken strains can adapt to high temperatures albeit to varying degrees. Habituation is the reduction in physiological responses elicited by exposure to a repeated stressor. It is important, therefore, to investigate the extent to which different strains and even sexes can cope with heat stress.

2.6.4 Humidity

High humidity is often always accompanied with high ambient temperature. The challenge of high humidity to thermoregulation and welfare of chickens is important (Lin *et al*., 2005) as it has a remarkable influence on the ability of chickens to efficiently thermoregulate. At high temperature, heat production decreases while heat dissipation increases (Lin *et al*., 2005). The amount of evaporative heat loss depends on air humidity and is suppressed when humidity is high. The effects of humidity on thermoregulation and the performance of chickens, however, depend on the ambient temperature (Lin *et al*., 2005). Non-evaporative heat loss takes place at the surface of bare skin and also the plumage. Birds thermoregulate by behavioural changes exposing a larger body surface area to encourage heat loss and body temperature is elevated (Warriss *et al*., 2005) and humid conditions reduce the effectiveness of these mechanisms (Warriss *et al*., 2005).

2.6.5 Nutrition

Under natural conditions, a chicken's diet is a very mixed one, comprising seeds, fruits, herbage and invertebrates that is indigenous chickens largely rely on scavenging for their nutrient needs. Birds browse on herbage and forages by scratching at the ground exposing small food items (Miao *et al*., 2005). Under free-range conditions, birds may be able to compose a diet that is adequate for all their requirements (Hughes, 1984) depending on the resource endowment of the free-range environs. Feed resources vary depending on local conditions (Mtileni *et al*., 2011). In addition, there is seasonal variation that occurs in feed quality and quantity and this has a direct influence on growth performance, egg production and flock health. Local slow-growing strains have lower dietary protein requirements than imported birds. Imported strains generally have higher body weights and egg production potential (King'ori *et al*., 2010). Chickens scavenge for feed and water for an average of 11 h per day between 0500 and 1800 h (Maphosa *et al*., 2004). Farmers provide some form of feed supplement for their flocks (Okeno *et al*., 2012; Nyoni and Masika, 2012; Dana *et al*., 2010; Hailemariam *et al*., 2010).

Where dietary supplementation is done during the dry season (Mapiye *et al*., 2008), supplements are indiscriminately rather than preferentially fed. Different classes of chickens, including chicks, therefore, compete for the supplement (Maphosa *et al*., 2004; Muchadeyi *et al*., 2004). The communal provision of dietary supplements is likely to result in a mismatch between absolute nutrient requirement and supply resulting in older birds being overfed at the expense of the chicks. Feeding strategies, thus, need to be developed by matching feed supply with nutrient requirement.

2.7 Estimating nutrient requirements of free-range chickens

Unlike the requirements of foraging birds, the nutrient requirements of intensively managed birds have been extensively investigated and documented. The foraging activity and variable environmental conditions of free-range poultry makes it difficult to apply the nutritional management guidelines recommended for intensively managed birds (Miao *et al*., 2005). The requirement under free-range management is higher than in intensive management conditions. Theoretically, the amount of feed offered to foraging birds must be the amount of feed required minus intake from foraging (Miao *et al*., 2005). The protein requirement of free-range chickens in the tropics has been estimated at 11g/bird/day.

Crop content analyses has been used to characterise the diet composition for free-range birds. Foraging birds consume worms, insects, seeds, leaves and other unidentified materials. Owing to seasonality, the forage base for free-range birds is, thus, highly variable. For example, the proportions of seeds, worms and insects are higher during the rainy season. While an understanding of the seasonal forage intake of free-range birds is essential for developing supplementary feeding regimes, it remains difficult to measure the intake of foraging birds due to the lack of an appropriate methods (Miao *et al*., 2005). The visual appraisal of crop contents provides guidelines on diet composition but cannot further quantify the pasture species ingested by foraging birds (Miao *et al*., 2005).

Another approach has been to assess the contribution of the scavenging feed resource base (SFRB) in addition to crop content analyses (Rashid *et al*., 2004; Sonaiya, 2004; Momoh *et al*., 2010). Even then, the amount of scavengeable feed resources consumed by chickens is affected by factors that include locality, season and farming system (Goromela *et al*., 2008), all of which present omplications.

2.8 Gut morphology

Diet composition affects gastrointestinal tract development in chickens. It has been demonstrated that insoluble fiber is advantageous to gastrointestinal tract development (Jimenez-Moreno *et al*., 2009). In addition, lowering the crude protein (CP) or crude fat (EE) content of poultry diets affects, not only the growth rate and reproduction, but also the ontogeny and morphology of the gastrointestinal tract (Incharoen *et al*. 2010). Intestinal villi and absorptive epithelial cells play significant roles in the final phase of nutrient digestion and assimilation (Incharoen *et al*., 2010). Awad and co-workers (2008) demonstrated that an improvement in gut morphology in chickens is accompanied by increased digestive and absorptive function of the intestines due to increased absorptive surface area, expression of brush border enzymes and nutrient transport systems. Effort should, thus, be directed to investigating fine gut morphology to gain better insights on the dynamics of nutrient uptake and subsequent utilization. In view of the fluctuation in both quality and quantity of feed resources available for foraging chickens, there is a need to investigate the effects of changes in nutrient availability on GIT development in free-range slow-growing chickens. It is vital to pay attention to the minute changes that occur in the gut, which are often overlooked because the damage is subtle and usually characterized by microscopic changes in gut mucosal architecture (Choct, 2009).

2.9 Water

Water is essential for chicken production, especially during hot ambient temperature (Dai *et al*., 2009) conditions. Farmers generally pay little attention to the water requirements of their fowl. In most cases, chickens acquire water from succulent feed resources during scavenging. Occasionally, they may obtain water around the homestead as remains after doing dishes and related household chores. Stagnant, transient water pools that form following rainfall also provide water for chickens, albeit momentarily. As a result, water deficiency persist for most parts of the year.

Birds are exposed to unhealthy conditions that may lead to heavy worm burdens. Furthermore, climatic conditions that prevail for most of the year are hot and dry and coincide with peak periods of water and feed scarcity (Chikumba *et al*., 2013). Owing to limited water and succulent foraging resources, birds are, therefore, prone to dehydration and this negatively impacts feed intake, immune response and overall performance. This is only likely to worsen given the trends in various weather phenomena, hence the need for farmers to make efforts to ensure adequate clean water availability for their flocks.

Almost 80 % of indigenous chicken producers in Southern Africa live in fragile and marginal environments where there is lack of adequate potable water for human and livestock consumption (Swatson, 2003). The situation is worsened in the hot dry periods of the year when availability of water and succulent scavenging resources is low (Chikumba and Chimonyo, 2014). Most free-range producers in rural Africa do not provide water to recently weaned chickens. They often subsist on unpalatable, detergent tainted waste-water from bathrooms and kitchens (Mwale and Masika, 2011; Chikumba and Chimonyo, 2014). There are views, however, to the effect that some slow-growing strains demonstrate a high degree of tolerance to water scarcity and can survive on low water intake. Lower water intake was observed in NN chickens on *ad libitum* water intake in comparison to OV chickens (Chikumba and Chimonyo, 2014). This was attributed to either lower water requirements, a greater dependence on metabolic water to maintain hydrational homeostasis or a greater capability to economically budget body water in the former. Birds also reduce their feed intake so as to preserve body water by reducing faecal water loss and body heat increment (Chikumba and Chimonyo, 2014). Needless to say, however, that water consumption by birds is a function of numerous other factors.

2.10 Parasites

Parasites are a major health problem for organic poultry producers (Permin *et al*., 1999). Free-range scavenging chickens are in direct contact with parasite vectors, soil and faeces (Abdelqader *et al*., 2008) coupled with unhygienic physical environmental components that provide a conducive environment for thriving endo- and ecto-parasite populations. The high susceptibility to parasites, coupled with poor biosecurity measures, compounds the problem of poor flock health translating to reduced vitality. Free-range chickens are exposed to both ecto- and endo-parasites and GIT worms cause poor feed conversion and utilization (Jinga *et al*., 2012). The closer contact between faeces, parasites and hosts may increase the incidence of existing infections, and potentially result in emergence or re-emergence of new parasitic infections (Thamsborg and Roepstorff, 2003).

Though the impact of parasitic diseases in farm birds reared on cage systems has diminished due to modernization and improved biosecurity, farm birds maintained on deep litter and free-range systems remain susceptible to parasitic infections via droppings and scavenging habits (Puttalakshmamma *et al*., 2008). Inadequate nutrition and improper management compels chickens to scavenge for feed in contaminated environments, which predisposes them to arthropod-borne helminth infections (Mukaratirwa *et al*., 2001). Parasitism, thus, ranks high among factors that negatively impact village chicken production. Of the diseases that reduce productivity of free-range poultry, parasitic diseases come first (Ashenaf and Eshetu, 2004). Intensification of organic systems has to be balanced by an increase in awareness of the risks involved accompanied by measures to reduce parasitic infections and the possibility of subsequent transmission of diseases to humans.

There are practices that can be incorporated into free-range or organic systems to reduce parasitic infections. Pasture rotation is one way to prevent the building up of coccidia and helminth infections in the outdoor environment. This requires adherence to good management practices like cleaning, disinfection, avoiding water spillage and prompt medication, if needed. Future internal parasite control options may include selection of appropriate strains and perhaps breeding for parasite resistance and tolerance.

2.10.1 Gastrointestinal parasites

The most common gut worms in scavenging chickens include cestodes, nematodes and trematodes. Nematodes constitute the most important group of helminth parasites of poultry both in number of species and the extent of damage they cause. The main genera include *Capillaria, Heterakis*, and *Ascaridia* (Jordan and Pattison, 1996).

Most foraging chicken production systems allow for intimate interaction of birds and their faecal material. This leads to high prevalence rates of parasites in free-range chickens. In few operations, producers use elevated slatted floors that allow manure to drop through as a method of separating birds from their waste (thus breaking the life-cycle of parasites) and consequently reducing contact.

A high prevalence of gastrointestinal helminth infections was reported in free-range, organic and backyard systems, especially the incidence of *Heterakis gallinariu, Ascaridia galli*, *Capillaria obsignata* and *Capillaria anatis* (Permin *et al*., 1999). A higher occurrence of parasitic diseases was observed in laying hens reared in free-range compared with cage systems (Fossum *et al*., 2009). Coccidiosis caused by *Eimeria* spp. is probably the most important parasitic poultry disease in most production systems but poultry reared on litter with outdoor access is particularly at risk (Thamsborg and Roepstorff, 2003). A study of organic egg layers revealed high prevalences of infection with the nematode species *Ascaridia galli*, *Heterakis gallinarum* (vector of *Histomonas meleagridis*) and *Capillaria* spp. (Permin *et al*., 1999). In Zambia, helminth prevalence of 95 % has been reported (Phiri *et al*., 2007) while in South East Nigeria, 41 % and 71 % were reported for ecto-parasites and endo-parasites, respectively. In Northern Nigeria, a helminth prevalence of about 70 % was reported (Yoriyo *et al.,* 2008) while Komba and co-workers (2013) reported a prevalence rate of 87 % in Tanzania. Mwale and

Masika (2011) reported a 99 % prevalence in Centane District of the Eastern Cape Province of South Africa while 93.3 % helminth prevalence was reported in Kenya (Mungube *et al*., 2008). Table 2.2 shows some of the most prevalent internal parasites of chickens in selected African countries.

Table 2.2. Prevalence (%) of helminths in selected African countries

Helminth species	Komba *et al*. (2013)		Mukaratirwa and Khumalo (2010)		Mwale and Masika (2010)		Mungube *et al*. (2008)
Cestodes							
Raillietina tetragona	31	34.2	16.7	5.7*	-	-	-
Raillietina echinobothrida	34.5	38.2	0	*	-	-	33.3
Choanotaenia infundibulum	1.2	1.3	0	-	0	1.43	6.9
Raillietina celticullus	2.4	2.6	-	*	1.43	1.43	-
Hymenolepsis cantaniana	1.2	1.3	-	-			-
Davainea proglottina	-	-	-	3.45	0	1.43	19.4
Amoebataenia spp	-	-	-	1.15	1.43	0	8.3
Suburula brumpti	-	-	-	2.3	0	1.43	-
Nematodes							
Ascaridia galli	9.5	10.5	22.2	17.2	14.28	31.43	33.3
Heterakis isolonche	3.6	3.9	-	-	-	-	
Capillaria spp	2.4	2.6	-	5.7	22.86	28.57	5.6
Heterakis gallinarium	4.8	5.2	94.4	12.6	25.72	27.14	22.8
Syngamus treachea	-	-	-	4.6	1.43	0	5.6
Tetrameres americana	-	-	66.7	-	-	-	37.7
Gongylonema ingluvicola	-	-	72.2	-	-	-	5.3
Acuaria hamulosa	-	-	27.8	-	-	-	-
Dispharynx nasuta			11.1	-	-	-	-
Trematodes							
Postharmostomum gallum	-	-	-	-	*2.86*	*35.71*	
Postharmostomum communtatum	-	-	-	-	*18.57*	*2.86*	

* *The prevalence is for Raillietina spp. in general; Prev Prevalence n Sample size*

2.10.2 Ectoparasites of chickens

External parasites are widely distributed in free-range chickens. Most ecto-parasites of birds are blood-suckers. Only the *Ischnocera* lice and some species of mites subsist on skin components. Ecto-parasites such as fleas, lice and mites cause anaemia and, depending on the degree of infestation, may lead to egg abandonment in brooding hens. The distribution of ecto-parasites on the host varies with the parasite concerned. Table 2.3 shows the prevalence for some common ectoparasites.

Table 2.2. Common ectoparasites of chickens

Ecto-parasite name	Common name	Site	Prevalence (%)
Echidnophaga gallinacean	Flea	Comb, wattle, eyes, ears	76.7
Dermanyssus gallinae	Red poultry mite	Entire body	60
Menacanthus stramineus	Poultry body louse	Skin of thigh, breast & areas near cloaca	71.4
Knemidocoptes mutans	Scaly leg mite	Lower limbs	13.3
Argas persicus	Fowl tick/ fowl tampan	Cracks of houses	11.1

Source: Mungube *et al*. (2008)

2.11 Predators

Free-range birds are under the risk of predation from foxes, wild cats, eagles and hawks (Miao *et al.,* 2005). It is not clear whether this should be a welfare issue for free-range poultry since birds are subject to similar risk even under natural conditions. What is clear, however, is that the mere presence of predators induces fear in birds (Faure *et al*., 2003; Campo *et al*., 2008).

2.12 The behaviour and welfare of birds

The welfare of an animal is defined as its state as regards its attempts to cope with its environment (Broom, 1986) including the extent of failure to cope, which may lead to disease and injury. It also involves ease or difficulty of coping and the possible associated disease and injury (Europen Commission (EC, 2000). Some animals adapt better to different environments and adapting is vital for survival. Adapting means the animal is in an environment that allows it to perform adequate reactions and to get sufficient feedback from its behaviour. If such adaptation is prevented or compromised, welfare will be poor. Deviations from the behaviour, which is normal for the species, age and sex considered may therefore be important signs of welfare risks (EC, 2000). Significant efforts have been made to establish welfare indices to give indications of the extent of the effects of stressors on a bird's welfare (EC, 2000) ranging from simple indicators like respiratory rate and rectal or cloacal temperature to fairly elaborate phenomena like tonic immobility, changes in enzyme concentrations and blood osmolarity.

2.13 Assessing stress in birds

There are various forms of stressors that impact free-range chickens from climatic, environmental, nutritional, physical, to social or physiological factors. Public concern regarding farm animal well-being has skyrocketed in the past few years (Quintero-Filho *et al*., 2010). Many countries presently have laws and welfare codes meant to protect farm animals, including poultry, from distress and fear (Main *et al*., 2009; Bonafos *et al*., 2010). Stress in birds, as well as other animals, invokes changes often involving a cascade of physiological adaptive responses (Thaxton and Puvadolpirod, 2000a). Parga *et al*. (2001) investigated the effect of transporting hawks and falcons on haematological parameters. They noted that transport is usually associated with other stressors like; catching, handling, loading, motion, acceleration, impact, withdrawal of water, fasting, restriction of behaviour, social disruption, extremes of temperature and noise, and many more. All through their rearing life, scavenging chickens are exposed to most, if not all, of these stressors.

Heat stress is one of the most important stressors particularly in the hot regions of the world (Altan *et al*., 2003) and to that effect, the influence of hot and cold regimes on birds have been investigated. Injections with various pharmacological preparations e.g. norepinephrine, social groupings and alterations in population density, feeding various nutritional formulations have also been tested (Thaxton and Puvadolpirod, 2000a). While elevated plasma corticosterone and increased circulating H/L ratio (Gross and Siegel, 1983; Quintero-Filho *et al*., 2010; Altan *et al*, 2010; Puvadolpirod and Thaxton, 2000b; Gross and Chickering, 1987; Cravener *et al*., 1992) are the two most widely accepted indicators of the stress condition in birds, other measures such as tonic immobility (TI) have also been applied.

2.13.1 Duration of tonic immobility

Also known as animal hypnosis, tonic immobility (TI) is a phenomenon found in many different species which is induced by a brief period of physical restraint, typically administered by holding an animal down on a flat surface (Gallup, 1974). It is a behavioral state characterized by lack of movements and an apparent lifeless position (Gallup and Rager, 1996; Miyatake *et al*., 2009; Edelaar *et al*., 2012). Tonic immobility is a fear-potentiated response induced by physical restraint. The technique has been applied in various species including beetles, crabs, lizards, pheasants, chickens and sharks. Fear is an important component of stress and can potentially reduce welfare in birds and duration of TI is widely accepted and applied as a measure of fearfulness in birds (Altan *et al*., 2003; Hrabcakova *et al*., 2012). The more fearful birds show longer immobility reactions when tonic immobility is induced (Altan *et al*., 2003). The TI test is based on a natural defensive reaction used by birds living in the wild when escaping a predator (Hrabcakova *et al.*, 2012). The principle behind this defense strategy is to remain completely motionless and appear virtually lifeless in order to dissuade the predator from attacking.

Although heat stress influences fearfulness, it is as yet unclear how slow-growing free-range chicken behaviour, particularly physiological responses, is influenced by environmental factors such as high temperatures. In addition, interspecific differences in response to various environmental stimuli have not been evaluated under outdoor rearing conditions. Outdoor conditions are becoming increasingly popular partly because housing of chickens in conventional cages with high stocking densities is associated with increased fearfulness (Keeling and Gonyou, 2001). This triggers welfare concerns. Animal well-being can be improved by housing animals in species-specific, natural or near-to-natural environments (Hrabcakova *et al*., 2012) but there is need to further investigate salient complications associated with the same.

2.13.2 Heterophil to lymphocyte (H/L) ratio

Physiological variables can be used to measure the condition of thermal comfort of animals. Any variation in them is indicative of attempts to maintain thermal equilibrium, and consequently, if an animal is or not under stress (Nascimento *et al*., 2012). The main haematological response is a change in the heterophil/ lymphocyte (H/L) ratio. This is premised on the fact that the numbers of heterophils and lymphocytes per unit of blood increase and decrease, respectively, in birds under stress, but their ratio is less variable. The H/L ratio is thus a better indicator than individual cell numbers (Gross and Siegel, 1983).

Physiological and physical stressors such as fasting, water deprivation, frustration, crowding, stocking density, high temperature and housing increase the H/L ratio (Cravener *et al*., 1992). Extensive research has been done on the effects of stress on H/L ratio in various livestock species including chickens (Askar and IsmaeI, 2012; Guémené *et al*., 2010; Altan *et al*., 2003; Thaxton and Puvadolpirod, 2000a), hawks and Peregrine falcons (Parga *et al*., 2001) and various other species. For birds, a normal ratio is about 0.42 but this can rise to 8 under severe stress. Changes in H/L have been observed in response to thermal stress and treatment with corticosterone (EC, 2000). Exposure of birds to heat stress results in an increase in the H/L ratio (McFarlane and Curtis, 1989; al-Murani *et al*., 1997; Altan *et al*., 2000a). Fear is an important component of stress and prolonged or intense fear can markedly reduce welfare and performance. The influence of high temperature on H/L ratio in slow-growing chickens has not been evaluated, let alone effect of cyclic exposure to such temperatures.

2.13.3 Other indicators of stress

Total white blood cell counts have been used as indicators of stress in birds. Other factors that have been applied in the study of stress responses in chickens include; mediation of the adrenal glands directly by exogenous administration of adrenocorticotropin (ACTH) and exogenous administration of steroid moieties, including corticosterone (Gross and Siegel, 1983), cortisone, cortisol, deoxycorticosterone, and dexamethasone (Thaxton and Puvadolpirod, 2000b). Plasma enzymes have also been widely used as indicators of stress. Common examples include creatine kinase (CK), aspartate transaminase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) (EC, 2000).

2.14 Carcass and meat quality

Selection of meat-type chickens has previously focused, not only on increased growth performance but also, carcass quality (Le Bihan-Duval *et al*., 1999). Interest has centred on better body composition, with higher breast meat yield and lower abdominal fat. According to Le Bihan-Duval and co-workers (1999), profitability is therefore largely determined by the possibility of increasing the proportion of prime parts in the carcass, mainly breast meat, and by reducing fat content.

Consumers have become increasingly interested in products that are perceived as naturally produced or environmentally friendly (Küçükyilmaz *et al*., 2012). As a result, organic and free-range systems have gained in popularity over age-old conventional methods. There is a common belief that organic chickens are safer and more nutritious than conventional ones, and an increasing number of consumers are willing to pay a premium for free-range or certified organic products (Castellini *et al*., 2008; Crandall *et al*., 2009). One of the principal expectations from outdoor access and feeding on organic diets is improvement in the functional quality of meat (Küçükyilmaz *et al*., 2012).

The poultry industry aims to increase carcass yield and reduce fatness, mainly the abdominal fat pad (Fouad and El-Senousey, 2014). This is as a result of the link that exists between fat intake and problems such as cardiovascular conditions. Total fat intake, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), or polyunsaturated fatty acid (PUFA) intake are independent risk factors for prospective all-cause, cardiovascular and cancer mortality (Leosdottir, 2005).

Exposure to high ambient temperatures has detrimental effects on poultry production efficiency and meat yield (Sandercock, 2001). These negative effects have generally been studied in relation to long-term or chronic heat stress associated with rearing in hot climates or seasonal changes in environmental temperatures. They, therefore, reflect the consequences of physiological adaptations to prolonged exposure to elevated temperature conditions (Sandercock, 2001) and can generally be regarded as applicable to rural chicken production in developing countries. Though the common strains are perceived as adapted to harsh environments, prolonged exposure to heat stress, among other environmental stressors, compromises productivity and meat quality. This, therefore, warrants investigation and documentation.

2.15 Summary

Chickens play a vital role in the livelihoods of almost all households in the developing world. They are mainly produced under extensive management where scavenging is expected to meet all or most of their nutrient needs. Free-range and other outdoor rearing systems have gained popularity in recent years and this has been driven by the need to ensure good bird welfare as well as the production of safe meat and eggs. Outdoor systems often entail exposing birds to factors that negatively impact performance, including thermal stress, predation, mortality, parasites, diseases and various others. Important environmental stressors in outdoor conditions include heat and relative humidity. The increasing popularity and demand for free-range and organically-produced meat and eggs presents an opportunity for intensified free-range chicken production. The increased awareness on animal welfare and humane bird management practices, coupled with certified organic products having a niche market, present significant scope for the promotion of free-range/ organic systems. Potential environmental stressors should be evaluated in view of their effects on bird behaviour, physiology, gut morphology, welfare and hence overall performance of free-range chickens. This would pave way for the identification of appropriate strains and practices to be used in order to improve bird welfare, productivity and meat quality.

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Chapter 3: Effects of strain and sex of bird on the behaviour of free-range slow-growing chickens

(Published in *the Journal of Applied Animal Research, appendix 3*)

Abstract

Behaviour is a good indicator of the wellbeing of chickens. The objective of the study was to compare foraging behaviour of 3 slow-growing chicken strains under free-ranging conditions in a hot environment. Behavioural activities were monitored in 21-week old NN, OV and PK chickens. Birds were separated by sex and allocated to 4 free-range pens of *Chloris gayana*. Three birds per pen were randomly chosen and marked with paint on the tail 20 minutes before observation. Ambient temperature and relative humidity were used to compute a temperature humidity index (THI) and the main effects were analysed using Proc GLM. A linear regression model was fitted to test the relationship between THI and time spent on each activity. Strain influenced time spent walking but not other behaviours. Sex of bird affected ($P < 0.05$) foraging, standing and walking behaviours. Time of day influenced ($P < 0.05$) time spent foraging, drinking and preening. Week of observation influenced ($P < 0.05$) time spent foraging and standing. THI had an effect on foraging, standing and preening behaviours. There was negative correlation between THI and time spent foraging. Females spent more time ($P < 0.05$) than males on the same activity. Foraging and drinking behaviours were prominent in the morning whereas preening and dust-bathing were dominant around mid-day. It was concluded that strain, sex and environmental factors influence behaviour in free-range chickens.

Keywords: Behaviour, Chickens, Free-range, Humidity, Temperature, Sex, Strain

3.1 Introduction

Animal welfare activists campaign for the use of natural or near-natural environments for chickens. This has stimulated an increase in the popularity of free-range systems across the world. Free-range chickens have limited access to feed additives and artificial ingredients and are grown in an environmentally friendly manner (ERS, 2002). Free-range or organic systems allow birds access to an outside area promoting foraging, feed selection and activity thus theoretically improving their welfare (Ponte *et al*., 2008). These outdoor production systems could decrease stress and allow selection of strains that may increase comfort and bird welfare (Wang *et al*., 2009) particularly in the wake of production conditions that are only likely to worsen with predicted trends in climate change. Conventional systems limit the expression of normal behaviour and have become unpopular. Conventional cage systems for laying hens were banned in the European Union (EU) as from January 2012 according to an EU Council Directive 1999/74/EC on the welfare of laying hens (CEC, 1999). In the developed world, free-range and organic livestock production are well defined with products derived from such systems getting certified (ERS, 2002). Free-range products are perceived to be safer and healthier and may carry several health benefits to consumers (Midmore *et al*., 2005).

Meteorologic elements constitute a major variable for outdoor operations (Sossidou *et al*., 2011) and the concern and emphasis for such elements in recent years are due to the fact that they are not constant, but change continuously (Ayo *et al*., 2011). Factors such as temperature (Kristensen *et al*., 2007) and humidity influence life cycles, reproductive ability, growth rates and thus body weights (BW) of birds. Direct meteorologic factors affecting birds include, especially, high ambient temperature and relative humidity, and may result in severe heat stress (Ayo *et al*., 2011). High humidity impacts thermoregulation and welfare of chickens (Lin *et al*., 2005) in that humid conditions reduce the effectiveness of heat dissipation (Warriss *et al*., 2005). This impairs normal body functions as efficiency is achieved if body temperature is kept constant or maintained within a narrow range (Ayo *et al*., 2011). The normal body temperature of an adult chicken is 40.6 to 41.7°C while the thermo-neutral zone (TNZ) is 18 - 24°C (Fanatico *et al*., 2007).

Free-range systems make use of slow-growing strains which are more suitable for these production systems (Castellini *et al*. 2002; Gordon and Charles 2002). Utilisation of the slow-growing indigenous strains enhances sustainability of chicken production systems. Slow-growing strains are preferred because of their pigmentation, taste, flavour and leanness (Moreda *et al*., 2013). Popular strains in Southern Africa include Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) (Grobbelaar *et al*., 2010; Nthimo *et al*., 2004) which are dual purpose strains. The NN and OV are closely associated with rural livelihoods where they are used to meet nutritional and economic needs of households (Mapiye *et al*., 2008). They are considered hardy and adaptable to harsh local climatic conditions which are important attributes since predominant systems often entail exposing birds to adverse environmental conditions. Such exposure influences the behaviour of birds in various ways and behavioural responses are the most pertinent indicators of the well-being of an animal (Moura *et al*., 2006). In hot weather, birds thermoregulate behaviourally by exposing a larger body surface area to encourage heat loss and body temperature is elevated (Warriss *et al*., 2005). Thermal stimulation, among other factors, influence behaviours such as dust-bathing (Orsag *et al*., 2011).

Literature show that there are strain differences in response to heat stress and that slower growing strains range more (Altan *et al*., 2003; Nielsen *et al*., 2003). Even among slow-growing strains, thermoregulatory capabilities vary. It is thought that the thermoregulatory ability of NN chickens at high temperature is slightly better than that of normally feathered birds (Yahav *et al.,* 1998). Naked Necks are a light-weight multi-coloured strain with white, red and black feather combinations. They reach sexual maturity at 155 d of age, with males weighing about 1.95 kg and females 1.40 kg (Chikumba and Chimonyo, 2014).

The reduced feather cover in the NN strain may be of advantage in thermoregulation at high ambient temperature (Eberhart and Washburn, 1993). The strain carries a gene which results in reduced overall plumage cover (Rajkumar *et al*., 2010; Fathi *et al*., 2013). The OV is a predominantly dark coloured fairly heavy strain that attains sexual maturity at average weights of 2.16 kg for males and 1.54 kg for females at about 140 d of age (Nthimo *et al*., 2004). It is generally regarded as adapted to high temperatures though the degree of thermal tolerance does not match NN owing to darker plumage colour and fairly heavier body weight (BW). The PK is a composite strain developed by crossing Black Australorp cockerels with White Leghorn hens and the Plymouth Rock (Grobbelaar *et al*., 2010). It is a heavy strain with an average adult BW varying from 3 - 4 kg for cocks and 2.5 - 3.5 kg for hens. Birds in this group reach sexual maturity at 130 d. Though bred to be adaptive and to survive under low input conditions, little is known about adaptability to high ambient temperature, particularly differences between sexes which exhibit clearly defined sexual dimorphism in plumage colour intensity. The strain has a characteristic black and white speckled colour pattern described as barred. The barred appearance is darker in females. The pattern is a sex-linked character that is useful for colour sexing in breeding for egg producing hens suitable for medium input production systems (Grobbelaar *et al*., 2010). This is vital as it may have direct influence on the fate of male PK after the females are selected for egg production.

Despite being adapted to harsh environmental conditions, the productivity of free-range slow-growing chickens is low. Given that numerous factors affect the behaviour of birds under free-range conditions, investigating the influence of environmental factors and their interaction with bird factors, on the behaviour of birds is essential. This would inform the designing of efficient management techniques aimed at improving productivity. In this study, it was anticipated that birds would adjust their behaviour in order to cope with changes in environmental conditions (Bertin *et al*., 2013) and that the degrees of adaptability would vary with strain and sex. Based on plumage colour and BW, better behavioural adaptation to high ambient temperature and humidity were anticipated in the NN, more-so in females which are lighter than males. It was, thus, expected that NN would be the least affected by heat stress, hence more time spent on feeding-related behaviours and consequently higher BW in comparison to other strains. It was rather difficult to predict the relative adaptabilities of the OV and PK strains due to darker plumage colour in one strain and higher BW in the other.

Comparison of foraging habits and behaviour of indigenous chicken strains, and sexes of the same, is useful in view of the increasing importance of outdoor systems across the world. The objective of the study was, therefore, to establish and compare the foraging behaviour of PK, OV and NN chicken strains under free-range conditions. Behaviour in these strains of chickens has not been studied before.

3.2 Materials and Methods

3.2.1 Animal ethics

The care, use and management of birds were according to internationally accepted standards for welfare and ethics of research animals (National Research Council, 2011). Specific approval was granted by the University of KwaZulu-Natal Animal Ethics Research Committee (Reference Number: 039/15/Animal).

3.2.2 Study site description

The study was conducted at Cedara College of Agriculture, in Pietermaritzburg, South Africa (SA). The area is located in an upland savanna zone on latitude 29.53°S and longitude 30.27°E at altitude 613 m. The area is characterised by a varied yet verdant climate owing to its diverse and complex topography. The lowest temperatures are experienced between June and July, averaging 6°C whereas the highest temperatures in the area occur between November and February with a mean of 31°C. The minimum and maximum temperatures recorded over the trial period were 17°C and 39.7°C, respectively. Two separate experiments were conducted at the site. Laboratory analyses were performed at the University of KwaZulu-Natal (UKZN), Discipline of Animal and Poultry Science, School of Agricultural, Earth and Environmental Sciences (SAEES), Pietermaritzburg, KwaZulu-Natal Province of SA.

3.2.3 Treatments and experimental design

A total of 144, 20-week old PK, OV and NN chickens were used in the study. Birds were separated by sex and allocated to 4 free-range pens such that there were 12 males × 3 strains on each of 2 pens and 12 females × 3 strains on each of 2 separate pens. Strains were mixed to enable comparison of their responses under exactly the same management conditions. The pens, measuring 900 m^2 each (Figure 3.1), were demarcated by 2.2 m high wire mesh reinforced by wooden and steel poles. *Chloris gayana* (Katambora Rhodes grass) was the dominant grass species on the pens. The birds were weighed individually on a digital crane scale, model UME CCS-150K, S/N: NXC 100020, to determine initial body weights (BW).

3.2.4 Housing, feeding and health management

Day-old chicks of OV, NN and PK strains were obtained from parent flock kept at the Agricultural Research Council (ARC), Irene, Pretoria, SA. From d 1 to d 49, chicks of each strain were reared in 2 × 1.5 m pens in a closed well ventilated poultry house which was 4 × 10 m. The house floors were covered with a 10 cm thick layer of wood shavings. Heat and light was provided using 75 W infrared lamps. The day-old chicks were maintained at a temperature of 32°C which was gradually reduced to 21°C by 21 d old. A thermometer was kept in the house just above the level of the birds and was used to monitor changes in temperature.

A broiler starter diet was offered *ad libitum* from standard tube feeders. Potable tap water was offered *ad libitum* through 4 L plastic founts. Chicks were vaccinated against Newcastle disease at 10 and 35 d of age. A foot bath drenched with disinfectant (Virukill®, Hygrotech South Africa (Pty) Ltd, Pretoria, SA) was placed at the entrance to the brooding house.

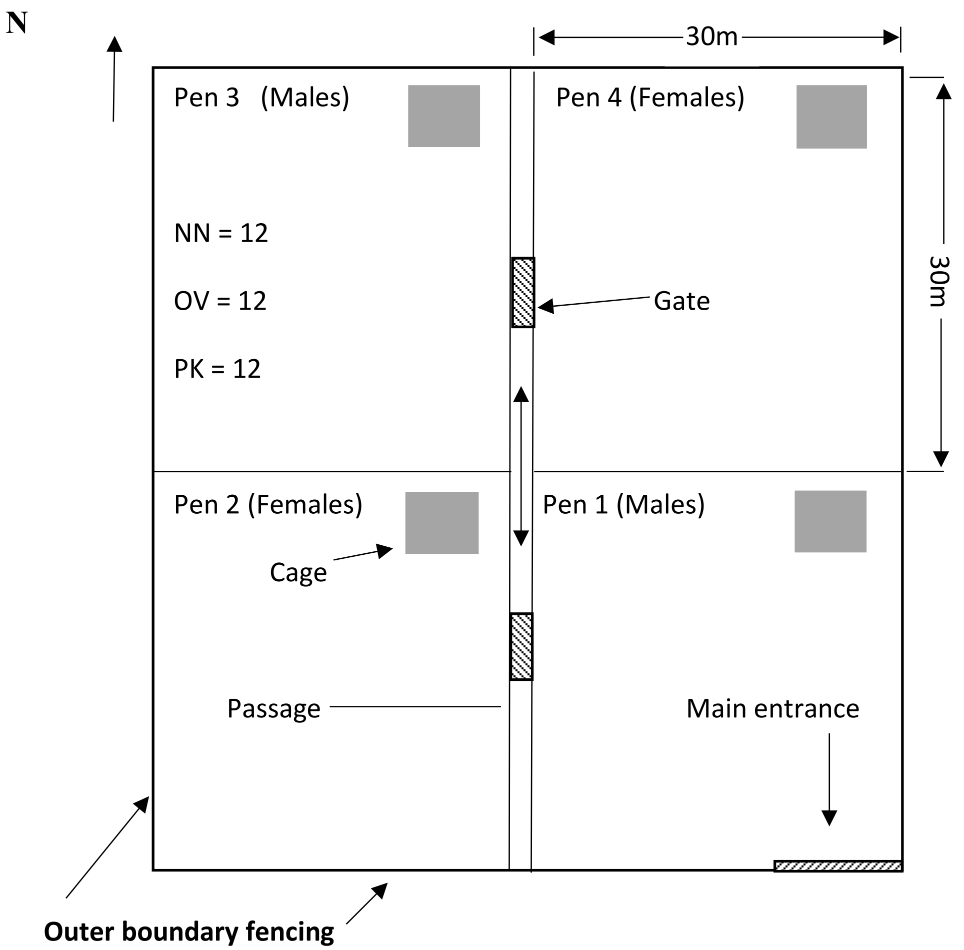

Figure 3.1. Free-range pen lay-out as used in the study

NN: Naked Neck; OV: Ovambo; PK: Potchefstroom Koekoek

From d 50, birds were given a grower meal supplied by Meadow feeds, South Africa. The nutritional composition of the feeds is shown in Table 3.1. At 20 weeks old, selected birds were moved from the poultry house and assigned to 4 free-range pens as described earlier. The Rhodes grass paddocks were located side by side and separated by fencing. During establishment, they were watered regularly and were rain-fed once established.

Table 3.1. Chemical composition (label values) of commercial broiler starter and grower feeds

Composition	Starter	Grower
Crude protein (g/kg)	200.0	180.0
Metabolisable energy (MJ/g)	12.8	13.0
ME/CP ratio (MJ/g)	0.1	0.1
Fat (g/kg)	25.0	25.0
Fibre (g/kg)	50.0	60.0
Moisture (g/kg)	120.0	120.0
Calcium (g/kg)	12.0	12.0
Phosphorus (g/kg)	6.0	5.5
Lysine (g/kg)	12.0	10.0

Feed supplied by Meadow Feeds, Pietermaritzburg, South Africa.

Weeds and other invader grass species were hand-picked and eliminated from the pens. Cattle manure was used to fertilize the grass paddocks. Wooden cages measuring 2.5 × 2 m were placed in one corner of each pen to provide shelter for the birds overnight. The cages, with slatted floors elevated 1 m above the ground surface, were fitted with wire mesh doors to deter predators. Doors were left open during the day and closed at night. A standard plastic drinker was placed under shade near each cage to provide clean water. The drinkers were inspected, washed and replenished at least twice a day to ensure *ad libitum* access to clean water.

3.2.5 Observations and data collection

After placement onto the pens, the birds were allowed a 7 d adaptation period before commencement of data collection. For behavioural observations, 3 birds, one of each strain, in each of the 4 pens were randomly chosen and marked with paint on the tip of the tail 20 minutes before being let out of the cage at 07:00 h. Paint of a different colour was used each time. Two trained observers recorded the activities of one bird each, simultaneously, in 2 pens purposively chosen to represent males and females for each observation session. Two pens of males and females were observed simultaneously for 30 minutes as a result. Birds in the other 2 pens were observed immediately after. The observers switched from pens with males to females and *vice-versa*.

Birds in each pen were observed 3 times a day, once a week, for a total of 3 weeks (1, 3, 5) from 0700 to 0800 h; 1200 to 1300 h and 1600 to 1700 h. During behaviour observation, a distance long enough to avoid disrupting the expression of normal behaviour by the birds was maintained. The time spent on each of the following behaviours was recorded:

1). Drinking behaviour (standing over a drinker with the head towards the drinker)

2). Foraging (pecking on vegetation in the paddock or scratching the ground)

3). Preening (cleaning of feathers)

4). Dust bathing (the act of rolling or moving around in dirt)

5). Hunting (chasing after insects)

6). Standing (remaining still in inactivity)

7). Other activities

Other general behavioural activities like panting were also noted. A stopwatch, model 870A Century clock-timer, was used to time and record specific time intervals devoted to a particular activity. Ambient temperature (°C), and relative humidity (%) were recorded on the same days that behaviour observations were made. As a result, meteorological data reported in the current study correspond to measurements made on the day of observation. These were used to compute a temperature humidity index (THI). In addition to these observations, BW were measured weekly using a digital scale (± 1 g sensitivity) as part of a separate experiment.

3.3 Statistical analyses

Data on the daily activities and BW of the free-range chickens were analysed using the PROC GLM of the Statistical Analysis System (SAS, 2010). Least square means were generated by the LSMEANS and separated using the PDIFF option of SAS (2010). The model $Y_{ijklmn} = \mu + B_i + S_j + W_k + TD_l + O_m + THI_n + (S \times B)_{ij} + \varepsilon_{ijklmn}$ was used for behavioural activities, where; $Y_{ijklmno}$ = the response variable (time spent on a particular activity); μ = overall mean common to all observations; B_i = effect of the i^{th} strain (*i* = NN, OV, PK); S_j = effect of the j^{th} sex (*j* = Male, female); W_k = effect of the k^{th} week (*k* = 1, 3, 5); TD_l = effect of the l^{th} time of day (*l* = 07:00 h, 12:00 h, 16:00 h); O_m; observer effect (*m* = 1, 2); THI_m = combined effect due to ambient temperature and humidity; $(B \times S)_{ij}$ = effect of the interaction between strain and sex of bird and $\varepsilon_{ijklmno}$, the random residual error. A linear regression model was fitted to test the relationship between time spent on feeding-related activities and THI. Significance was considered at the 5 % level of probability in all cases.

Data on BW were subjected to analysis of variance using the model $Y_{ijkl} = \mu + B_i + S_j + W_k + (B \times S)_{ij} + \varepsilon_{ijkl}$, where Y_{ijkl} = the response variable (BW); μ = overall mean common to all observations; B_i = effect of the i^{th} strain (*i* = NN, OV, PK); S_j = effect of the j^{th} sex (*j* = male, female); W_k = effect of the k^{th} week (*k* = 1, 3, 5); $(S \times B)_{ij}$ = effect of the interaction between sex and strain of bird, and Y_{ijkl}, the random residual error. All interactions that had no effect at the 5 % level of probability were dropped from the model.

3.4 Results

3.4.1 Meteorological observations

Overall minimum and maximum temperatures of 17 and 35°C, respectively, were recorded in the first week of study. Mean, minimum and maximum humidity recorded were 61, 35 and 87 %, respectively. Ambient humidity was highly variable, particularly in week 1 (SD = 22.81). The weekly average, minimum and maximum temperature and humidity experienced over the trial period are given in Table 3.2. Temperature humidity index means ranged from 68 to 79.2. The overall mean THI value for the observation period was 73.2.

3.4.2 Body weights

Strain, sex of bird and wk of observation had effects ($P < 0.01$) on BW. Significant interactions were observed between strain and sex of bird on this parameter. Of the three strains studied, OV chickens with average BW of 1892.8 ± 24.99 g/ bird were the heaviest ($P < 0.05$), followed by PK, average BW of 1787.3 ± 23.88 g/ bird and the NN chickens with a mean BW of 1687.9 ± 47.10 g/bird. Sexual dimorphism was observed in BW for NN and OV chickens. Males were heavier ($P < 0.05$) in the NN strain with average BW 1906.0 ± 45.45 g/ bird and OV with average BW 1672.7

± 41.28 g/ bird. There was no significant difference (P > 0.05) in BW between male and female PK (Figure 3.2). Marginal weight losses were observed in birds across all three strains over the period of observation.

3.4.3 Time spent foraging and drinking water

Significance levels for time spent on activities studied are presented in Table 3.2. Week, sex of bird and time of day all influenced (P < 0.05) time spent foraging. Birds spent the most time foraging in the third wk as shown in Table 3.3. Females spent more time foraging (P < 0.05) than their male counterparts. In addition, birds spent the most time foraging at 07:00 h followed by 16:00 h (Figure 3.3). There was a significant negative correlation (t (100) = -2.69, P < 0.01) between time spent foraging and THI. It was estimated that for a unit increase in THI, time spent foraging would decrease by 47.99 s. Strain did not influence time spent foraging (Table 3.2). Time of day had an effect (P < 0.01) on time spent drinking water (Figure 3.3) while strain, sex, week, observer and THI did not have an effect (P > 0.05). Figure 3.3 shows that the most time was spent drinking water in the morning compared to the other two periods of observation.

3.4.4 Time spent standing and walking

Strain and time of day did not affect time spent standing (P < 0.05). Week and sex of bird influenced (P < 0.05) time spent standing by the birds. The effect of week on time spent standing is shown in Figure 3.3. There was interaction (P < 0.001) between strain and sex of bird on time spent standing (Table 3.3). Strain and sex of bird were the only factors that affected (P < 0.05) time spent walking. Males of all strains spent more time (P < 0.05) walking than females while the NN spent the most time walking relative to the other strains (Table 3.3). No observer effect (P > 0.05) was oserved these parameters.

Table 3.2. Weekly and overall environmental temperature and humidity values recorded during the study period.

		Temperature (°C)				Humidity (%)			
Week	**n**	**Min.**	**Max.**	**Mean**	**SD^2**	**Min.**	**Max.**	**Mean**	**SD**
1	33	17	35	25	5.9	35	87	66	22.8
3	33	22	30	26	2.0	45	81	56	9.6
5	33	18	35	25	14.4	42	82	63	14.5
Overall		17	35	25	4.6	35	87	61	16.3

n = number of observations

SD^2 = standard deviation

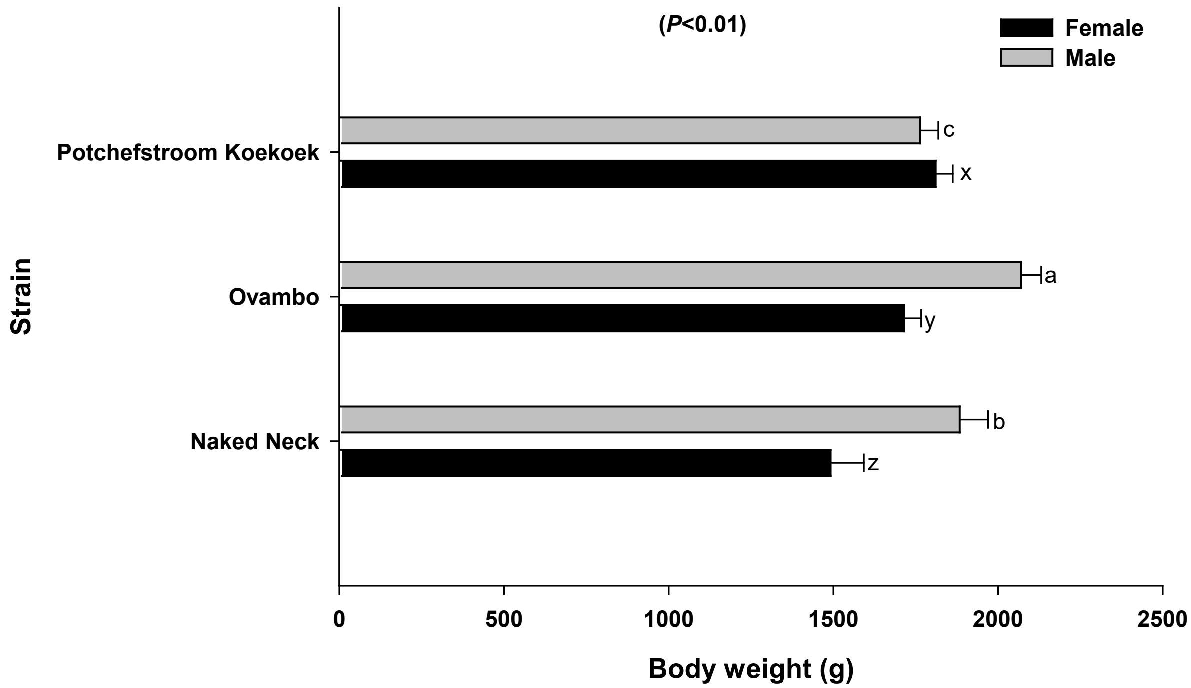

Figure 3.2. Effect of strain and sex of bird on final body weights of Potchefstroom Koekoek, Ovambo and Naked Neck chickens

Significant differences (P < 0.05) in body weights (BW) within a particular sex are shown by different letters (a, b, c) for males and (x, y, z) for females of different strains

Table 3.3. Effects of strain, sex, week and time of day on time spent foraging, drinking water, preening, dust-bathing, walking and standing by free-range slow-growing chickens

	Time spent (s)											
Effect	**Foraging**		**Drinking water**		**Preening**		**Dust-bathing**		**Walking**		**Standing**	
	F value	***P*-value**	**F value**	***P*-value**	**F value**	***P*-value**	**F value**	***P*-value**	**F value**	***P*-value**	**F value**	***P*-value**
Strain	0.57	NS	1.31	NS	1.77	NS	0.07	NS	3.87	*	0.19	NS
Sex	6.50	*	1.85	NS	1.60	NS	0.23	NS	13.28	***	10.52	**
Week	9.67	***	0.72	NS	1.49	NS	0.12	NS	0.64	NS	15.71	***
Time of day	5.57	**	5.23	**	8.00	***	2.48	NS	1.37	NS	2.27	NS
Sex × Strain	1.08	NS	0.81	NS	1.14	NS	0.71	NS	1.54	NS	11.69	***

**** P < 0.001; * P < 0.01; * P < 0.05; NS Not significant*

Table 3.4. Least square means for time spent on different activities by Potchefstroom Koekoek (PK), Ovambo (OV) and Naked Neck (NN) chickens

Effects		n	Time spent on activity (s)					
			Foraging	Standing	Drinking water	Preening	Dust-bathing	Walking
	NN	36	265.4 ± 28.00	136.9 ± 19.37	25.6 ± 8.88	74.4 ± 17.83	42.5 ± 21.77	77.1 ± 8.29[a]
Strain	OV	36	297.1 ± 28.91	140.8 ± 19.37	43.7 ± 10.27	41.6 ± 18.10	54.6 ± 26.06	46.1 ± 8.29[b]
	PK	36	252.9 ± 28.91	152.7 ± 19.66	35.8 ± 10.27	88.07 ± 18.70	42.6 ± 29.17	69.6 ± 8.41[a]
P value			0.5375	0.8370	0.4083	0.1862	0.9208	0.0253
Sex	Male	36	230.4 ± 23.95[b]	176.2 ± 15.81[a]	28.3 ± 8.09	54.9 ± 14.71	53.8 ± 21.44	81.7 ± 6.77[a]
	Female	36	313.3 ± 22.86[a]	110.7 ± 15.97[b]	41.8 ± 7.96	81.1 ± 15.03	39.3 ± 20.90	46.7 ± 6.83[b]
P value			0.0139	0.0044	0.2223	0.2156	0.5966	0.0004
Week	1	36	187.8 ± 29.98[b]	207.0 ± 19.66[a]	48.7 ± 12.38	101.3 ± 19.0	54.4 ± 38.14	69.9 ± 8.41
	3	36	364.7 ± 28.00[a]	67.7 ± 19.37[b]	20.6 ± 8.75	45.5 ± 17.83	36.1 ± 21.05	65.8 ± 8.29

	5	36	263.0 ± 28.00[b]	155.8 ± 19.37[a]	35.8 ± 8.75[a]	57.2 ± 17.83[a]	49.2 ± 21.05[a]	57.0 ± 8.29[a]
P value			0.0002	0.0001	0.1642	0.0866	0.8725	0.5369

[a, b, c]Values within a column, separated by a horizontal line, with different superscripts differ significantly (P < 0.05).

n Number of observations, NN Naked Neck, OV Ovambo, PK Potchefstroom Koeokek

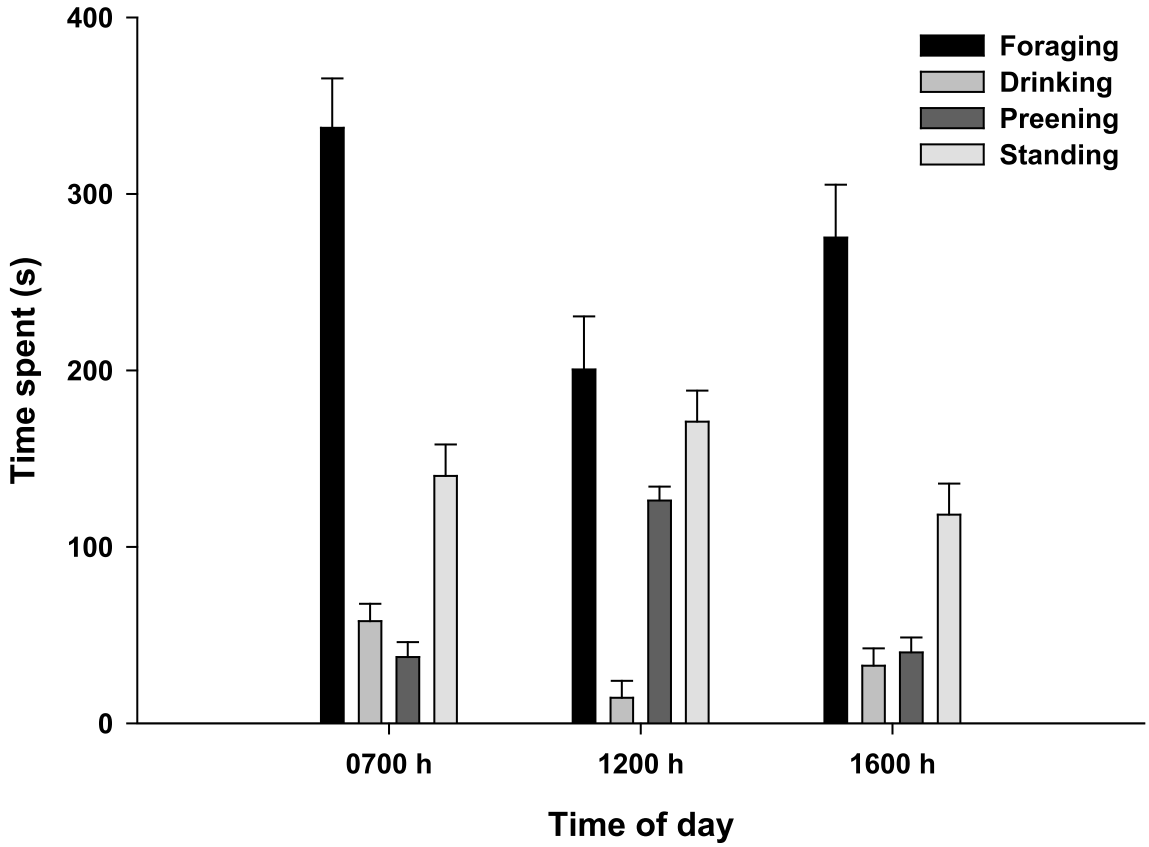

Figure 3.3. Time spent foraging, drinking water, preening and standing at different observation periods by Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) chickens

Females dominated most of the activities in terms of the time dedicated to a particular activity.

Strain, observer and time of day did not affect time spent standing ($P > 0.05$). Week, sex of bird and THI influenced ($P < 0.001$) time spent standing by the birds. There was significant positive correlation between THI ($t(105) = 3.24$, $P = 0.0016$) and time spent standing. For every unit increase in THI, time spent standing increased by approximately 39.56 s. The effect of week on time spent standing is shown in Figure 3.4. There was interaction ($P < 0.001$) between strain and sex of bird on time spent standing (Table 3.2). Strain and sex of bird were the only factors that effected ($P < 0.05$) time spent walking. Males of all strains spent more time walking than females while the NN spent the most time walking relative to the other strains (Tables 3.3 and 3.5). Females dominated most of the activities in terms of the time dedicated to a particular activity.

3.4.5 Other observations

The mean time spent drinking water, dust-bathing, foraging and preening were higher for females than males. It was rather interesting to note that male OV and PK dominated in standing while males of all 3 strains spent more time walking than females (Figure 3.5). Four PK females and two NN males were attacked by a hawk, as a result 6 birds were lost over the observation period.

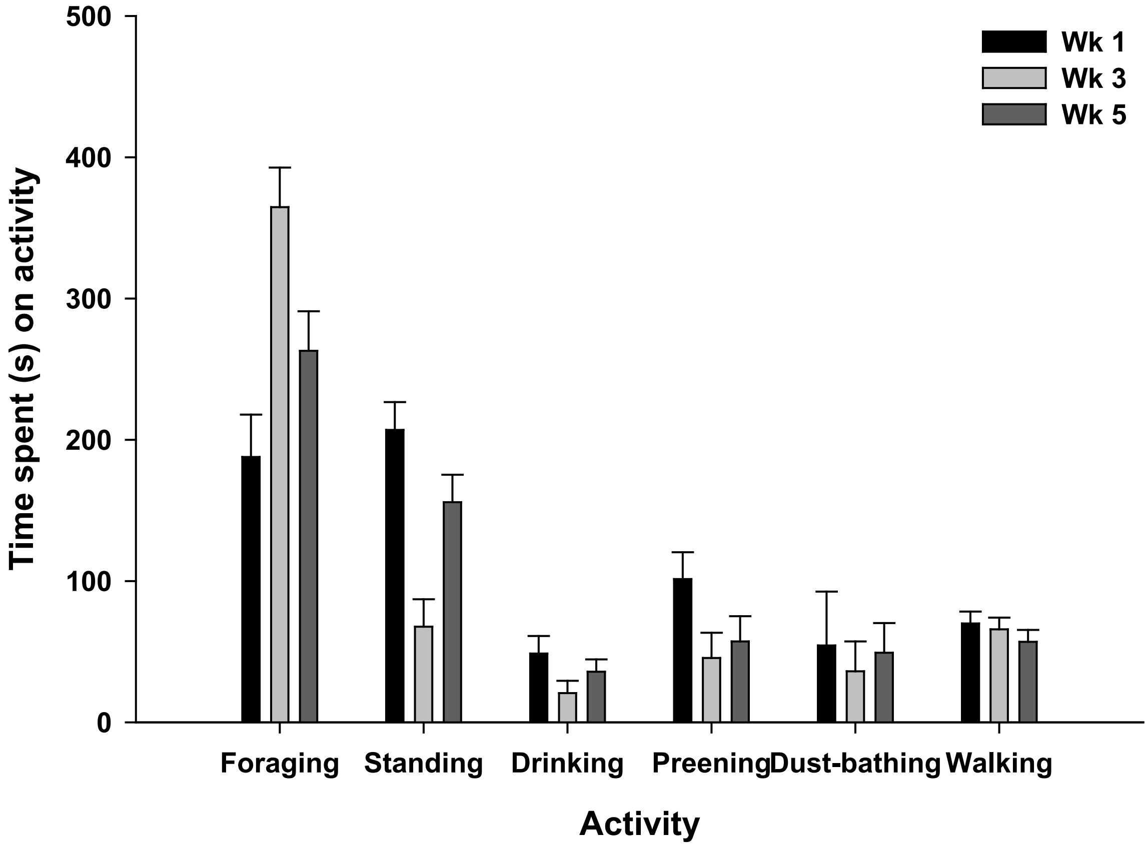

Figure 3.4. Time spent on various activities by Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) chickens by week (Wk) of observation

Table 3.5. Least square means on the interaction effects of strain and sex of bird on time spent standing by birds

	Strain, time (s)		
Sex	**Naked Neck**	**Ovambo**	**Potchefstroom Koekoek**
Male	100.6 ± 24.84^{b}	212.1 ± 24.84^{a}	215.9 ± 24.84^{a}
P-value	*0.0001*	*<.0001*	*<.0001*
Female	173.2 ± 24.84^{a}	69.4 ± 24.84^{b}	87.6 ± 25.59^{b}
P-value	*<.0001*	*0.0063*	*0.0009*

$^{a, b, c}$*Values in the same row with different superscripts differ significantly (P < 0.05).*

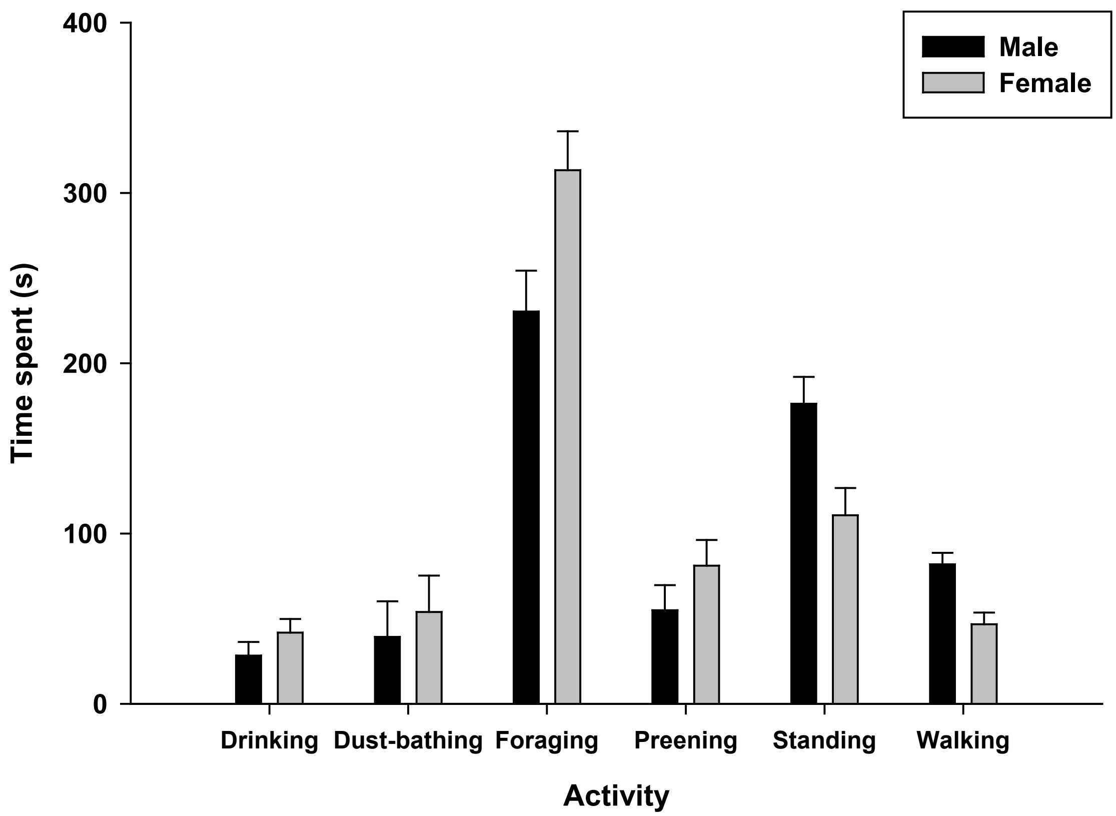

Figure 3.5. Time spent on various activities by the two sexes in Potchefstroom Koekoek, Ovambo and Naked Neck chickens

3.5 Discussion

Bird behaviour frequently switched amongst the major activity categories namely; foraging, drinking, preening, dust-bathing, standing, walking and lying down. Similar observations were made in broilers (Merlet *et al*., 2005). Literature reports strain differences in response to heat stress (Atlan *et al*., 2003), free-ranging behaviour (Nielsen *et al*., 2003) and BW (Nthimo *et al*., 2004) in chickens. Strains used in this study differ in BW and plumage cover and colour thus, contrary to our findings, we anticipated strain differences in foraging behaviour. Our expectation was that the NN would forage for longer than the other strains owing to their reported better thermoregulatory ability. The NN is a colourful strain in which reduced feather cover may be of advantage in thermoregulation at high ambient temperature (Eberhart and Washburn, 1993). Reduced plumage cover is effective in minimising heat stress where birds have to dissipate excess heat (Deeb and Cahaner, 2001; Raju *et al*., 2004). The NN chickens possess better post weaning heat tolerance than OV and PK due to the reduced plumage cover (Cahaner *et al*., 1993; Fathi *et al*., 2013). The NN strain carries an autosomal incompletely dominant gene (*Na*) which results in a 30 % reduction in overall plumage for heterozygotes and 40 % for homozygotes (Raju *et al*., 2004; Rajkumar *et al*., 2010; Fathi *et al*., 2013). The resultant reduction in total plumage cover is associated with increased thermal tolerance (Raju *et al*., 2004; Rajkumar *et al*., 2010; Fathi *et al*., 2013). In the current study, one behaviour that perhaps shows marginal advantage of the NN is the difference in time spent walking, with NN spending the most time walking perhaps suggesting greater adaptability.

Ovambo chickens, which were the heaviest in the study, an observation consistent with earlier studies (Chikumba and Chimonyo, 2014; Nthimo *et al*., 2004) but not Grobbelaar *et al*. (2010), are predominantly dark coloured. It was, thus anticipated that OV would be most affected by high THI. The observation on females spending more time foraging than males is also not consistent with previous research (Nthimo *et al*., 2004). The females, which were at point-of-lay, probably had greater nutrient demands to meet egg production requirements. Males were expected to forage for a longer time to meet greater nutrient requirements, conversely, they have higher BW which might mean potential susceptibility to heat stress. Birds did not seem to forage much in the first week probably as a result of the dramatic change in conditions including the transition from indoor to an outdoor environment. The first wk of observation had the highest average maximum temperature (35°C) and humidity (87 %) hence a high THI. It was shown in this study that environmental conditions influence time spent foraging with increases in THI leading to reduced time spent foraging. Time spent standing was also influenced, where an increase in THI resulted in an increase in time spent standing. The times spent standing in the current study are higher than 20.3 ± 30.80 s reported by Spencer (2013). The discrepancy could be a reflection of the differences in strains as well as rearing conditions. Regression analyses showed that there was no relationship between time spent on all other activities and THI. Females spent more time on most activities with the exception of standing and walking.

Strain differences observed in BW in the current study are consistent with previous observations (Chikumba and Chimonyo, 2014) where differences were recorded in 16-week BW of OV and NN chickens. Similar BW observations were made by Nthimo *et al*. (2004). Sexual dimorphism observed in the BW of NN and OV strains is in agreement with Nthimo *et al*. (2004) who noted differences on 26-week BW of OV, PK and NN chickens. The weight loss that occurred during the study period is probably related to the change in rearing conditions. The same might have been worsened by predominant environmental conditions which discouraged foraging. Literature reports reduced feed intakes at high temperatures so as to preserve body water by reducing faecal water loss and body heat increment (Mashaly *et al*., 2004; Chikumba and Chimonyo 2014). Reduced feed intake by birds is an adaptive strategy to survive under hot environmental conditions. Reduced BW were recorded in broilers exposed to high temperatures and humidity, perhaps indicating depressed feed intake (Lin *et al*., 2005).

The observation that birds foraged and drank water more in the cooler hours of the day agrees with previous reports (Dawkins *et al*., 2003; Horsted *et al*., 2007; Spencer, 2013). Contrary to the same researchers, birds foraged much longer in the morning than other periods in our study. Dawkins *et al*. (2003) observed chickens to be most active right before sunset. Chickens forage more during the cooler hours of the day as they are less likely to struggle with thermoregulation. Higher temperatures that are commonly experienced around mid-day to early afternoon also depress appetite thus compromising feed intake (Dawkins *et al*., 2003). The THI range from 68 to 79.2 shows that, at some point, birds were exposed to some degree of heat stress. At THI values of between 72 and 79, mild heat stress occurs while THI values of 80 - 89 indicate heat stress (Pennington *et al*., 2004). The current findings suggest that foraging, hence dry matter intake, is perhaps more important at influencing drinking behaviour than other factors since invariably more time was spent drinking water during periods when birds foraged more actively than otherwise.

Water intake in chickens is, therefore, primarily related to feed intake and may have a secondary role in cooling when these two are considered simultaneously. Chickens go off-feed if water intake is restricted (Chikumba and Chimonyo, 2014). Feed consumption in NN chickens given *ad libitum* water access was 52 % and 8 % higher than that of birds given water at 40 % and 70 % of *ad libitum*, respectively (Chikumba and Chimonyo, 2014). The times spent drinking water were similar to those reported by Murphy and Preston (1988). The strains used in the two studies differ, suggesting that the important factor driving drinking behaviour is dry matter intake.

Spending the most time preening during the 12:00 h period perhaps indicates more than just a simple trade-off between preening and foraging by birds. It is during the hottest period that birds clean their feathers and wad external parasites (Clayton *et al*., 2010). A previous study noted that the preferred time of dust-bathing, a similar behaviour driven by thermal stimulation, is the middle of the day (Wichman and Keeling, 2009; Orsag *et al*., 2011). Our observation on dust-bathing contradicts the report by Murphy and Preston (1988) where absence of dust-bathing was noted in broilers. Preening and dust-bathing are important parts of normal bird behaviour (Orsag *et al*., 2011). They help to remove stale oil from feathers. These behaviours are particularly important in free-range chickens which are often exposed to various edaphic and biotic factors, hence parasite infestation.

The main pathway of heat dissipation for birds under a hot environment is respiratory evaporation (Hillman *et al*., 1985), especially when ambient temperature approaches body temperature. The maximum environmental temperature recorded in the current study was much higher than 18 and

24°C which is the TNZ for chickens (Fanatico *et al*., 2007). At ambient temperatures within the TNZ, chickens are able to maintain their body temperature. Any increase in temperature above this zone initiates heat dissipation mechanisms. The body temperature of an adult chicken should be between 40.6 and 41.7°C (Fanatico *et al*., 2007). At high THI, heat production decreases while heat dissipation increases (Lin *et al*., 2005). High THI values experienced in the current study discouraged foraging due to increased heat load (Lin *et al*., 2005). The high THI positively influenced standing and preening behaviours as birds normally thermoregulate by behavioural changes. Transmission of heat from the body core to the skin is less effective when humidity is above 60 %. High ambient humidity exacerbates the effects of high temperatures by reducing the effectiveness of panting to induce evaporative cooling from the respiratory tract (Warriss *et al*., 2005). This largely agrees with behavioural trends observed in the current study.

3.6 Conclusions

Strain and sex influenced foraging behaviour and NN chickens and females in general, spent more time foraging. The OV strain achieved higher BW than NN and PK in the current study while sex also influenced walking and standing behaviours. Foraging and drinking behaviours were more prominent in the morning while preening and dust-bathing occurred mostly around mid-day. Other behaviours were more sporadic in their distribution. There was negative correlation between time spent foraging and THI but time spent standing and preening increased with increasing THI. Ambient temperature and humidity are, therefore, important factors influencing free-ranging behaviour and hence overall performance of slow-growing chickens. Physiological responses of slow-growing chickens to environmental temperature and humidity also need to be understood.

Investigating such responses under both intensive and extensive rearing conditions could help identify the more appropriate rearing system.

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Chapter 4: Physiological responses in slow-growing chickens under diurnally cycling temperature and humidity in a hot environment

(Under review*, Brazilian Journal of Poultry Science*)

Abstract

It is vital to minimise thermal stress and associated welfare problems for chickens in hot environments. The study was conducted to determine physiological responses in Potchefstroom Koekoek (PK), Ovambo (OV) and Naked Neck (NN) chickens to cycling temperature and humidity. Body weight (BW), rectal temperature (RT), breathing rate (BR) and heart rate (HR) were determined, weekly for 4 weeks, in NN, OV and PK chickens under cyclic environmental conditions. A total of 288, 20-week old PK, OV and NN chickens were separated by sex and allocated to free-range and confined rearing pens. Temperature and RH were used to compute a temperature humidity index (THI). Proc MIXED was used to analyse fixed effects and a linear regression model was fitted to test the relationship between THI and physiological response parameters. All factors studied influenced ($P < 0.05$) BW while none affected ($P > 0.05$) RT. Higher BW ($P < 0.05$) were obtained with OV in both rearing systems. Sex influenced ($P = 0.0021$) HR but not BR ($P > 0.05$). Week of observation affected ($P > 0.05$) BR. There was significant correlation between THI and BR and HR. THI was higher in intensive than free-range rearing. Physiological responses of PK, OV and NN are comparable under similar rearing conditions.

Key words; Breathing rate, Humidity, Heart rate, Strain, Temperature

4.1 Introduction

Free-range chicken production is increasing due to increased awareness on animal welfare. In some parts of the world, there is widespread promotion of free-range and organic production of livestock. Conventional cage systems for laying hens were banned in the European Union (EU) as from January 2012 according to an EU Council Directive 1999/74/EC on the welfare of laying hens (CEC, 1999). In the developing world, free-range production systems are predominant. Often, free-range systems entail exposure of birds to environmental conditions such as high ambient temperature (T_a) and humidity. The climatic environment is one of the main limiting factors of production efficiency (Renaudeau *et al*., 2011) and thermal stress is amongst the most important environmental stressors, particularly in hot regions of the world (Altan *et al*., 2003). It is vital to understand the effects of high temperatures on livestock performance, in view of anticipated increase in global average surface temperature by between 1.88°C and 4.08°C in the next 60 years (Renaudeau *et al*., 2011). High T_a and relative humidity (RH) are some of the direct meteorologic factors affecting birds that may result in severe heat stress (Ayo *et al*., 2011).

In chickens, when T_a increases, heat production decreases while heat dissipation increases (Lin *et al*., 2005). Chickens, as homoeotherms, maintain constant body temperature within the thermoneutral zone (TNZ) of 18 to 24°C (Cahaner *et al*., 2008; Soleimani *et al*., 2008). For that to happen, excess heat must be lost and heat exchange could be assessed directly from physiological measurements e.g. rectal and skin temperatures, respiratory rate, panting, heat production and growth rate. Rectal temperature, breathing rate and heart rate can be used as indices of thermal stress in birds.

While slow-growing strains are known to be hardy, little is known of the effects of thermal stress on homeostatic responses of free-range chickens common to Southern Africa. The Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) chickens are closely associated with rural livelihoods in Southern Africa where they are used to meet household nutritional and economic needs (Mapiye *et al*., 2008). Naked Necks are a light-weight multi-coloured strain with white, red and black feather combinations. They are known to be thermal tolerant (Fathi *et al*., 2013) and generally adapted to harsh environmental conditions. They carry a gene which results in reduced overall plumage cover (Rajkumar *et al*., 2010; Fathi *et al*., 2013). The reduced feather cover is thought to be advantageous in thermoregulation at high temperature (Eberhart and Washburn, 1993). It is thought that the thermoregulatory ability of this strain at high temperature is slightly better than that of normally feathered birds (Yahav *et al.*, 1998). The OV is a predominantly dark coloured fairly heavy strain that attains sexual maturity at average weights of 2.16 kg for males and 1.54 kg for females at about 140 d of age (Nthimo *et al*., 2004). It is generally regarded as adapted to high temperatures though the degree of thermal tolerance does not match NN owing to darker plumage colour and fairly heavier body weight (BW). The PK is a composite strain developed by crossing Black Australorp cockerels with White Leghorn hens and the Plymouth Rock (Grobbelaar *et al*., 2010). It is a heavy strain with an average adult BW varying from 3 - 4 kg for cocks and 2.5-3.5 kg for hens. Though bred to be adaptive and to survive under low input conditions, little is known about its adaptability to high T_a, particularly differences between sexes which exhibit clearly defined sexual dimorphism in plumage colour intensity.

Investigating and comparing physiological responses of these strains is important in making decisions on appropriate genotypes to recommend for farmers in areas experiencing harsh environmental conditions. Fluctuations in environmental conditions have implications of productivity since birds cannot stay in thermal equilibrium with the environment. This results in physiological and behavioural changes as chickens are particularly sensitive to heat stress (Renaudeau *et al*., 2011; Lara and Rostagno, 2013; Fathi *et al*., 2013) which could result in reduced performance (de Souza *et al*., 2015). In the previous Chapter, it was observed that strain and sex of bird influence behaviour in slow-growing chickens. It is therefore possible that physiological responses of NN, OV and PK chickens might vary under similar rearing conditions hence potential differences in actual performance. The current study was, therefore, designed to investigate the effect of strain, sex of bird and rearing system on BW, RT, BR and HR of PK, OV and NN chickens raised in a hot environment.

4.2 Materials and Methods

4.2.1 Animal ethics

Animal care and handling were as previously described in section 3.2.1.

4.2.2 Description of study site

The study site was as previously described in section 3.2.2.

4.2.3 Treatments and experimental design

A total of 288, 20-week old dual purpose slow-growing chickens comprising PK, OV and NN strains were used in the study. Birds were allocated to four pens each of free-range and confined rearing systems. There were 12 males/ strain and the same number of females/strain in each pen on each rearing system. The free-range pens measured 900 m^2 each and were demarcated by 2.2 m high wire mesh reinforced by wooden and steel poles. *Chloris gayana* (Katambora Rhodes grass) was the dominant grass species on the free-range pens. Similarly, males and females of the 3 test strains were separated by wire mesh in a poultry house measuring 4 × 10 m. The birds were weighed individually on a digital scale, model UME CCS-150K, S/N: NXC 100020, to determine initial body weights.

4.2.4 Bird management

Wooden cages measuring 2.5 × 2 m were placed uniformly in one corner of each pen, under extensive rearing, to provide shelter for the birds. The stocking densities were 6.6 birds/ m^2 and 3 birds/m^2 in the extensive and intensive systems, respectively. The cages, with slatted floors elevated 1m above the ground surface, had louvered walls approximately 2.2 m above the floor. Cages were fitted with wire mesh doors to deter predators. Cage doors were left open during the day and closed at night after all birds had voluntarily climbed into the cages. Birds climbed into the cages between 17:30 and 18:30 h. The photoperiod during the observation period was approximately 10 h long. A standard plastic drinker was placed under shade near each cage to provide cool clean water. The drinkers were inspected, washed and replenished at least twice a day to ensure *ad libitum* access to clean water.

The poultry house for intensive rearing was fitted with two roof air-vents and side curtains on both sides to enable adequate ventilation and had corrugated iron sheet roofing. Fluorescent lamps were used for lighting. Under intensive rearing, birds were raised on a deep litter system with wood shavings as bedding. The litter, which was regularly inspected for wetness, was maintained between 8 and 10 cm thick. Feed and potable tap water were supplied *ad libitum* through 2 standard plastic feeders and 2 standard 12 L plastic drinkers, respectively.

4.2.5 Brooding, feeding and health management

Day-old chicks of OV, NN and PK strains were obtained from a parent flock kept at the Agricultural Research Council (ARC), Irene, Pretoria, SA. From d 1 to d 49 chicks of each strain were reared in 2 × 1.5 m pens in a well ventilated 4 × 10 m poultry house. The house floors were covered with a 8-10 cm thick layer of wood shavings. Infrared lamps (75 W) were used as a source of heat and light. Day-old chicks were maintained at 32°C which was gradually reduced to 21°C by 21 d old by adjusting the height of the infrared lamps from the floor.

Broiler starter mash and potable tap water were offered *ad libitum* from standard tube feeders and 4 L plastic founts, respectively. Chicks were vaccinated against Newcastle disease (ND) at 10 and 35 d of age. A foot bath drenched with disinfectant (Virukill®) was placed at the entrance to the brooding house. From d 50, birds were given a grower meal. Feeds were supplied by Meadow Feeds, SA. The nutrient composition of the feeds is shown in Table 3.1 (Chapter 3).

4.2.6 Data collection and measurements

4.2.6.1 Meteorological measurements

Meteorological measurements were recorded daily over the duration of the trial period. Ambient temperature (T_a, °C) and relative humidity (RH, %) were recorded automatically after every 5 minutes throughout the trial period using HOBO data loggers (Onset Computer Corporation, Pocasset, MA, USA). During measurements, care was taken not to expose the instrument to direct rays from the sun. The recorded temperature and RH values were extracted and used to estimate the temperature humidity index (THI) as follows;

$$\mathrm{THI} = \mathrm{T_d} - \left[0.55 \times \frac{\mathrm{RH}}{100}\right] \times [\mathrm{T_d} - 58]$$ (Spencer, 1995)

Where THI is the temperature humidity index; T_d is the ambient temperature and RH is the relative humidity.

4.2.6.2 Body weights

A total of 72 birds representing all 3 strains were randomly selected and weighed using a digital scale and weekly live body weights (BW) recorded. Three birds/strain were sampled/pen on each rearing system. Altogether, 72 birds comprising 36 cocks and the same number of hens were weighed. Weighing was done on the same day that physiological response parameters were measured to minimize handling on the birds. The birds were weighed weekly at 0900 h and the time was maintained throughout the study period.

4.2.6.3 Physiological responses

Heart rate (HR) in bpm, breathing rate (BR) in breaths/ min and rectal temperature (RT) in °C, were determined. Measurements were made immediately after weighing the birds. Heart rate was determined with the aid of a 3M™ Littmann® Classic III™, USA, stethoscope and stop watch, model 870A, Century clock-timer. It was achieved by counting the number of beats in 30 s multiplied by two. The stethoscope was placed on the left side of the breast of an inverted bird after feathers were separated in order to expose as much skin as possible. Rectal temperature was measured using an Omron digital clinical thermometer, model MC-246 (± 0.1°C accuracy), inserted 3cm into the rectum (Altan *et al*., 2003) and left until a constant reading followed by a repeated beeping tone was reached. The thermometer was wiped using fresh clean cotton wool moistened with methylated spirit between subsequent measurements in order to prevent possible cross infection among birds. With the bird still in an inverted position, the abdominal region was observed to count respiratory movements within 1 minute and counting was done with the aid of a stop watch to determine BR.

4.2.7 Statistical analyses

Data were subjected to analysis of variance using PROC GLM of SAS ver 9.3 (SAS, 2010). Means were generated by the LSMEANS and compared using the PDIFF options of SAS (2010). Significance was considered at the 5 % level of probability. The following was used to model the data; $Y_{ijklmn} = \mu + B_i + S_j + WK_k + H_l + THI_m + (B \times S)_{ij} + \varepsilon_{ijklmn}$, where; Y_{ijklmn} = response variable (BW, RT, HR and BR), μ = overall mean, B_i = effect of the i^{th} strain (i = NN, OV, PK), S_j = effect of the j^{th} sex (j = male, female), WK_k = effect of the k^{th} week (k =1, 2, 3, 4), H_l = effect of l^{th}

rearing system (l = Intensive, extensive) and THI_m = combined effects due to environmental temperature and humidity, $(B \times S)_{ij}$ = effect of the interaction between strain and sex of bird, and ε_{ijklmn} = the random residual error. A linear regression model was used to test the relationship between THI and the physiological response parameters. Interactions that had no effect on parameters under study were dropped from the model.

4.3 Results

4.3.1 Body weight changes

All factors studied influenced ($P < 0.05$) BW (Figure 4.1, Table 4.1). There was interaction ($P < 0.05$) between strain and sex of bird on this parameter. Sexual dimorphism was observed on BW with the highest weights in the extensive system being recorded among males. Males were significantly heavier ($P < 0.05$) in the NN and OV (Figure 4.1). Among the three strains, the OV chickens were the heaviest ($P < 0.05$), followed by PK and lastly, NN strain.

During the study period, the T_a ranged between 17 and 39.7°C. The lowest mean T_a (17.6°C) was recorded in wk 3 in the extensive system while the highest (38.8°C) was observed in the first week of study. The overall average temperatures recorded were 24.7 ± 0.98°C and 22.7 ± 2.88°C in the free-range and intensive systems, respectively. Higher RH was recorded inside at 63.6 ± 11.9 %, compared to 55.9 ± 0.06 % observed on free-range. Temperature humidity index means ranged from 68 to 86.1 and 68.0 to 73.2 for the two rearing systems. The overall mean THI values were 70.0 ± 3.55 and 74.0 ± 4.95 inside and outside, respectively. The highest maximum THI of 86.1 was recorded inside *vs* 73.2 observed outside. Overall mean THI was consistently higher in the intensive system as shown in Figure 4.2d.

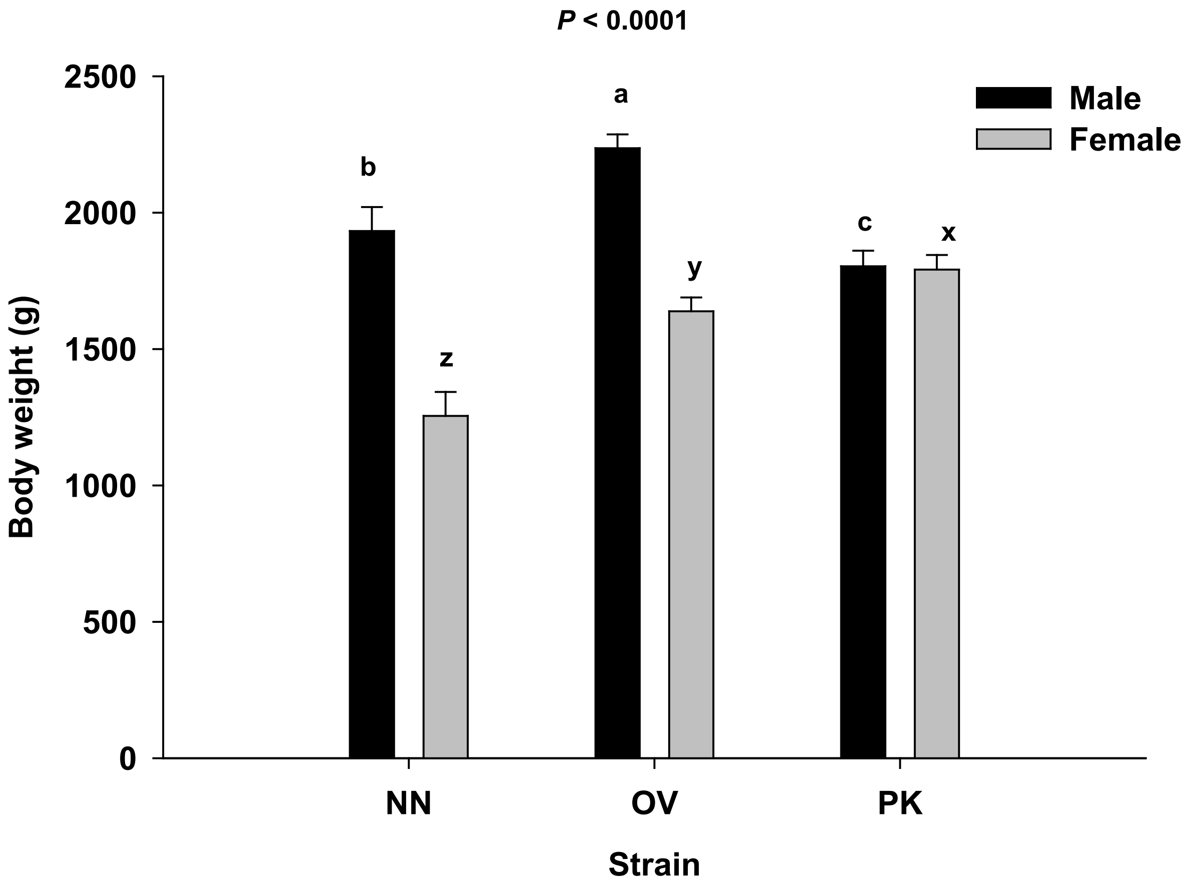

Figure 4.1. Body weight (BW) by strain and sex of bird for Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) chickens used in the study

[a b c, x y z] Live body weights (BW) that differ significantly ($P < 0.0001$) within a particular sex are shown by different letters (a, b, c) for males and (x, y, z) for females of different strains

Table 4.1. Changes in live body weights (BW) of Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) chickens

Age of bird (Weeks)	Strain, BW (g)				
	Naked Neck	Ovambo	Potchefstroom Koekoek	SEM†	*P-value*
21	1646.5^{c}	1981.2^{a}	1877.6^{b}	46.59	< 0.0001
22	1649.8^{c}	1919.4^{a}	1757.1^{b}	49.40	< 0.0001
23	1539.8^{c}	1862.1^{a}	1734.8^{b}	49.06	< 0.0001
24	1537.3^{c}	1986.2^{a}	1818.1^{b}	52.17	< 0.0001

$^{a, b, c}$ *Values in the same row with different superscripts differ significantly (P < 0.05)*

BW: Body weight

SEM: Standard error of the mean

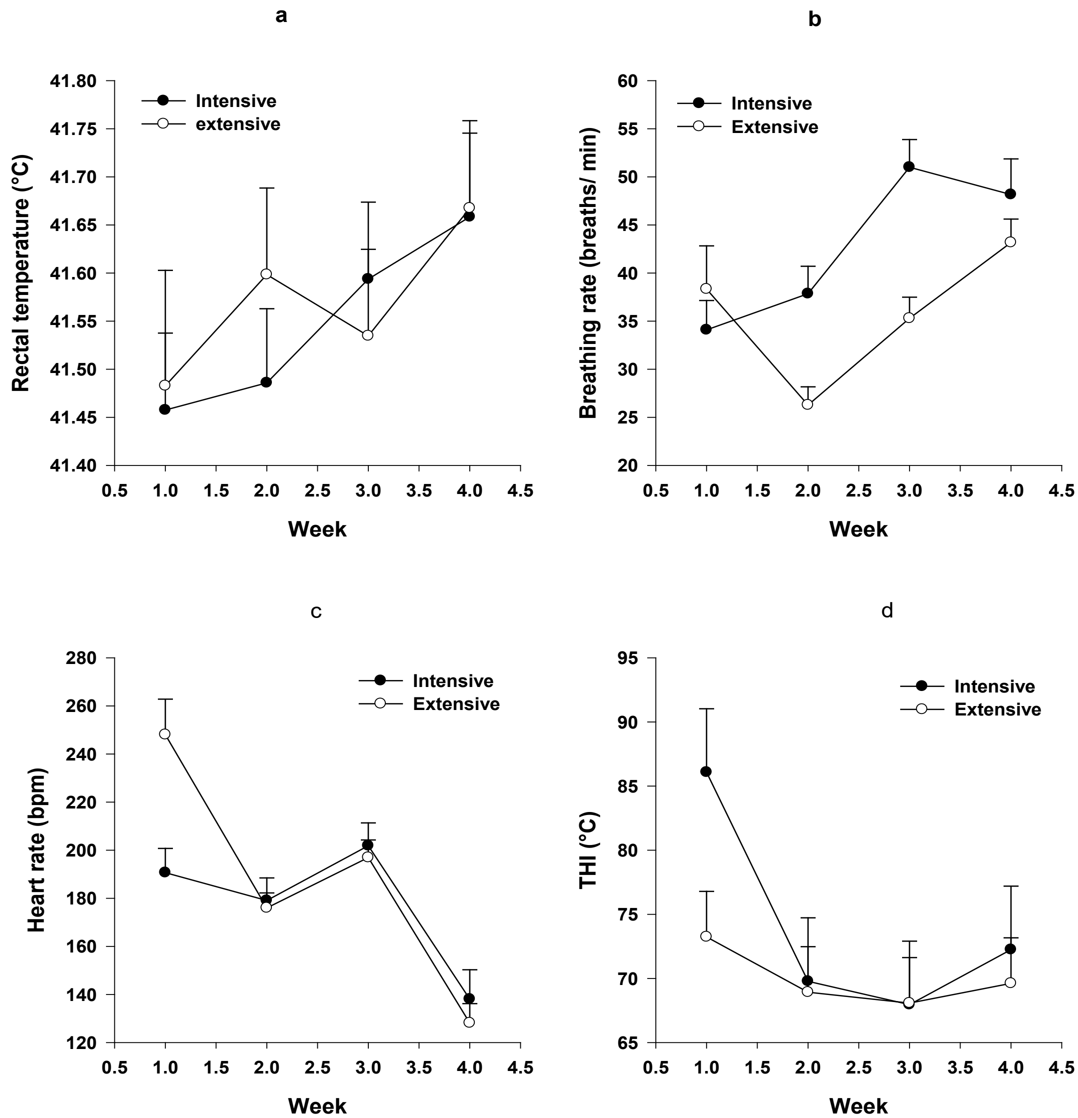

Figure 4.2. Rectal temperature (RT), breathing rate (BR), heart rate and temperature humidity index (THI) distribution in PK, OV and NN chickens used in the study

4.3.2 Physiological responses

4.3.2.1 Rectal temperature

None of the factors studied influenced RT ($P > 0.05$; Figure 4.2a). An overall mean RT of 41.6°C was recorded over the duration of the study period.

4.3.2.2 Breathing rate

Strain and sex of bird had no effect ($P > 0.05$) on BR. Rearing system and week influenced ($P < 0.001$) BR (Figure 4.2b). No interactions were observed on BR. Breathing rate was higher ($P < 0.001$) in birds under the intensive than extensive system. The lowest and highest BR were 26.3 ± 3.06 breaths/ minutes and 43.2 ± 2.44 breaths/ minute, respectively, for birds in the extensive system. There was significant positive correlation (Table 4.2) between BR and THI under extensive rearing system. For a unit increase in THI, BR increased by 0.56 breaths/ minute ($P = 0.01$).

4.3.2.3 Heart rate

Sex of bird and week significantly influenced HR. Significant interaction was observed between sex and strain of bird on HR. Figure 4.2c shows that HR was highest in the first wk of study and generally decreased up to wk 4. Interaction effects between strain and sex of bird and HR are shown in Figure 4.3. The mean HR was significantly higher ($P < 0.05$) in males at 198.1 ± 5.20 than 176.4 ± 5.73 bpm observed for females. The HR was significantly higher ($P < 0.05$) in females in the paddocks. An increase in T_a and humidity from 20 to 25°C and 64 to 68 %, respectively, led to a 35 % reduction in HR from 197 to 128 bpm on free-range.

Table 4.2. Effect of rearing system on RT, BR and HR in Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) chickens

Response	**Rearing system**							
	Free-range				**Intensive**			
	Estimate	**t-value**	**P-value**	**Error DF**	**Estimate**	**t-value**	***P-value***	**Error DF**
RT	0.00	0.49	0.64	36.0	0.00	0.31	NS	50
BR	0.56	3.26	0.01	36.0	0.73	1.45	NS	50
HR	1.90	2.83	0.01	36.0	1.20	0.51	NS	50

RT: Rectal temperature; BR: Breathing rate; HR: Heart rate; DF: Degrees of freedom

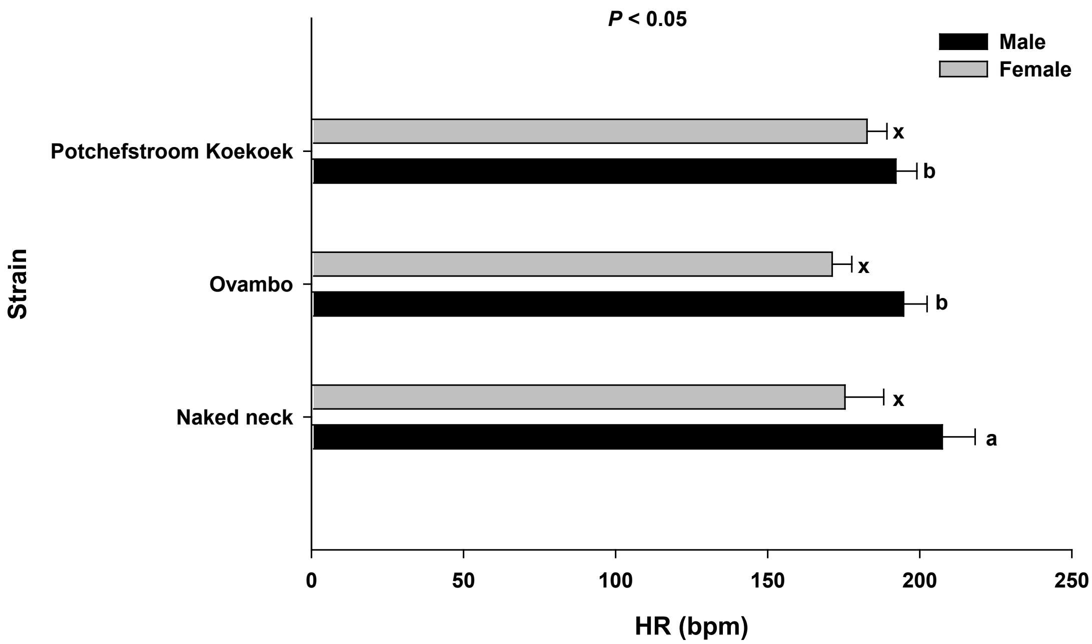

Figure 4.3. Influence of strain and sex of bird on heart rate (HR) in beats/ minute (bpm)

a, b, x: Heart rates (HR) that differ significantly (P < 0.05) within a particular sex are shown by different letters (a, b) for males and (x) for females of different strains

**BW: Body weight*

4.4 Discussion

From the above observations, it appears that birds in the extensive system were able to thermoregulate more efficiently compared to birds under the intensive system. Core temperature, as reflected by RT, was not significantly variable for both systems. The same is true among strains. The current results suggest that free-range systems enable, to a certain degree, more efficient thermoregulation in birds.

It was not surprising that birds under intensive rearing were significantly heavier than birds in the extensive system. In addition to restricted space allowances in the house minimizing energy lost due to walking longer distances, housed birds had *ad libitum* access to feed. In contrast, birds reared in the extensive system had to forage to meet their nutrient requirements which might have meant lower levels of nutrition. In addition to absence of unlimited feed access, mean T_a on the extensive system fluctuated between 17.6 and 38.8°C implying possible cyclic exposure of birds to varying degrees of heat stress. Exposure to moderate chronic heat induces a decline in performance and birds tend to decrease their heat production by limiting feed consumption (Collin *et al*., 2012) leading to reduced BW. Decreases in BW gain were recorded in broilers exposed to; periods of 31 and 36°C (Quinteiro-Filho *et al*., 2010), 28 to 36ºC (Mazzi *et al*., 2002) and general heat stress (Mashaly *et al*., 2004). Furthermore, Emery *et al*. (1984) showed that birds under cycling temperatures ranging between 21.1 to 37.7°C lost more BW than birds at a constant temperature of 23.9°C. This was largely attributed to reduced feed consumption.

Strain differences observed in BW at the end of the trial are consistent with literature (Chikumba and Chimonyo, 2014; Nthimo *et al*., 2004). In their studies, OV chickens were significantly heavier than PK, which, like in the current study, were heavier than NN at 26 week of age. In the current study, male NN and OV were heavier than females of the same strains. This is consistent with the findings of Nthimo *et al*. (2004) who reported significant differences between sexes on 26-week BW of OV and NN, among other local strains.

The lowest mean T_a recorded in the current study is comparable to 17.9°C observed by Chikumba and Chimonyo (2014) in the same study area. The same is not true, however, for the highest mean T_a Chikumba and Chimonyo (2014) recorded 25.4°C while 38.8°C was recorded in the current study and this might be as a result of the differences in the years studied. Remarkable variability was observed in weather during the observation period.The THI range shows that, at one point or another, birds were exposed to varying degrees of heat stress in both rearing systems. At THI values of 72 - 79, mild heat stress occurs while THI values of 80 - 89 indicate heat stress (Pennington *et al*., 2004). Regression results indicate that free-range birds were affected more by changes in THI. Conversely, THI was lower under free-range conditions, suggesting that perhaps it is the variability that triggered fluctuations in BR and HR as birds tried to maintain equilibrium.

The observation that none of the variables studied influenced RT is contrary to observations of Donkoh (1989) and Lin *et al*. (2005). These researchers reported an increase in RT at 30 and 35°C. Similarly, mean RT increased from 40.36°C at 25°C to 42.41°C at 35°C (Darre and Harrison, 1986; Yahav *et al*., 1998; Mazzi *et al*., 2012). The discrepancy might be as a result of strain differences and duration of exposure. Broilers, as fast-growing birds tend to suffer higher thermal loads compared to slow-growing birds. The strains used in our study are probably adapted to the conditions prevailing in the study area and were as such only narrowly affected by the prevailing environmental conditions. The birds were also reared in a similar environment prior to introduction to the outside pens such that possible acclimation cannot be ruled out. This, together with the 7 d adaptation window, might have enabled the birds to acclimatize to ensuing study conditions. This might explain why even the combined effects of T_a and RH did not have a significant suppressive effect on heat dissipation mechanisms in the birds as reflected in the narrow core (RT) temperature range.

In broilers, ideal RT values vary between 41 and 42°C for a comfort condition (Elson, 1995). We hypothesize that the range could be considerably wider for local strains. It appears the strains used in the current study were able to efficiently thermoregulate and maintain core temperature within a narrow range even at T_a above the TNZ. The TNZ is the interval of thermal environment, usually characterized by T_a over which heat production is relatively constant for a given energy intake (Renaudeau *et al*., 2011). It is defined as a T_a range in which the metabolic rate is minimal and the best performance is achieved (de Souza *et al*., 2015). Any variation in RT indicates that heat exchange mechanisms on the body surface are not sufficient for the maintenance of thermal equilibrium (Nascimento *et al*., 2012).

Heart rate, along with other cardiovascular parameters such as blood pressure and cardiac output, are subject to rapid changes in response to thermal stress (Darre and Harrison, 1986). Average HR from our study was lower than values reported in literature (Darre and Harrison, 1986), a possible consequence of strain differences. The study of Darre and Harrison (1986) did not report the possible effects of increasing THI. Evaporative heat loss increases along with T_a and decreases with increasing RH (Lin *et al*., 2010). This explains the positive linear relationship between THI and HR as observed in this study. Our observation on the reduction in HR with increasing T_a, thus, agrees with earlier observations. Heart rate decreased by 15.5 % from 301 bpm at 25°C to 254, at 35°C (Darre and Harrison, 1986).

Heart rate was highest in the first week of study and decreased progressively. The high HR in the first week may have resulted from the high relative humidity. Literature reports show that direct meteorologic factors affecting birds include elevated T_a and high RH resulting in heat stress leading to elevated HR (Zhou *et al*., 1996; Ayo *et al*., 2011). The subsequent HR decreases observed in this study could also be a consequence of habituation due to repeated exposure to similar environmental conditions as well as handling. Following handling, Eider Ducks *Somateria mollissima* display an elevated HR for 2 - 3 minutes (Cabanac and Guillemette, 2001) afterwhich HR decreases. Habituation is the reduction in physiological responses elicited by exposure to a repeated stressor. Overall, birds showed sexual dimorphism with higher HR in females. This agrees with findings of Sturkie and Chillseyzin (1972) who studied white leghorns. They reported that HR of 211.5 ± 6.56 and 168.0 ± 7.28 bpm were observed for female and male chickens, respectively.

Several factors interact to influence HR in any given environment. Heart rate varies with the method of determination, time of day, sex and age, among other factors. Faster HR are obtained when birds are restrained than when they are free to move about. Measurements are probably most meaningful when made while the birds are free to move about in their normal surroundings. In a hot environment, homeothermic animals increase heat dissipation, reduce heat production and absorption from their environment. A reduction in heat generation often follows a reduction in feed intake. It has also been postulated that thermoregulatory responses start with a decreased HR and peripheral vasodilation and leads to decreased blood pressure (Darre and Harrison, 1986; Chaiyabutr, 2004). These cardiovascular changes occur before thermal panting, which is primarily dependent upon core temperature and begins at about 42°C in chickens. Panting was not observed even at the highest mean T_a of 38.8°C recorded in the current study. This probably indicates a higher degree of thermal tolerance for the strains used. Panting allows poultry to increase evaporative heat loss during heat stress, however, it reduces production efficiency as metabolic energy is diverted from growth and development to maintaining homeothermy (Purswell *et al*., 2012).

Although a higher mean T_a was observed in the free-range system, RH and THI were higher under the intensive system. This probably explains the higher BR observed under intensive management. Humidity suppresses evaporative heat loss such that when body temperature increases, as reflected by an increase in RT, the BR also increases. The observation that there were no strain differences in BR is consistent with the study of Yahav *et al*. (1998) although our expectation was that NN strain would better withstand the effects of high T_a and RH. It is thought that reduced feather cover may be advantageous for thermoregulation at high T_a (Eberhart and Washburn, 1993a) by increasing sensible heat loss. However, no genotype benefit was observed during exposure to temperature cycles in the study of Yahav *et al*. (1998). In other studies, BR increased with increasing T_a (Darre and Harrison, 1986). Increases in BR of up to 165 breaths/ minute were observed in broilers at 42 d of age under high temperatures (Silva *et al*., 2007). Zhou *et al*. (1996) reported increased BR in birds under heat stress. The effect of therml stress is more pronounced in specialized strains with high growth potential compared to the slower-growing chickens. The mean BR range observed in this study, is wider but generally lower than the 40 to 60 breaths/ minute observed in broiler chickens (Nascimento *et al*., 2012). When the thermal requirement of chickens is not satisfied, heat stress may occur, depending on the strain, feathering and nutrition (Lin *et al*., 2010).

4.5 Conclusions

The NN, OV and PK strains appear to exhibit comparable thermal tolerance as they were able to maintain a fairly constant core temperature as reflected in the RT. Both free-range and confined flocks suffered some degree of thermal stress as shown by increases in HR and BR. Based on the differences observed in the preceding chapter, and slight differencies in physiological parameters, it was necessary to further investigate stress indicators in NN, OV and PK chickens exposed to similar rearing conditions.

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Chapter 5: Gut morphology and histological parameters of ileal mucosa in three slow-growing chicken strains under intensive and extensive rearing systems

(Under review*, Animal Science Journal*)

Abstract

A well developed and healthy gut is a pre-requisite for efficient feed digestion and nutrient absorption in birds. The study was designed to investigate effects of strain, sex and feeding of provitamin-A biofortified maize on the gut morphology of Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekok (PK) chickens. Experiment 1 tested the effect of provitamin A bio-fortified maize (PABM) on gut morphology in OV chickens using a white maize (WM)-based diet as the control. At 18 and 21 weeks of age, 16 birds were slaughtered and gut organ weights, ileal villus height (VH), villus density (VD), villus width (VW) and apparent villus surface area (aVSA) were assessed. Neither dietary treatment nor strain influenced ($P > 0.05$) ileal villus morphological measurements. Sex of bird influenced ($P < 0.05$) VH, aVSA, VW and gizzard weight. Age at slaughter affected ($P < 0.01$) muscularis externa (ME) thickness, liver and gizzard weights. Villi were taller, wider, hence greater aVSA in males than females on WM and PABM while ME thickness decreased ($P < 0.01$) between 18 and 21 weeks of age. In Experiment two, 288 NN, OV and PK chickens were allocated to either free-range or confined rearing system and measurements were similar to experiment 1. Strain influenced ($P < 0.05$) VW, aVSA, ME thickness, intestine length, liver, gizzard, pancreas and heart weights. Sex of bird influenced *(*$P < 0.05$) carcass weight (CW), heart, proventriculus and abdominal fat pad (AFP) weight. Rearing system had significant effects on heart, proventriculus, crop, intestine weights as well as large intestine length (LIL), aVSA and ME thickness. The heart, liver and pancreas weights were significantly heavier in OV than PK and NN. Strain and sex of bird influenced gut macro and microstructure. Ileal villus characteristics of OV and PK chickens were comparable. It was concluded that strain and sex of bird influence gut and ileal villus morphology, hence absorptive capacity in slow-growing chickens.

Key words: Chickens, Free-range, Villus, Morphology, Body weight

5.1 Introduction

Poultry rearing in semi-confined and free-range systems has received significant attention in recent years. Legal statutes have been gazetted in different parts of the world to encourage humane production of chickens. In Brazil, policies concerning the criteria for production, supply, processing, distribution and certification of bird quality (DOI/DIPOA 007/99 of 05/19/1999) were enacted in 1999 (Santos *et al*., 2005). In the European Union (EU), conventional cage systems for laying hens were banned in January 2012 according to an EU Council Directive 1999/74/EC on the welfare of laying hens (CEC, 1999). Free-range systems allow birds access to an outside area to promote foraging and expression of normal behaviours such as dust-bathing (Orság *et al*., 2011) which decrease stress thereby increasing comfort (Ponte *et al*., 2008; Wang *et al*., 2009). Birds on free-range systems forage on fibrous materials of plant origin and insects which have high fibre concentration that improves nutrient digestion and gut morphology (Awad *et al*., 2008). Fresh forage also supplies vitamins which are needed for various functions. Vitamin A for instance, is required for animal growth, development, maintenance of health and normal mucus secretion (Karadas *et al*., 2005). Common sources include green leafy materials. It is not clear whether

Vitamin A supplementation enhances gut morphology, however, retinoic acid, a Vitamin A metabolite, is a critical mediator of mucosal immune response and homeostasis (Kunisawa and Kiyono, 2013).

The gastrointestinal tract (GIT) is the largest immunological organ in the body (Choct, 2009). The GIT lining forms the first protective layer against invasion by exogenous pathogens (Ao and Choct, 2006). Material ingested by birds can contain beneficial as well as potentially harmful organisms thus maintenance of gut health is vital for the welfare and productivity of birds (Choct, 2009). Dietary composition, gut microflora and their interaction influence GIT development, mucosal architecture and gut mucus composition (Apajalahti *et al*., 2004) which influence digestive, absorptive and assimilation of the digested nutrients (Incharoen *et al*., 2010). The functional surface area of the intestine is increased by villi and microvilli on cells (Zhang *et al*., 2015). Each villus surface is covered by simple columnar epithelial cells, with cuticular borders, and resting upon connective tissue. Adjacent villi are separated by crypts which are deep pits extending to the muscularis mucosae (Choct, 2009).

Slow-growing chicken strains are more suitable for free-range systems (Castellini *et al*., 2002; Gordon and Charles 2002; Fanatico *et al*., 2007; Moreda *et al*., 2013). Popular strains in Southern Africa include Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) (Nthimo *et al*., 2004; Mapiye *et al*., 2008; Grobbelaar *et al*., 2010). These strains are considered hardy and adaptable to harsh local climatic and environmental conditions. The gross and microscopic GIT structure in these birds are poorly understood. The current study is the first effort aimed at examining gut morphology and villus characteristics of NN, OV and PK strains. It was hypothesised that PABM inclusion would indirectly improve gut development, hence a more developed gut and better nutrient digestion and absorption compared to WM-based diets. It was also predicted that rearing system would influence gut development, particularly ileal villus morphological properties, hence nutrient uptake and general performance.

5.2 Materials and Methods

5.2.1 Animal ethics

Animal care and handling were as previously described in section 3.2.1.

5.2.2 Study site description

The study site was as previously described in section 3.2.2.

5.2.3 Experiment 1: Effect of feeding provitamin A bio-fortified maize on body weights, gut morphology and ileal villus parameters of Ovambo chickens

5.2.3.1 Birds, diet and management

Experiment 1 used 200 unsexed OV chicks that were hatched from parent stock held at the Agricultural Research Council (ARC), Irene, Pretoria in SA. Birds were raised in a well-ventilated floor area of 2 × 2.5 m under a deep litter system where cement floors were adequately covered with a 8 - 10cm layer of wood shavings. A commercial standard broiler starter meal was fed *ad libitum* to the chickens from d 1 to 49. Day-old chicks were maintained at 32°C which was gradually reduced to 21°C by 21 d old by adjusting the height of the infrared lamps from the floor. A thermometer was kept in the house just above the level of the birds and used to monitor changes in ambient temperature. From d 50, birds were given a commercial grower meal. The feeds were provided in tube feeders made of standard gutter material. Water was offered *ad libitum* in 4L plastic founts. The broiler starter and grower feeds were supplied by Meadow Feeds, SA. Light and heat were provided continuously using 70 W infra-red lamps. The birds were vaccinated against Newcastle disease at 14 and 35 d of age while a Gumboro vaccine was administered at 6 weeks old. The vaccines were administered orally through drinking water. A foot bath drenched with disinfectant (Virukill®) was placed at the entrance to the brooding house.

Two dietary treatments were used namely a control diet formulated with 100 % white maize (WM) and the test diet, a provitamin A bio-fortified maize (PABM)-based diet formulated with 100 % HP326-6 maize variety (Table 5.1). The PABM was obtained from the Makhathini Research Station, Jozini, KwaZulu-Natal, SA. The aim of bio-fortification of maize with provitamin A was to increase the concentration of β-carotene in the endosperm of the maize.

At 12 weeks old, 48 male and 48 female birds were randomly selected. The birds were acclimatized to the experimental pen environment for 7 d prior to the commencement of data collection at 13 weeks of age. During this period, the birds were fed on a common proprietary grower diet. The pens were placed in open sided houses with cement floor on a 15 cm deep wood shavings littering. The pens were 230 cm long, 143 cm wide and 120 cm in height. Each experimental unit, represented by a pen, had six birds. Eight pens were allocated for each diet, with four pens for each sex. A minimum of 15 h of light was provided daily throughout the experimental period. No antibiotic or growth promotant was administered during the observation period. Water and feed were supplied *ad libitum* through 4 L plastic founts and suspended 10 L plastic feeders, respectively. The bedding was monitored daily and wood shavings were changed fortnightly. The initial body weight for birds were 1.5 ± 0.5 and 1.0 ± 0.5 kg for males and females, respectively.

Table 5.1. Nutrient composition of experimental diets containing provitamin A bio-fortified maize (PABM) and white maize (WM)

Ingredients (kg)	**WM**	**PABM**
Provitamin A bio-fortified maize	0.0	417.7
White maize	417.7	0.0
Soya meal	175.4	175.4
Vegetable oil	23.8	23.8
Limestone	12.3	12.3
Declaim phosphate	6.9	6.9
Salt	1.9	1.9
DL-Methionine	1.2	1.2
L-Lysine	0.1	0.1
Vit.-min. premix (excluding vit A)	3.2	3.2
Nutrient composition	0.0	0.0
Metabolizable energy (MJ/kg)	12.6	13.0
Crude protein (g/kg)	199.0	198.0
Fat (g/kg)	35.2	50.9
Ash (g/kg)	110.0	97.3
Calcium (g/kg)	10.0	11.0
Phosphorus (g/kg)	7.4	8.1
Provitamin A carotenoids(mg/kg)	0.1	0.5

One kg of feed contained the following: cholecalciferol,60 mg; all-rac-tocopheryl acetate, 30 mg; menadione, 3 mg; thiamine, 22 mg; riboflavin, 8 mg; pyridoxine, 5 mg; cyanocobalamin, 11 mg; folic acid, 1.5mg; biotin, 150 mg; calcium pantothenate, 25 mg; nicotinic acid, 65 mg; Mn, 60 mg; Zn, 40 mg; I, 0.33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0.15 mg; ethoxyquin, 150mg.

5.2.3.2 *Laboratory analyses of feeds*

The fat content of dry milled maize flour was determined using Soxhlet extraction method (Association of Official Analytical Chemists (AOAC), 1990). To determine the crude protein (CP), the total nitrogen content was determined by the Kjeldahl procedure, according to the AOAC (1995). The percentage ash content was calculated as: $\% \, Ash = \frac{Weight\ of\ ash \times 100}{Weight\ of\ sample}$ (AOAC, 1980). The gross energy values were estimated by multiplying the crude protein, fat and carbohydrate by their water values of 4, 9 and 4 kcal/ g, respectively. Calcium and phosphorus were determined by atomic absorption spectrophotometry and calorimetrically, respectively, according to AOAC (1984). Carotenoid analysis was carried out using a Hewlett Packard 1100 HPLC (Agilent Technologies Incorporated, Loveland, CO, USA) consisting of a binary pump, autosampler, column thermostat, diode array detector and ChemStation software (Revision B.03 02, Agilent Technologies Incorporated, Loveland, CO, USA).

5.2.3.3 *Data collection*

At 18 and 21 weeks, 16 birds from each dietary treatment, comprising 8 females and 8 males and 2 birds/replicate, were randomly selected for carcass, internal organ weights and ileal villus measurements. Feed was withdrawn 5 h before slaughter. The birds were weighed on a digital scale, euthanized by cervical dislocation and slaughtered by exsanguination. The carcasses were scalded in hot water at about 60°C for approximately 63 s and the feathers plucked manually. Carcasses were eviscerated and portions cut and separated. Digestive tract organs (empty crop, proventriculus, gizzard, liver (without gall bladder), pancreas, intestines (duodenum + jejunum + ileum) and abdominal fat pad)) were weighed, using a digital electronic scale (Jadever JPS-1050, Micro Preciso Calibraton Inc, USA; ± 1 g sensitivity). The length of the entire tract was determined with the aid of a flexible tape (± 0.1 mm) on a glass surface to prevent inadvertent stretching. Determination of bird and organ weights was done within 10 minutes of dissection. No chick deaths were recorded during the entire experimental period.

For ileal villus measurements, the ileum was separated from the rest of the segments at the Meckel's diverticulum to ileocecal-colonic junction. Segments, 2 - 3 cm in length, were collected from the midpoints of the ileum (Incharoen, 2013) and flushed with Phosphate Buffered Saline (PBS) to remove intestinal contents. The segments were immediately fixed in buffered formalin for storage pending further analyses. Thereafter, they were cut into 1 cm sections, dehydrated through a graded series of ethanol and embedded in paraffin wax. Embedded samples were transversely cut at 4 µm, mounted onto glass slides and stained with haematoxylin-eosin. Slides were observed under a Trinocular Research Microscope, Model B-5127, India, fitted with an IS camera model S300, at × 40 magnification.

Gut mucosa morphometric measurements were made with the aid of a B&L Olympus eye-piece graticule, calibrated using an Olympus B&L stage micrometer (0.01 mm) at the same magnification. Histological measurements (villus height (VH), villus density (VD), villus width (VW), muscularis externa (ME), and submucosa (SM) thickness) were assessed. Procedures described previously (Incharoen *et al*., 2010) were followed with minor modifications. Villus density was defined as the number of villi encountered within the entire range of the eye-piece graticule. For measurement of VH and area, two villi were randomly selected from each cross-section. The VH was considered as the distance from tip to base, excluding the intestinal crypt. Villi widths were determined at the basal and apical points of each villus. Basal and apical widths were measured approximately one-third and two-thirds from each selected villus, respectively. Apparent villus surface area (aVSA) was calculated by the following modified formula of Iji *et al*. (2001):

$$\mathrm{aVSA} = [(\mathrm{VW}_1 + \mathrm{VW}_2) \times 2^{-1} \times \mathrm{VH}],$$

Where; aVSA = apparent villus surface area; VW_1 = villus width at $\frac{1}{3}$ of the villus; VW_2 = villus width at $\frac{2}{3}$ of the villus and VH = villus height. The calculations of villus area were expressed as the mean for each bird.

5.2.4 Experiment 2: Effect of strain and sex of bird on gut and ileal villus morphology in NN, OV and PK chickens

A total of 288, 20-week old dual purpose slow-growing chickens comprising PK, OV and NN strains were used in the study. Birds were allocated to four pens each of intensive and extensive systems. There were 12 males of each strain and the same number of females/ strain in each of the 8 pens. The pens on the extensive system, measuring 900 m^2 each (Figure 3.1), were demarcated by 2.2 m high wire mesh reinforced by wooden and steel poles. *Chloris gayana* (Katambora Rhodes grass) was the dominant grass species. Similarly, males and females of the 3 test strains were separated by wire mesh in a poultry house measuring 4 × 10 m. The birds were weighed individually on a digital scale, model UME CCS-150K, S/N: NXC 100020, to determine BW.

5.2.4.1 Bird management

Wooden cages measuring 2.5 × 2 m were placed uniformly in one corner of each pen to provide shelter for the free-range birds. The cages, with slatted floors elevated 1 m above the ground surface, had louvered walls approximately 2.2 m above the floor. Cages were fitted with wire mesh doors to deter predators. Doors were left open during the day and closed between 1730 h and 1830 h after all birds had voluntarily climbed into the cages. The photoperiod during the observation period was approximately 10 h long. A standard plastic drinker was placed under shade near each cage to provide cool clean water. The drinkers were inspected, washed and replenished at least twice a day to ensure *ad libitum* access to clean water.

The poultry house, fitted with 2 roof air-vents and side curtains on both sides to ensure adequate ventilation, had corrugated iron sheet roofing. Fluorescent lamps were used for lighting. The housed birds were raised on a deep litter system with about 10 cm wood shavings layer as bedding. The litter was constantly inspected for wetness and changed fortnightly or as necessary.

5.2.4.2 Brooding, feeding and health management

Day-old chicks of OV, NN and PK strains were obtained from a parent flock kept at the Agricultural Research Council (ARC), Irene, Pretoria, SA. Chicken management and rearing were as described in Experiment 1.

5.2.5 Data collection

5.2.5.1 Body weight

A total of 72 birds across all 3 strains were randomly selected and weighed using a digital scale and BW recorded. Three birds/ strain were sampled per pen to give a total of 36 birds on each system. Altogether, 72 birds comprising 36 cocks and 36 hens were used. The birds were weighed weekly at 0900 h each time throughout the study.

5.2.5.2 Sampling procedure

Thirty-two, 26-week old NN, OV and PK chickens, comprising 16 of each sex were randomly selected. The chickens were subjected to a 5 h feed withdrawal period and weighed individually. Birds were euthanized by cervical dislocation and slaughtered by exsanguination. Carcasses were immersed in water at 60°C for 63 s and plucked manually.

5.2.5.3 Gut and intestinal anatomy

Gut and intestinal morphology measurements were conducted as previously described in Experiment 1.

5.2.6 Statistical analyses

The data were tested for normality using the Shapiro-Wilk test and log_{10}-transformed wherever data were not normal. Data were analyzed using the General Linear Models (GLM) procedure of the Statistical Analysis System, ver 9.3 (SAS, 2010) with strain, sex as the main effects. The model $Y_{ijkl} = \mu + T_i + S_j + A_k + (B \times S)_{ij} + (B \times A)_{ik} + \varepsilon_{ijklmn}$ was used, where; Y_{ijkl} = response variable (BW, CW, DC, internal organ weights, VD, ViH, aVSA, VW, ME, serosa and submucosa thickness); μ = general mean common to all observations; T_i = effect of the i^{th} dietary treatment (*i* = WM, PABM); S_j = effect of the j^{th} sex of bird (*j* = Male, female); A_k = effect of the k^{th} age at slaughter (*k* = 18, 21); $(T \times S)_{ij}$ = effect of the interaction between dietary treatment and sex of bird; ε_{ijkl} = random error term.

The following model was used for data from Experiment 2; $Y_{ijkl} = \mu + B_i + S_j + R_k + (B \times S)_{ij} + \varepsilon_{ijkl}$, where; Y_{ijkl} = response variable (BW, VH, VW, aVSA); μ = general mean common to all observations; B_i = effect of the i^{th} strain (*i* = NN, OV, PK); S_j = effect of the j^{th} sex of bird (*j* = Male, female); R_k; effect of the k^{th} rearing system (*k* = Intensive, Extensive); $(B \times S)_{ij}$ = effect of the interaction between strain and sex of bird; ε_{ijkl} = random error term. Least square means (LSMEANS) were compared using the PDIFF options of SAS (2010). Statistical significance was considered at the 5 % level of probability and interactions that had no effect were dropped from analyses.

5.3 Results

5.3.1 Experiment 1

The levels of significance for fixed effects on parameters studied are shown in Table 5.2. PABM inclusion had no effect ($P > 0.05$) on any of the parameters studied (Table 5.3). Sex of bird affected ($P < 0.001$) BW, CW and DC. Males had higher BW and consequently, higher CW and DC. Sex of bird influenced ($P < 0.05$) VH, aVSA, VW and gizzard weight. Age at slaughter affected ($P < 0.01$) ME thickness as well as liver and gizzard weights. Significant treatment × sex interactions were observed on BW, CW, DC and ME thickness.

Females had a higher dressed carcass weight ($P = 0.0002$) than males (Table 5.4). There was a significant difference ($P < 0.05$) in VH between males and females. Villi were taller, wider hence greater surface area in males than females (Figure 5.1) on both dietary treatments. Muscularis externa thickness decreased ($P < 0.01$) between 18 and 21 weeks of age (Table 5.4). However, liver and gizzard weight significantly increased from 18 to 21 weeks of age.

Table 5.2. Effect of provitamin A bio-fortification on various response parameters

Parameters	Effect			
	Treatment	**Sex**	**Age at slaughter**	**Treatment × Sex**
Body weight	NS	***	NS	*
Carcass weight	NS	***	NS	*
Dressed carcass	NS	**	NS	*
Villus density	NS	NS	NS	NS
Villus height	NS	*	NS	NS
aVSA	NS	*	NS	NS
Basal VW	NS	*	NS	NS
Basal VW	NS	*	NS	NS
Crypt depth	NS	NS	NS	NS
ME thickness	NS	NS	*	NS
Serosa	NS	NS	NS	NS
Submucosa	NS	NS	NS	NS
Liver	NS	NS	**	NS
Pancreas	NS	NS	NS	NS
Crop	NS	NS	NS	NS
Proventriculus	NS	NS	NS	NS
Gizzard	NS	*	***	NS
Entire tract	NS	NS	NS	NS
SI length	NS	NS	NS	NS
LI length	NS	NS	NS	NS

****P < 0.0001; **P < 0.001; *P < 0.05; NS: Not significant; aVSA: Apparent villus surface area;*

VW: Villus width; ME: Muscularis externa; SI: Small intestine; LI: Large intestine

Table 5.3. Effect of feeding PABM and WM on body, carcass, dressed weights and ileal villus characteristics

	Diet		
	PABM	**WM**	***P value***
Body weight	2.0±0.06	2.0±0.05	NS
Carcass weight	1.4±0.04	1.4±0.03	NS
Dressed weight	0.7±0.00	0.7±0.00	NS
Villus density	4.4±0.16	4.1±0.12	NS
Villus height	7.0±0.45	7.5±0.35	NS
Basal VW	2.2±0.09	2.4±0.07	NS
Apical VW	1.8±0.07	1.9±0.05	NS
aVSA	14.4±1.51	16.7±1.17	NS
Muscularis externa	13.2±0.56	13.6±0.44	NS

PABM: Provitamin A bio-fortified maize; WM: White maize; NS: Not significant; VW: Villus width; aVSA: apparent villus surface area

Females had a higher dressed carcass weight (P = 0.0002) than males. There was a significant difference (P < 0.05) in VH between males and females. Villi were taller, wider hence greater aVSA in males than females (Figure 5.1) on both dietary treatments. Muscularis externa thickness decreased (P < 0.01) between 18 and 21 weeks of age. However, liver and gizzard weight significantly increased between weeks 18 to 21 of observation. Diet × sex interaction effects on BW, CW and DW are shown in Table 5.5.

5.4 Experiment 2

5.4.1 Strain differences

Strain influenced (P < 0.05) BW, VW, aVSA, muscularis externa thickness, large intestine length (LIL), entire intestinal length, liver, gizzard, pancreas and heart weights. Sex of bird had an effect (P < 0.05) on BW, CW, heart, proventriculus and AFP weight. Sex marginally influenced (P = 0.0511) gizzard weight. Levels of significance for parameters studied are shown in Table 5.5.

5.4.2 Internal organ weights and ileal villus morphology

BW, heart, liver and pancreas weights were significantly heavier in OV than PK and NN (Table 5.6). The same strain, however, had the smallest gizzard among the three strains. Body weight, heart and gizzard weights were significantly higher in males compared to females (Table 5.7). Proventriculus and AFP weights were higher in females. On ileal villus morphology, muscularis externa thickness and aVSA were significantly higher in confined birds. Crop weight was, however, significantly higher in the free-range birds.

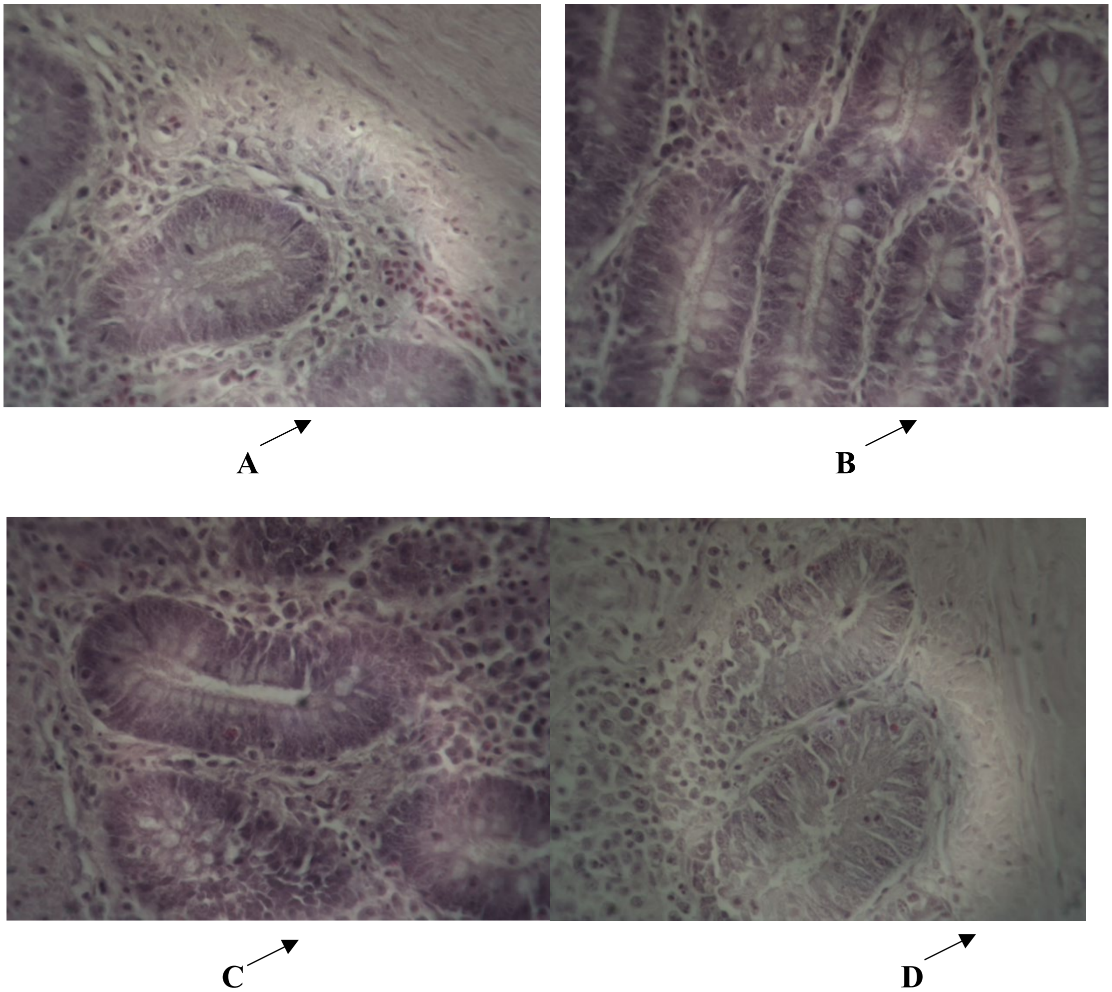

Figure 5.1 Ileal villi appearance at ×40 magnification for female (L) and male (R) Ovambo chickens on WM (A and B) and PABM (C and D) at 18 weeks

Table 5.4. Effects of interaction between diet and sex of bird on weight and ileal villus morphological characteristics

Fixed effects		Response parameter				
		Weight			Ileal villus	
Sex	Treatment	Body (kg)	Carcass (kg)	Dressed carcass (%)	Height (µm)	Surface area (μm^2)
Female	PABM	1.8±0.11	1.3±0.08	0.7±0.01	6.0±0.82	11.4±2.77
Female	WM	1.5±0.07	1.0±0.05	0.7±0.01	7.2±0.49	14.1±1.65
Male	PABM	2.2±0.06	1.5±0.04	0.7±0.00	8.1±0.42	17.3±1.42
Male	WM	2.4±0.07	1.7±0.05	0.7±0.01	7.7±0.49	18.5±1.65
P-value		*0.0049*	*0.0018*	*0.0863*	*0.2148*	*0.0322*

PABM: Provitamin A bio-fortified maize

WM: White maize

Table 5.5. Effect of rearing system, strain ans sex of bird on response parameters

	Fixed effect			
Parameter	**Rearing system**	**Strain**	**Sex**	**Strain × Sex**
Body weight	NS	*	*	NS
Carcass weight	NS	NS	*	NS
Dressed weight	NS	NS	NS	NS
Heart	*	*	*	NS
Spleen	NS	NS	NS	NS
Pancreas	NS	*	NS	NS
Proventriculus	*	NS	*	*
Gizzard	NS	*	NS	NS
Liver	NS	*	NS	*
Crop	*	NS	NS	NS
Intestine weight	*	NS	NS	**
Intestine length	NS	*	NS	*
SI length	NS	NS	NS	*
LI length	*	*	NS	*
Abdominal fat pad	NS	NS	*	NS
Villus density	NS	NS	NS	NS
Villus height	NS	NS	NS	NS
Basal VW	NS	NS	NS	NS
Basal VW	NS	*	NS	NS
aVSA	**	*	NS	NS
Muscularis externa	*	*	NS	NS
Serosa	NS	NS	NS	NS
Submucosa	NS	NS	NS	NS

****P < 0.0001; **P < 0.001; *P < 0.05; NS: Not significant; aVSA: Apparent villus surface area; VW: Villus width; SI: Small intestine; LI: Large intestine*

Table 5.6. Effect of strain of bird on BW, internal organ weight (g) and intestine length (cm)

	Strain		
	NN	OV	PK
BW	1580.6±99.85^{b}	2072.5±108.53^{a}	1965.8±108.53^{ab}
Heart	7.9±0.97^{b}	11.5±1.06^{a}	11.1±1.06^{a}
Gizzard	62.7±3.22^{a}	54.0±3.5^{b}	67.9±3.5^{a}
Liver	26.1±2.19^{b}	37.4±2.38^{a}	32.3±2.38^{ab}
Pancreas	1.8±1.14^{b}	6.5±1.23^{a}	5.0±1.23^{a}
Proventriculus	6.7±0.56^{b}	7.4±0.61^{b}	8.9±0.61^{a}
Intestinal tract†	119.3±4.84^{b}	139.3±5.26^{a}	140.3±5.26^{a}
Large intestines†	22.5±0.56^{b}	23.9±0.61^{ab}	24.9±0.61^{a}
Ileal villus morphological characteristics			
Apical VW	2.1±0.08^{a}	1.6±0.09^{b}	1.7±0.09^{b}
aVSA	17.7±0.46^{b}	18.4±0.50^{a}	19.7±0.50^{a}
ME thickness	21.7±1.59^{b}	29.4±1.73^{a}	29.9±1.73^{a}

BW: Body weight; NN: Naked Neck; OV: Ovambo; PK: Potchefstroom; VW: Villus width; aVSA: Apparent villus surface area; ME: Muscularis externa

†Represent length and as such units of measurement were cm and not g as for the other parameters

Table 5.7. Effect of sex of bird on body weight, carcass weight, heart, proventriculus, gizzard and abdominal fat pad weight

	Sex	
	Male	**Female**
Live body weight	2252.5±114.43^{a}	1493.4±114.43^{b}
Carcass	1918.8±115.76^{b}	1239.2±115.76^{a}
Heart	13.8±1.11^{a}	6.5±1.11^{b}
Gizzard	68.4±3.69^{a}	54.8±3.69^{b}
Proventriculus	5.6±0.65^{b}	9.7±0.65^{a}
Abdominal fat pad	16.4±1.39^{b}	21.8±1.39^{a}

$^{a, b}$*Values in the same row with different superscripts differ significantly (P < 0.05)*

Table 5.8. Effect of rearing system on internal organ weights and ileal apparent villus surface area (μm^2) and muscularis externa thickness (μm) in slow-growing chickens

	Rearing system	
	Extensive	**Intensive**
[1]BW	1801.7±179.11[b]	2158.1±95.12[a]
Heart	9.3±1.74[b]	13.6±0.93[a]
Proventriculus	7.5±1.01[b]	8.2±0.54[a]
Crop	12.8±1.09[a]	10.3±0.58[b]
Intestines	63.2±3.84[b]	69.9±2.04[a]
*Large intestines	23.4±1.01[b]	25.2±0.54[a]
[2]ME thickness	7.5±1.01[b]	8.2±0.54[a]
Apparent villus surface area	9.3±1.74[b]	13.6±0.93[a]

[1]*Body weight*

**Represents length and as such units of measurement were cm and not grams as for the other parameters*

[2]*Muscularis externa*

5.5 Discussion

The observation that feeding provitamin A bio-fortified maize to the birds did not influence any of the parameters studied is surprising. It was anticipated that BW, for instance, would be influenced by inclusion of vitamin A in the diet. Vitamins positively influence gut development and health, in general, as well as nutrient absorption in the gut specifically. Lack of noticeable changes can be attributed to the age at which the dietary treatments were introduced. The timing and form of nutrients available to chicks soon after hatch is critical for development of intestines (Yegani and Korver, 2008). The observation on higher BW in males is consistent with literature reports (Chikumba and Chimonyo, 2014; Nthimo *et al*., 2004). Perhaps the broader and taller villi in males are among the factors accounting for the higher BW in males.

Muscle accretion is influenced by hormones and is more extensive in males hence the sexual dimorphism observed in the current study. A broad and tall villus translates to a high villus surface area, thus increased surface area for nutrient absorption. Lengthening of intestinal villi increases the surface area for nutrient absorption (Jiang *et al*., 2012). Also, changes in intestinal morphology, such as shorter villi and deeper crypts, have been associated with the presence of toxins (Choct, 2009). The increase in gizzard weight with increasing age was expected. Feed intake is likely to increase with increasing BW, thus, an increase in gizzard size is in response to the need to grind more feed particles in preparation for further digestion in the lower parts of the GIT (Yegani and Korver, 2008). An increase in muscularis thickness has been reported in literature (Gunal *et al*., 2006), and was attributed to increased gram negative bacteria counts. During bacterial infection, there is lymphocyte proliferation in order to kill bacteria and cause inflammation, and this increases muscularis thickness.

Intestinal surface area is a key determinant of the overall hydrolytic capacity of membrane bound digestive enzymes and absorptive capacity of the same (Zhang *et al*., 2015). Shorter and lighter intestines were observed under free-range rearing where birds fed mainly on fibrous material. The difference in intestine weight observed in Experiment 2 can be attributed to reduced muscularis externa thickness (Gunal *et al*., 2006). The morphology of the intestinal epithelium is particularly affected by both diet composition (Jiang *et al*., 2012) but also, the nature of the diet. A decrease in the relative length of all components of the GIT as grain particle size increased was reported by Amerah *et al*. (2007). Besides dietary influence on GIT development, the observed decrease in intestinal weight and/or length is thought to contribute to improved feed efficiency due to reduced maintenance cost (Xu *et al*., 2015). Incharoen *et al*. (2010) concluded that a reduction in dietary CP would also affect intestinal morphology. The diet composed by free-range chickens is likely to have been deficient in CP. The same study by Incharoen and co-workers (2010) noted an increase in duodenal and jejunal weight and length in response to a low-CP diet. This was attributed to a compensatory mechanism meant to increase absorptive capacity in an attempt to assimilate any available nutritional benefit from the hypo-protein diet. The absence of differences in AFP yield, in the current study, is consistent with findings of Santos *et al*. (2005).

Strain differences in final BW have been reported before (Chikumba and Chimonyo, 2014; Nthimo *et al*., 2004; Mikulski *et al*., 2011) but contradicts the findings of Zhao *et al*. (2015). Strain differences in selected parts weights e.g. liver and proventriculus were observed between 2 broiler strains (Santos *et al*., 2005). The observation that sex of bird influenced BW in this study agrees with earlier studies (Chabault *et al*., 2012; Zhao *et al*., 2015) where males were heavier than females. Cocks were 15 - 20 % heavier than hens at slaughter age (Mignon-Grasteau *et al*., 1998). Similar sexual dimorphism was reported by Remesˇ and Sze´kely (2010). Sexual dimorphism in relation to BW can be ascribed to differences in feed intake as well as hormonal influence on tissue accretion. The observation on males yielding less abdominal fat than females was expected and is consistent with literature (Hrnčár *et al*., 2010). Higher fat yield in females was also observed in the study of Santos *et al*. (2005). Generally, females deposit more fat compared to males. Bigger organs in the heaviest bird is consistent with expectations. Gut and other organs are expected to grow proportionate to BW in order to support tissue and cellular demands for nutrients and oxygen, among other requirements.

Mean BW, heart, proventriculus, crop and intestinal weight observed in the study were higher in confined than free-range birds. Similar results on BW were observed by Wang *et al*. (2009) contrary to findings of Santos *et al*. (2005) who compared growth performance between confined and semi-confined birds. The former reported that BW and weight gain of free-range chickens were significantly lower than those of confined chickens. The differences observed may be due to the inherent variability typical of free-range systems. Birds are exposed to factors that are inherently variable including light intensity, photoperiod, and temperature (Li *et al*., 2016) and humidity.

The exposure of birds to cyclic temperatures and humidity and increased exercise raises their energy requirement thus influencing their feed conversion and hence, overall growth performance (Li *et al*., 2016) resulting in inferior growth performance. The same was also echoed by Wang *et al*. (2009). In a separate study, rearing system had no effect on BW and fatty acid profile of lipids in abdominal fat (Mikulski *et al*., 2011). The absence of differences in gizzard weight between rearing systems was surprising. It was anticipated that free-range birds would yield heavier gizzards. Our observation contradicts that of Santos *et al*. (2005) where gizzard weight was higher in semi-confined compared to confined birds resulting from greater intake of fibre and grit. The difference could probably be a consequence of access to wood shavings by confined birds. The physical form of dietary structural components, such as dietary fibre, may affect the morphological and physiological characteristics, hence GIT development and function (Xu *et al*., 2015) and this influences the growth of chickens (Brunsgaard, 1998; Engberg *et al*., 2004). Also, gizzard weight was higher in males than females in the current study, contrary to findings of Santos *et al*. (2005).

Crop weight was significantly higher on the free-range system, a possible consequence of distension in order to increase holding capacity. Fibre digestion is slow and this may lead to diastrophy in the crop, an organ adapted for temporary feed storage. The abdominal fat yield of chickens in the free-range system was expected to be significantly lower than that of chickens raised in confinement. This was not observed in this study, contrary to observations of Li *et al*. (2016) but consistent with findings of Mikulski *et al*. (2011). Free-range birds are exposed to variable environmental conditions which could increase the birds' metabolic rates leading to extensive use of energy thus reduction in abdominal fat deposition. The higher muscularis externa thickness and aVSA in free-range birds are possible compensatory responses to hypo-caloric and hypo-protein diets, meant to increase the efficiency with which available nutrients are extracted from the lean diets. It is important to note that there are several key factors that have been inconsistent among studies on the effects of different raising systems on bird performance (Li *et al*., 2016) hence the need for more research in this area particularly focusing on the small intestine since changes in its fine morphology can alter absorption rate, weight gain, hence performance (Rezaian and Hamedi, 2012).

5.6 Conclusions

Strain and sex of bird influenced the gut macro- and microstructure, particularly intestine length and weight, ileal villus height and surface area, hence absorptive capacity. The PK and OV chickens are comparable in ileal villus characteristics. Lower GIT weights and shorter intestines were observed in free-range birds. Free-range birds had heavier crop sizes compared to confined birds. Based on the differences observed in the preceding chapters, and slight differencies in physiological parameters, it was necessary to further investigate stress indicators in NN, OV and PK chickens exposed to similar rearing conditions.

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Chapter 6: Effect of strain, sex of bird and rearing system on duration of tonic immobility, heterophil to lymphocyte ratio and organ weights in slow-growing chickens

*(*Published in*, the Journal of Applied Poultry Research, appendix 3)*

Abstract

Effects of sex and strain of bird on factors influencing welfare in chickens were investigated in 288, twenty-one week old Potchefstroom Koekoek (PK), Ovambo (OV) and Naked Neck (NN) chickens. The birds were allocated to 4 free-range pens of *Chloris gayana* and a house subdivided into 4 pens. There were 12 males/ strain and 12 females/ strain in each pen. Twelve birds, 4 each of NN, OV and PK, were randomly selected per pen and tonic immobility (TI) was determined. Ambient temperature and humidity were recorded and fitted into a PROC MIXED model as random effects, with strain and sex as main effects. On the last day of the trial, blood samples were collected from nine (3 of each strain) randomly selected birds per pen, via brachial venepuncture, using 5 mL syringes and 22 gauge needles. At slaughter, the liver and spleen were recovered and weighed immediately. Organ weights were expressed relative to body weight (BW) of each bird. Sex of bird influenced ($P < 0.05$) BW, spleen and relative liver weights and heterophil to lymphocyte (H/L) ratio. Strain did not influence ($P > 0.05$) TI but affected ($P < 0.05$) all other parameters. Strain × sex interactions were significant ($P < 0.05$) on all organ weights. Males appeared more stressed than females. The free-range system could minimise stress in birds though mechanisms should be devised to prevent attacks by predators. Perhaps future research could be designed to study similar parameters over a longer observation period using younger slow-growing chickens.

Key words: Chickens, Strain, Stress, Temperature, Tonic immobility

6.1 Introduction

The demand for meat from alternative systems requires that birds are raised under management conditions that uphold and promote animal welfare, with minimal use of medical treatments (Hangalapura *et al*., 2003; Quinteiro-Filho *et al*., 2010; Sossidou *et al*., 2011). The main focus is to allow outdoor access to birds. This provides birds with ample space, fresh air, direct sunlight, and allows them to express natural behaviours such as dust bathing, scratching, foraging, running and flying (Fanatico, 2008).

Hot and semi-arid conditions prevalent in most parts of sub-Saharan Africa present numerous challenges to chicken production. Birds cannot stay in thermal equilibrium with the environment causing physiological and behavioural changes, which reduces performance (de Souza *et al*., 2015). Chickens are particularly sensitive to heat stress (Renaudeau *et al*., 2011; Fathi *et al*., 2013; Lara and Rostagno, 2013). Free-ranging chickens also face a variety of stressors including food deprivation, agonistic social interactions, human disturbances, predators, injury, endo- and ecto-parasites and diseases (Sossidou *et al*., 2011). Predators such as foxes, dogs, badgers, mink and birds of prey can be attracted to pastures. Their presence induces fear (Faure *et al*., 2003; Campo *et al*., 2008).

Duration of tonic immobility (TI), heterophil to lymphocyte (H/L) ratio as well as lymphoid organ weights are reliable indicators of stress in chickens (Altan *et al*., 2003). Tonic immobility, also known as thanatosis, death feigning or catatonia, is a behavioural state characterized by lack of movement and an apparent lifeless position (Gallup and Rager, 1996; Miyatake *et al*., 2009). Animals attacked by predators often enter a state of TI in which individuals appear to simulate death (Edelaar *et al*., 2012). It is an adaptive behavioural strategy and its duration is considered to be positively related to the antecedent fear state. The H/L ratio is also a reliable index for determining stress in poultry (McFarlane and Curtis, 1989). Stress leads to involution of immunological organs.

Free-range systems make use of slow-growing strains which are more suitable to these production systems. Popular slow-growing strains in sub-Saharan Africa (SSA) include Naked Neck (NN), Ovambo (OV), Potchefstroom Koekoek (PK) and Venda chickens (Grobbelaar *et al*., 2010; Nthimo *et al*., 2004) which are dual purpose strains. They are closely associated with rural livelihoods in Southern Africa where they are used to meet households' nutritional and economic needs (Mapiye *et al*., 2008). Hardiness and adaptability to high ambient temperature and humidity are important attributes since free-range systems often entail exposure of birds to inclement weather conditions. Despite the reported adaptability to harsh conditions, the productivity of slow-growing chickens remains low. Different strains and sexes are likely to respond differently to various stressors hence variation in productivity. Effect of these particular strains and sex on productivity, in addition to effects of interaction of bird factors with the environment, have not been studied. In the previous Chapter, it was noted that strain, sex and rearing system influence gut morphology in slow-growing birds. The current Chapter further interrogates effects of strain, sex and rearing system on stress indicators including duration of TI, H/L ratio and organ weights of free-range PK, OV and NN chickens reared in a high temperature environment. It was hypothesised that free-ranging, by providing a near-natural environment, could ensure better bird welfare hence performance.

6.2 Materials and Methods

6.2.1 Animal ethics

Animal care and handling were as previously described in section 3.2.1.

6.2.2 Study site

The study site was as previously described in section 3.2.1. The weekly mean, minimum and maximum ambient temperature and humidity experienced over the trial period were given in Table 3.2 (Chapter 3).

6.2.3 Treatments, experimental design and bird management

A total of 288, 21-week old PK, OV and NN chickens were used in the study. The birds were randomly allocated to four 900 m^2 free-range pens of *Chloris gayana* and a 4 × 10 m house subdivided into 4 pens. There were 12 males/ strain and 12 females/ strain in each pen. As a result, there were 36 males (12 each of NN, OV and PK) in any 2 pens and the same number of females in the other 2 pens per rearing system. Sexes were separated by wire mesh. Free-range pens were demarcated by 2.2 m high wire mesh and reinforced by wooden and steel poles. The birds were weighed individually on a digital scale, model UME CCS-150K, S/N: NXC 100020, to determine initial body weights.

6.2.4 Bird management

Wooden cages measuring 2.5 × 2 m were placed uniformly in one corner of each free-range pen to provide shelter for the birds. The cages, with slatted floors elevated 1 m above the ground surface, were fitted with wire mesh doors to deter predators. Doors were left open during the day and closed at night. A 12 L plastic drinker was placed under shade near each cage to provide cool clean water. The drinkers were inspected, washed and replenished at least twice a day to ensure *ad libitum* access to clean water.

6.4.5 Brooding, feeding and health management

Day-old chicks of OV, NN and PK strains were obtained from parent flock held at ARC, Irene, Pretoria, SA. From d 1 to d 49 chicks of each strain were reared in 2 × 1.5 m pens in a closed well ventilated poultry house which was 4 × 10 m in area. The house floors were covered with a 10 cm thick layer of wood shavings. Infrared lamps (75 W) were used as a source of heat and light. The day-old chicks were maintained at 32°C which was gradually reduced to 21°C by 21 d old by adjusting the height of the infrared lamps from the floor. A thermometer was kept in the house just above the level of the birds and to monitor changes in temperature. Broiler starter mash was offered *ad libitum* from standard tube feeders while potable tap water was offered *ad libitum* through 4 L plastic founts. Chicks were vaccinated against Newcastle disease at 10 and 35 d of age. A foot bath drenched with Virukill® was placed at the entrance to the brooding house. From d 50, birds were given a grower meal supplied by Meadow feeds, SA. The nutritional composition of the feeds is shown in Chapter 3, Table 3.1. At 20 weeks old, selected birds were assigned to 4 free-range pens located side by side and separated by a fence (Figure 3.1; Chapter 3). The Katambora Rhodes grass pens were rain-fed. The free-range pens were managed as described in Chapter 4. Weeds and other invader grass species were hand-picked and eliminated from the pens. Wooden cages described earlier were used to house the birds on each free-range pen.

6.4.6 Data collection and measurements

6.4.6.1 Tonic immobility

After seven days of acclimatization, TI was measured according to the modified protocol described earlier (Edelaar *et al*., 2012; Hrabcakova *et al*., 2012), once a week for 4 weeks. Twelve birds were randomly selected per pen to represent each of the 3 strains and both sexes. Duration of TI was determined in a separate enclosed area to avoid disruption of TI by sound or movement. All birds selected were caught and carried in an upright position to the room. Duration of TI was measured with the aid of a U-shaped wooden cradle and a stop watch, model 870A Century clock-timer. Tonic immobility was induced by placing the chicken on its back and exerting light pressure to the breast area for 15 s. After 15 s, the hand was gently removed and the stop watch started. During removal of the hand from the breast of the bird, the researcher retreated approximately 1 m within sight of the bird and remained silent and motionless carefully watching the bird. Duration of TI was determined with the aid of a stop watch.

Any bird that righted itself within 10 s had the induction process repeated, up to a maximum of 3 times. If a chicken stayed on its back for over 10 s, the time taken until the bird righted itself was recorded and was regarded as the duration of TI. The induction session was terminated if a bird remained in TI for 10 minutes and the bird was assigned the maximum duration of 600 s. If TI had not been induced after 3 attempts, the duration of TI would be considered 0 s. A maximum of 2 inductions was recorded in this study. Movements of the observer were minimized to avoid terminating TI. Tonic immobility was assessed four times, with seven days in between each assessment and tests were performed between 0930 and 1600 h each time.

6.4.6.2 Heterophil to lymphocyte (H/L) ratio

The heterophil to lymphocyte (H/L) ratio for each bird was determined according to Altan *et al*. (2003). On the last day of the trial, blood samples were taken from 6 randomly selected birds of each strain per pen via brachial venipuncture using 5 mL syringes and 22 gauge needles. The blood samples were collected into purple top, 5 mL ethylene diamine tetra-acetic acid (EDTA)-coated vacutainer tubes and placed in a cooler box with ice and transferred to the University of KwaZulu-Natal, Pietermaritzburg. Blood samples were stored in a refrigerator at -4°C, pending analyses.

Two glass slides and smears were prepared for each sample/ bird and stained with Wright stain for 15 minutes. The slides were then observed under a light microscope, model BX41TF, Olympus Corporation, Tokyo, Japan at × 100 magnification. One hundred leucocytes including heterophils, lymphocytes, monocytes, basophils and eosinophils were counted on each slide and H/L ratio calculated by dividing the number of heterophils by that of lymphocytes.

6.4.6.3 Organ weights

On the last day of the trial, all the birds were weighed using a digital scale (Jadever JPS-1050, Micro Preciso Calibraton Inc, USA; ±1 g sensitivity). Immediately after weighing, six birds per group, comprising two of each strain, were randomly selected and euthanized by cervical dislocation. At necropsy, the liver and spleen were recovered and weighed immediately. The weights were expressed relative to BW of each bird.

6.4.7 Statistical analyses

Data were checked for normality using the Shapiro-Wilk test. Duration of TI, relative spleen weight, H/L ratio and all leucocyte count data were $\log_{10}$-transformed to confer normality. For data on TI and BW, the main effects were analysed using the PROC MIXED of SAS (2010). The model $Y_{ijklmno} = \mu + B_i + S_j + R_k + W_l + T_m + H_n + (B \times S)_{ij} + \varepsilon_{ijklmno}$ was used for TI data where $Y_{ijklmno}$ = the response variable (duration of TI, BW); μ = the overall mean common to all observations; B_i = the effect of the i^{th} strain (*i* = NN, OV, PK); S_j = the effect of the j^{th} sex (*j* = Male, female); R_k = effect of the k^{th} rearing system (*k* = Intensive, extensive); W_l = the effect of the l^{th} wk (*l* = 1, 2, 3, 4); T_l = random effect of ambient temperature; H_m = random effect of ambient humidity; $(B \times S)_{ij}$ = interaction effect of strain and sex of bird and $\varepsilon_{ijklmno}$ = random residual error.

For the rest of the measurements, the General Linear Models procedure (Proc GLM) of SAS (2010) was applied and the model $Y_{ijkl} = \mu + B_i + S_j + R_k + (B \times S)_{ij} + \varepsilon_{ijkl}$ was used where; Y_{ijkl} = the response variable (liver and spleen weight, leucocyte count, H/L ratio, relative liver and spleen weight); μ = the overall mean common to all observations; B_i = the effect of the i^{th} strain (*i* = NN, OV, PK); S_j = effect of the j^{th} sex (*j* = Male, female); R_k = effect of the k^{th} rearing system; $(B \times S)_{ij}$ = interaction effect of strain and sex of bird and ε_{ijkl} = random residual error term. Least square means were generated using the LSMEANS option in SAS, and significance was considered at the 5 % level of probability (SAS, 2010). All interactions that had no effect at the 5 % level of probability were dropped from analyses.

6.5 Results

6.5.5 Levels of significance

Strain of bird affected ($P < 0.05$) all parameters except H/L ratio. Sex of bird had an effect on BW, spleen and relative liver weight, heterophil count and H/L ratio. There were significant strain × sex interactions on both absolute and relative liver- and spleen weights. The rearing system affected ($P < 0.05$) all parameters studied (Table 6.1). Values reported in this section, for log-transformed data, represent reverse-transformed analysis outputs.

6.5.6 Mortality and body weights

Raising birds in the outdoor system exposed them to predators. A total of 4 hens (PK) and 2 cocks (NN) were attacked by a hawk. Both strain and sex of bird influenced BW ($P < 0.001$). The highest body weight, 2165 ± 46.37 g/ bird BW, was observed among the housed birds. The OV had the largest BW, followed by PK, and the NN were the lightest of the 3 strains weighing 1660 ± 48.20 g/ bird BW. Males were heavier ($P < 0.001$) than females in the current study.

Table 6.1. Effect of duration of tonic immobility, body weight (BW), liver and spleen weight (g), relative liver and spleen weights (g/kg BW), H/L ratio and heterophil counts on slow-growing chickens

Factor	Parameters, levels of significance							
	Absolute weight (g)			Relative weights (g/kg BW)				
	BW	Liver	Spleen	Liver/ BW	Spleen/ BW	Duration of TI	H/L ratio	Heterophil
Strain	***	***	*	*	*	*	NS	*
Sex	***	NS	*	***	NS	NS	*	*
Rearing system	**	***	*	***	*	**	***	**
Week	-	-	-	-	-	***	-	-
Strain × sex	NS	***	***	*	***	NS	NS	NS

**P < 0.05; ** P < 0.001; *** P < 0.0001; NS: Not significant (P > 0.05); BW: Body weight; TI: Tonic immobility; H/L: Heterophil to lymphocyte*

6.5.7 Duration of tonic immobility

A maximum of two inductions was recorded in this study. Tonic immobility was influenced ($P < 0.05$) by strain, rearing system and week of observation. The TI durations ranged between 10 and 600 s. The duration of TI was lower ($P < 0.05$) for PK compared to NN and OV (Table 6.2), and the last 2 did not differ ($P > 0.05$). The overall TI duration for free-range birds was 275.5 ± 30.68 s compared to 349.8 ± 30.05 s for the housed birds. The distribution of the two environmental factors in relation to TI is presented in Figure 6.1. The overall TI durations for the 3 strains are presented in Figure 6.2.

6.5.8 Leucocyte and heterophil to lymphocyte (H/L) ratio

Strain and sex of bird influenced ($P < 0.05$) heterophil counts. No interactions were observed on lymphocyte counts in this study. Table 6.3 shows leucocyte counts by strain and sex of bird. The H/L ratio was affected ($P < 0.05$) by sex of bird. A higher H/L ratio was observed in housed birds (Table 6.4) compared to free-range birds. The overall H/L ratio for free-range birds was 0.63 ± 0.03 compared to 0.74 ± 0.02 for housed birds. Males across all strains had higher H/L ratios than females. The highest among the 3 strains was observed for PK hens (Table 6.5). No interactions were observed on this parameter.

Table 6.2. Effect of strain of bird on the duration of tonic immobility (TI) in Naked Neck (NN), Ovambo (OV) and Potchefsroom Koekoek (PK) chickens

Strain	Duration of TI (s)	*P*-value
NN	322.3±35.28[a]	***
OV	324.6±28.93[a]	***
PK	258.2±29.84[b]	***

[a,b]Values within a column with different superscripts differ (P < 0.05)

NN: Naked Neck; OV: Ovambo; PK: Potchefstroom Koekoek

**** P < 0.0001*

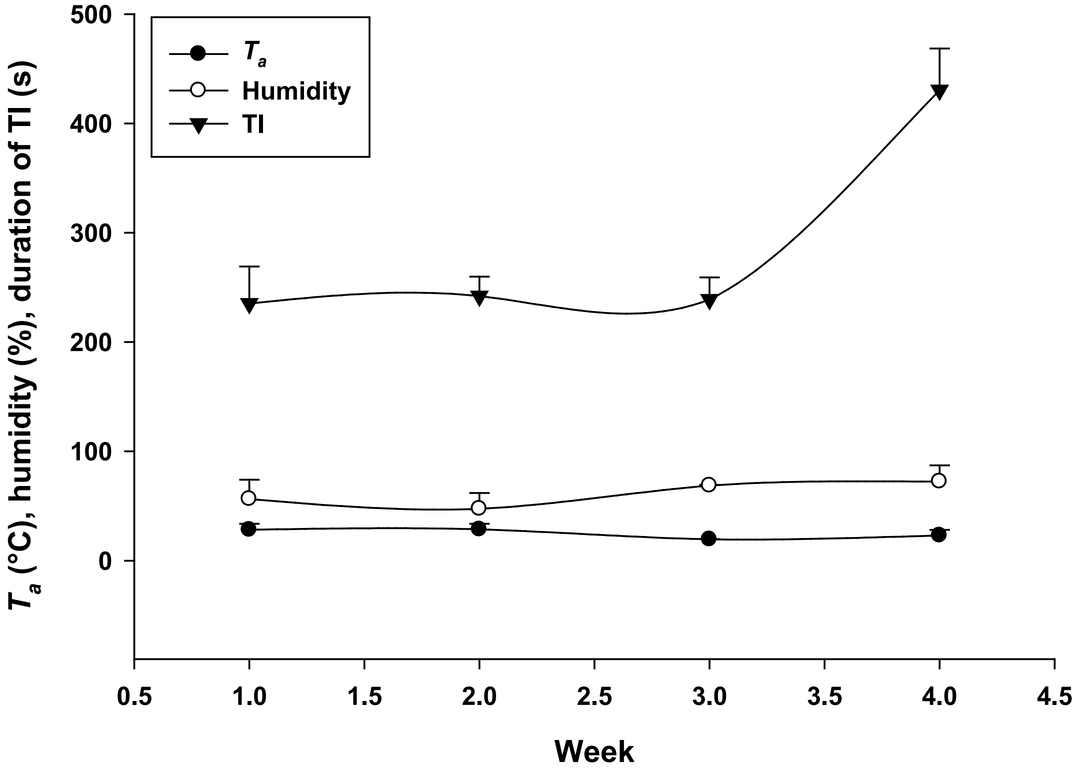

Figure 6.1. Ambient temperature (T_a), humidity and duration of tonic immobility (TI) distribution for Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) chickens over the study period

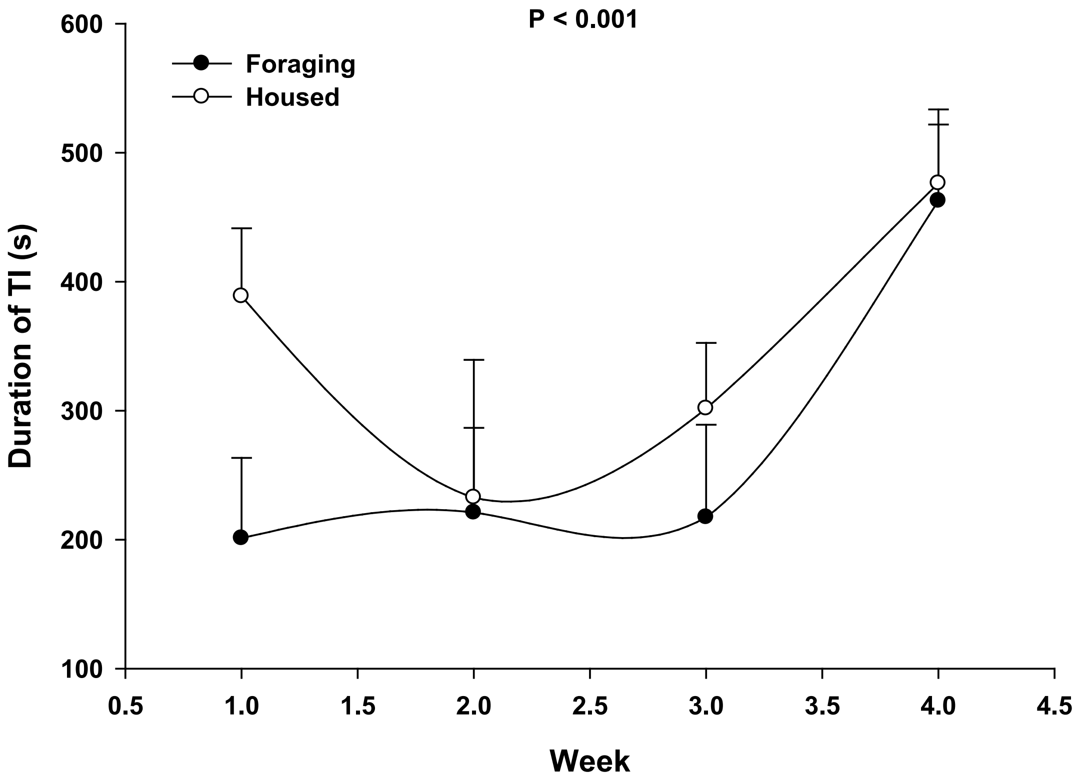

Figure 6.2. Effect of rearing system on the duration of tonic immobility (TI) in Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) chickens

6.5.9 Organ weights

Sex influenced ($P < 0.0001$) relative liver weight and so did strain ($P < 0.05$). There were significant strain × sex interactions on liver weights ($P < 0.001$). Relative liver weight was consistently higher in females across all strains (Table 6.3). All factors studied significantly influenced spleen weight. Hens had significantly larger ($P < 0.05$) spleens than cocks in the PK strain whereas spleens were heavier in males of the other 2 strains. The lowest relative spleen weight was observed in OV females. Significant interaction ($P < 0.05$) was observed between strain and sex of bird on this parameter. The spleen weights are as shown in Table 6.3**.**

Table 6.3. Effect of strain and sex of bird on LSMean leucocyte counts in Naked Neck, Ovambo and Potchefstroom Koekoek chickens

	LSMean				
Effect	**Lymphocyte**	**Heterophil**	**Basophil**	**Monocyte**	**Eosinophil**
Strain					
NN	48.0	31.0	3.5	5.5	6.0
OV	47.8	30.5	3.5	6.3	3.8
PK	51.3	33.8	3.0	5.8	4.3
SE	*1.02*	*0.55*	*0.87*	*0.72*	*0.61*
Sex					
Female	48.2	33.8	4.2	4.2	3.8
Male	49.8	29.7	2.5	7.5	5.5
SE	*1.30*	*0.69*	*1.10*	*0.92*	*0.78*

NN: Naked Neck; OV: Ovambo; PK: Potchefstroom Koekoek; SE: Standard error

Table 6.4. Effect of strain and sex on spleen weight (g), relative liver and spleen weights (g/kg BW) and H/L ratio

Strain	Sex	LSMean weight			
		Spleen	Liver	Spleen	H/L ratio
NN	Female	2.138 ± 0.866^{d}	1.952 ± 0.065^{ab}	0.142 ± 0.034^{b}	0.705 ± 0.039^{ab}
	Male	2.388 ± 0.866^{b}	1.500 ± 0.065^{c}	0.126 ± 0.034^{b}	0.593 ± 0.039^{bc}
OV	Female	1.512 ± 0.641^{d}	1.932 ± 0.048^{b}	0.007 ± 0.025^{c}	0.655 ± 0.029^{b}
	Male	8.291 ± 0.641^{a}	1.574 ± 0.048^{c}	0.304 ± 0.025^{a}	0.623 ± 0.029^{bc}
PK	Female	4.919 ± 0.641^{c}	2.077 ± 0.048^{a}	0.297 ± 0.025^{a}	0.766 ± 0.029^{a}
	Male	2.434 ± 0.641^{a}	1.218 ± 0.048^{d}	0.084 ± 0.025^{b}	0.560 ± 0.029^{c}

^{a,b,c}*Values within a column with different superscripts differ (P < 0.05)*

H/L: Heterophil to lymphocyte

NN: Naked Neck; OV: Ovambo; PK: Potchefstroom Koekoek

Table 6.5. Effect of rearing system and sex of bird on BW (g), absolute liver and spleen weight (g), relative liver and spleen weight (g/kg BW) and H/L ratio in free-range slow-growing chickens

Rearing system	Sex	BW, Organ weight (g)			Organ relative weight (g/kg BW)		
		BW	Liver	Spleen	Liver/ BW ratio	Spleen/ BW ratio	H/L
Free-range	M	1675.0^{c}	36.1^{a}	1.8^{b}	2.1^{a}	0.14^{b}	0.70^{a}
	F	1965.5^{b}	26.6^{b}	3.7^{a}	1.4^{b}	0.17^{b}	0.56^{b}
Housed	M	2165.0^{a}	35.3^{a}	4.6^{a}	1.7^{a}	0.20^{a}	0.74^{a}
	F	1920.0^{b}	28.3^{b}	4.2^{a}	1.5^{b}	0.20^{a}	0.56^{b}
SE		73.33	0.95	0.58	0.03	0.03	0.02

^{a,b,c}Values within a column with different superscripts differ significantly ($p<0.05$)

BW: Live body weight

H/L: Heterophil to lymphocyte (ratio)

M: Male

F: Female

SE: Standard error

6.6 Discussion

The thermal comfort zone for adult chickens is 18 to 20°C (Cahaner, 2008; Fanatico, 2007). This zone is defined as an ambient temperature range in which the metabolic rate is minimal and the best performance is achieved (de Souza *et al.*, 2015). The overall mean ambient temperature of 24.9°C experienced in the current study is above this zone. Thermal stress negatively impacts bird welfare mainly because birds are particularly sensitive to heat stress

(Lara and Rostagno, 2013). Heat stands out as one of the most important stressors in hot regions of the world (Altan *et al*., 2003).

The mortality experienced in the current study was mainly as a result of predation. Mortality is a common problem in outdoor poultry production systems. In the past, even the conventional poultry industry kept the birds with outdoor access and production moved indoors largely because of concerns of mortality due to predators among other factors (Sossidou *et al*., 2012). Free-range birds attract predators including foxes, dogs and birds of prey. In addition to attacks, the presence of predators can cause panic and smothering in the flock (Sossidou *et al*., 2012). The strain differences observed in BW are consistent with previous research findings (Chikumba and Chimonyo, 2014; Nthimo *et al*., 2004). There were no strain effects or interactions between treatment and strain in cold stressed chickens (Hangalapura *et al*., 2003).

Strain differences in duration of TI were observed implying differences in fearfulness among the strains. Ambient temperature and humidity did not influence duration of TI. Both observations contradict findings of Altan *et al*. (2003) who reported no strain differences in fearfulness between Ross and Cobb broilers. The same authors stated that TI durations differed significantly as influenced by heat stress. Several researchers have, however, observed results similar to the current study.

Campo *et al*. (2000; 2008) reported significant differences among strains in duration of TI in Spanish chickens while Edelaar *et al*. (2012) worked with yellow crowned bishops (*Euplectes afer*) and tree sparrows (*Passer montanus*). Level of fear seemed to differ in the two species and such consistent interspecific (or interpopulation) differences in fear are thought to have important consequences, for example, for adaptation to habitat changes (Carrete and Tella, 2011). Fear is an adaptive behavior whose biological role is to protect the animal from psychochemical damage (Gudev *et al*., 2011). Duration of TI has been used as a measure of fear, and several lines of evidence support this interpretation, especially for domesticated chickens (Edelaar *et al*., 2012). The TI durations recorded in this study indicate that the PK strain is less fearful compared to the NN and OV, respectively. TI durations in the current study are shorter than values reported for pheasants (Hrabcakova *et al*., 2012). The shortest and longest durations of TI were 111.4 ± 21.41 s and 361.6 ± 40.61 s, respectively, compared to 10 and 600 s in the current study. Besides species differences, the 2 experiments tested different effects on the subjects. Sex of bird did not influence TI durations, contrary to findings of Campo *et al*. (2000). Increased intensity of fear response can have deleterious consequences on poultry welfare and performance (Faure *et al*., 2003).

In this study, longer durations of TI were observed in housed birds and this is consistent with earlier reports (Campo *et al*., 2008; Hrabcakova *et al*., 2012). Duration of TI was longer with birds housed in deep litter than free-range birds (Campo *et al*., 2008). Even among housed birds, longer durations of TI were reported in hens housed in cages than hens kept on deep litter (Hrabcakova *et al*., 2008).

The observation on the absence of strain differences in H/L ratio agrees with previous research (Campo *et al*., 2000; Campo *et al*., 2008) but is not congruent with our expectations. We envisaged higher ratios in strains that recorded longer TI durations. Other factors may have influenced this observation. Other stressors like induced ACTH increased H/L ratio in chickens. In this study, temperature was not controlled and the highest temperature experienced was 35°C. Possible effects cannot be ruled out though this was not tested in this study. When temperature was maintained constant at 38°C for 3 h over two days in a separate study, increases in H/L ratio were reported (Puvadolpirod and Thaxton, 2000b). The 35°C experienced in this study is much higher than 18 to 24°C regarded as the thermoneutral zone for chickens (Hrabcakova *et al*., 2012; Edelaar *et al*., 2012). Free-range birds experience cyclic exposure to these conditions which might imply fluctuating responsiveness. Having said that, possible strain differences may occur in respect to thermo-tolerance. Literature reports that reduced feather cover in NN may be of advantage in thermoregulation at high ambient temperature (Eberhart and Washburn, 1993) by increasing sensible heat loss.

We observed significantly higher H/L ratios in housed birds compared to the free-range flock. This is consistent to a previous study (Campo *et al*., 2008) which evaluated H/L ratio in hens housed in deep litter and free-range systems. A H/L ratio of 0.42 indicates minimum or acceptable stress in chickens (Gross and Siegel, 1983). Higher ratios were observed in this study indicating potentially detrimental stress levels. The H/L ratio is a more sensitive indicator of stress than either the heterophil or the lymphocyte change alone in fowls (Gross and Siegel, 1983; McFarlane and Curtis, 1989) and is widely accepted as the best indicator of stress in chickens (Gross and Siegel, 1983).

Strain differences in absolute liver weights would be expected as the strains differ in BW in the first place. Females across the 3 strains had higher relative liver weights than males. Given that females are generally lighter in BW, this might imply that females were more stressed in this study. Hypertrophy of the liver, as evidenced by an increase in relative liver weight, is an indication of stress in birds (Puvadolpirod and Thaxton, 2000b). Literature reports that adrenocorticotropic hormone (ACTH) caused increases in relative liver weight in chickens (Puvadolpirod and Thaxton, 2000b) while tryptophan supplementation decreased the same (Moneva *et al*., 2008). A rather interesting observation was on the lowest and highest spleen and liver weights, respectively, in free-range birds. This may suggest spleen involution and liver hypertrophy, respectively, though the relative weight is more informative in that regard.

The spleen, together with the bursa of Fabricius and thymus, function in immune response in chickens. Stress is known to cause, among other effects, involution of primary lymphoid organs (Houshmand *et al*., 2012). Other forms of stress have also been found to lead to reduced spleen weights (Houshmand *et al*., 2012). The results seem to concur with previous reports (Puvadolpirod and Thaxton, 2000b) where ACTH and corticosterone administration resulted in reduced spleen weight. Reduced relative spleen weights were observed in heat stressed broilers in a separate study (Quinteiro-Filho *et al*., 2010). Tryptophan inadequacy is known to depress performance in poultry but supplementation did not significantly reduce relative spleen weight in stressed chickens (Moneva *et al*., 2008).

6.7 Conclusions

Strain affects duration of TI in slow-growing chickens while sex has no effect showing that strains may have different degrees of fearfulness. The H/L ratio was higher in females compared to males. Higher spleen weights were observed in housed birds and general liver hypertrophy in hens perhaps indicating stress. Based on H/L ratio, females appear more stressed than males, while results on duration of TI and organ weights are largely inconclusive. Based on findings of the current and preceding chapters, it was necessary to evaluate possible strain and sex effects on actual performance, by assessing meat and fat yield of slow-growing birds. Sexual dimorphism observed in terms of stress may translate to actual differences in BW and meat yield.

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Chapter 7: Effect of strain, sex and rearing system on carcass and fat yield of Naked Neck, Ovambo and Potchefstroom Koekoek chickens

(In press: *The Indian Journal of Animal Sciences*, appendix 3).

Abstract

Meat and fat yield influence the profitability of modern commercial poultry production. This study sought to determine the effect of strain, sex and rearing system on the carcass and fat yields of three slow-growing chicken strains reared in intensive and extensive systems. Two hundred and eighty-eight, 20-week old Potchefstroom Koekoek (PK), Ovambo (OV) and Naked Neck (NN) chickens, weighing 1710 ± 308.8 g, were allocated to 4 free-range pens of *Chloris gayana* and 4 pens in a poultry house. Final body weight (BW), carcass (CW), dressed weight (DW), cut, abdominal fat pad (AFP) yield and giblet weights were determined after a 4 week observation period. The weight of each parameter was expressed as a percentage of the eviscerated carcass weight of each bird and analysis of variance (ANOVA) was conducted. Strain influenced ($P < 0.05$) thigh, neck and giblets (pancreas, gizzard and crop) weight. Sex of bird influenced BW and males were significantly heavier, yielding heavier cuts and pancreases but lighter AFP than females. The OV yielded the heaviest portions among the three strains used in the study. Free-range birds experienced crop and gizzard hypertrophy and pancreatic atrophy. Further studies could focus on the characterisation of fatty acid composition of meat harvested from different rearing systems.

Key words: Abdominal fat, Carcass, Chickens, Sex, Strain, Weight

7.1 Introduction

Free-range systems allow access to an outside area, promoting expression of normal behaviour, thus theoretically improving bird welfare (Ponte *et al*., 2008). This is critical in view of the increasing demand for products that are produced under high welfare standards (Janczak and Riber, 2015). In addition to being more natural and better-tasting, such products may carry several health benefits (Midmore *et al*., 2005). Consumers prefer meat with low fat content since excessive fatness is associated with poor dietetic quality (Loh *et al*., 2000). This explains, in part, the preference for chicken meat to beef or pork (Haslinger *et al*., 2005). The poultry industry aims to increase carcass yield and reduce fatness, mainly the abdominal fat pad (AFP) (Fouad and El-Senousey, 2014)) and the latter is a reliable indicator of carcass fat content (Eits *et al*., 2003). Carcass and portion yield provide useful information to guide farmers as to how strain, sex and slaughter age influence consumer preferences (Faria *et al*., 2010).

Slow-growing lines are widely used for production of high quality free-range products (Chabault *et al*., 2012). The Naked Neck (NN), Ovambo (OV) (Mapiye *et al*., 2008) and Potchefstroom Koekoek (PK) chickens are closely associated with rural livelihoods in Southern Africa where they are used to meet household nutritional and economic needs. Meat and fat yield of these slow-growing chickens have not been examined despite their increasing popularity in recommended modern poultry rearing systems. In the previous Chapter, it was confirmed that strain and sex influence stress levels in birds hence the objective of the study was, to assess the effect of strain and sex on BW, carcass and fat yield of NN, OV and PK chickens. It was hypothesized that strain and sex of bird influence meat and fat yield of NN, OV and PK chickens.

7.2 Materials and Methods

7.2.1 Animal ethics

Animal handling and care were as previously described in Chapter 3, section 3.2.1.

7.2.2 Study site description

The study site was previously described in section 3.2.2. The ambient temperature and humidity recorded over the observation period ranged between 17.1 - 35°C and 23 - 93 % with means of 25°C and 61 %, respectively

7.2.3 Treatments, experimental design and bird management

A total of 288, 21-week old PK, OV and NN chickens were used in the study. The birds were randomly allocated to four 900 m^2 paddocks of *Chloris gayana* (Katambora Rhodes grass) and a 4 × 10 m house subdivided into 4 pens. There were 12 males/ strain and 12 females/ strain in each pen. As a result, there were 36 males (12 each of NN, OV and PK) in any 2 pens and the same number of females in the other 2 pens. Sexes were separated by wire mesh. The free-range pens were demarcated by 2.2 m high wire mesh and reinforced by wooden and steel poles. The birds were weighed individually on a digital scale, model UME CCS-150K, S/N: NXC 100020, to determine initial BW.

7.2.4 Bird management

Wooden cages measuring 2.5 × 2 m were placed uniformly in one corner of each free-range pen to provide shelter for the birds. The cages, with slatted floors elevated 1 m above the ground surface, were fitted with wire mesh doors to deter predators. Doors were left open during the day and closed at night. A 12 L plastic drinker was placed under shade near each cage to provide cool clean water. The drinkers were inspected, washed and replenished at least twice a day to ensure *ad libitum* access to clean water.

The poultry house, fitted with two roof air-vents and side curtains on both sides to enable adequate ventilation, had corrugated iron sheet roofing. Fluorescent lamps were used for lighting. The housed birds were raised on a deep litter system with wood shavings as bedding. The litter, which was constantly inspected for wetness, was maintained at about 10 cm thick. Feed and potable tap water were supplied *ad libitum* through 2 standard plastic feeders and two 12 L plastic drinkers, respectively.

7.2.5 Brooding, feeding and health management

Birds used in this trial were obtained from the same flock studied in the preceding Chapters 5 and 6, as such brooding, feeding and health management were the same as previously described in section 5.2.5. The nutritional composition of the feeds is shown in Chapter 3, Table 3.1.

7.2.6 Data collection

At the end of the trial, chickens were fasted overnight before slaughter. Two birds per strain were randomly selected per pen and weighed individually. Eight birds per strain were randomly selected from the pens. Body weights (BW) were measured on a digital scale. Birds were euthanized by cervical dislocation and slaughtered by exsanguination. Carcasses were immersed in water at 60°C for 63 s and plucked manually. Heads and feet were removed before weighing on a digital electronic scale (Jadever JPS-1050, Micro Preciso Calibraton Inc, USA; ± 1g sensitivity), to determine carcass weight. After evisceration, the AFP was removed with the aid of a scalpel and weighed. It comprised leaf fat surrounding the cloaca and abdominal muscles excluding fat around the gizzard.

Edible portions were removed and weighed individually and the respective weights recorded. The eviscerated carcass was cut into prime cuts (head, shanks, thighs, wings, back, breast and drumsticks) and individual cut weight expressed as a percentage of the eviscerated carcass. Gut organ weights were also measured individually for the crop, intestines, liver and gizzard.

7.2.7 Statistical analyses

All data were tested for normality using the Shapiro-wilk test and $\log_{10}$-transformed wherever the data were not normal. Data were subjected to ANOVA with strain and sex of bird as the main effects, using proc GLM of SAS version 9.3 (SAS, 2010). Least square (LS) means were generated by the LSMEANS and separated using the PDIFF options of SAS (2010) at the 5 % level of significance. The model $Y_{ijkl} = \mu + S_i + T_j + R_k + (S\times T)_{ij} + \varepsilon_{ijkl}$ was applied where Y_{ijkl} was the response variable (BW, DW, portion and giblet weights); μ, the general mean; S_i, effect of the i^{th} strain (i = NN, OV, PK); T_j, effect of the j^{th} sex of bird (j = male, female); R_k = effect of the k^{th} rearing system (k = Intensive, extensive); $(S \times T)_{ij}$, interaction effects of sex and strain of bird and ε_{ijkl}, the random residual error term.

7.3 Results

7.3.1 Body, carcass and dressed weights

Strain and rearing system had no effect ($P > 0.05$) on BW while males were significantly heavier ($P < 0.05$; Tables 7.2 and 7.3) than females in this study. All other factors did not influence BW and no interaction effects ($P > 0.05$) were observed. Strain and sex did not influence ($P > 0.05$) dressed weight (DW).

7.3.2 Portion and giblet weights

Strain significantly influenced ($P < 0.05$) back, pancreas, gizzard, neck and abdominal fat weight. Sex of bird had a significant effect ($P < 0.05$) on shank, back, thigh, drumstick, pancreas and gizzard weights. Shank, back, thigh, drumstick, pancreas, gizzard and abdominal fat weights were higher in males than females (Table 7.1) while the abdominal fat was heavier in females. Rearing system influenced ($P < 0.05$) back, pancreas, gizzard and AFP weight (Table 7.1). There was no interaction ($P > 0.05$) between sex and strain on portion weights. There was evidence of pancreatic atrophy among birds raised on the extensive rearing system (Table 7.2). Table 7.3 shows gizzard, pancreas and AFP weights for the 3 strains used in the study. The effects were more severe in the NN than the other 2 strains.

Table 7.1. Effect of sex of bird on live body weight (BW), selected cut and giblet weights in Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) chickens

	Sex			
Parameters	**Male**	**Female**	***SE***	***P*-value**
Body weight	2269.3[a]	1476.1[b]	96.71	0.0410
Back	357.4[a]	193.6[b]	6.83	0.0051
Drumstick	135.9[a]	56.7[b]	6.11	0.0171
Shank	274.3[a]	145.2[b]	9.96	0.0171
Thigh	138.4[a]	88.4[b]	6.67	0.0286
Wing	94.1	59.8	5.09	0.0576
Breast	345.4	269.5	18.17	0.1351
Neck	94.4	89.0	4.01	0.0541
Gizzard	61.4[b]	66.2[a]	1.92	0.0269
AFP	17.9[b]	18.6[a]	0.40	0.0091
Crop	13.6	12.0	0.66	0.1178
Pancreas	3.6	3.4	0.47	0.8833
Intestines	130.0	136.7	11.86	0.7742
Liver	33.2	31.0	3.00	0.7108
Heart	13.9	6.6	2.97	0.2919
Spleen	4.6	1.7	2.81	0.6188
Proventriculus	5.6	9.8	1.31	0.1973

[a, b]Values in the same row with different superscripts differ significantly ($P < 0.05$)

SE: Standard error; AFP: Abdominal fat pad

Table 7.2. Body weight (BW), back, neck, gizzard, pancreas, abdominal fat pad (AFP) and crop weights in foraging Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) chickens

Parameter	Strain, weight (g)		
	NN	**OV**	**PK**
BW	1578.0 ± 86.19^{a}	2036.0 ± 86.19^{a}	2004 ± 86.19^{a}
Back	241.0 ± 6.08^{c}	300.5 ± 6.08^{a}	285.1 ± 6.08^{b}
Neck	62.8 ± 3.57^{c}	84.7 ± 3.57^{b}	93.0 ± 3.57^{a}
Gizzard	61.0 ± 1.15^{b}	53.6 ± 1.15^{c}	69.5 ± 1.15^{a}
Pancreas	3.3 ± 0.28^{b}	5.9 ± 0.28^{a}	4.7 ± 0.28^{ab}
AFP	16.1 ± 0.24^{b}	21.2 ± 0.24^{a}	19.9 ± 0.24^{a}
Crop	11.6 ± 1.00^{b}	13.1 ± 1.00^{a}	13.7 ± 1.00^{a}

$^{a, b, c}$*Values in the same row with different superscripts differ significantly (P < 0.05)*

NN: Naked Neck; OV: Ovambo; PK: Potchefstroom Koekoek; AFP: Abdominal fat pad

Table 7.3. Effect of rearing system and sex of bird on back, AFP, crop and giblet weight in Naked Neck (NN), Ovambo (OV) and Potchefstroom Koekoek (PK) chickens

Portion	Rearing system, Sex of bird				*P*-value
	Extensive		Intensive		
	Male	Female	Male	Female	
Back	225.0 ± 10.14	303.5 ± 10.14	320.4 ± 10.14	294.9 ± 10.14	0.0414
Gizzard	61.4 ± 1.92	66.2 ± 1.92	51.7 ± 1.92	62.1 ± 1.92	0.0392
AFP	17.9 ± 0.40	18.6 ± 0.40	16.3 ± 0.40	22.0 ± 0.40	0.0126
Crop	13.6 ± 0.66	12.0 ± 0.66	10.1 ± 0.66	10.3 ± 0.66	0.0460
Pancreas	3.6 ± 0.47	3.4 ± 0.47	9.2 ± 0.47	2.9 ± 0.47	0.0261

AFP: Abdominal fat pad

7.4 Discussion

Management, environment and genetics all influence an animal's actual performance (Misztal and Lovendahl, 2012) while strains normally kept by farmers under free-range conditions, especially in SSA, are largely unimproved. The observation that sex of bird influenced BW in this study is in agreement with earlier studies (Zhao *et al*., 2015; Nthimo *et al*., 2004). Nthimo *et al*. (2004) reported significant differences between sexes on BW of OV, PK, NN among other strains while males recorded heavier BW in a study by Zhao *et al*. (2012; 2015). In all 3 studies, males were significantly heavier than females. The observation that strain did not influence BW in the current study, however, is contrary to earlier reports (Zhao *et al*., 2012; 2015; Chikumba and Chimonyo, 2014; Nthimo *et al*., 2004). Similarly, neither sex nor strain influenced DW. Chickens raised in cages were heavier with higher percentages of abdominal fat than those raised in pens (Zhao *et al*., 2012).

Deliberate selection over time has led to the development of chickens with a higher breast yield. This, to the best of our knowledge, has been confined to fast-growing specialized chicken strains while slower-growing strains have largely been neglected. The observation that strain influenced parts yield is consistent with findings of Fanatico *et al*. (2005). According to these researchers, fast-growing birds usually present higher breast yield as compared to slow-growing birds, which present higher drumstick and thigh yields.

In this study, sex of bird influenced shank, back, thigh, drumstick, gizzard, pancreas and gizzard weights and males across all strains had heavier shank, back, thigh, drumstick, gizzard, pancreas and gizzard weights. Similarly, Takahashi *et al*. (2006) observed higher thigh yields in male chickens while the current findings also largely agree with Hrnčár *et al*. (2010) except for back weight which was higher in females in the other study. Males in this study yielded less abdominal fat than females as was expected. Similar observations were made by other workers (Hrnčár *et al*., 2010). The fact that the NN strain was affected more may suggest poor or slow ability to adapt on the part of this strain. A peculiarity about the OV is the gizzard and pancreas weight. The OV has the highest body and pancreas weights but the smallest gizzard.

Differences in pancreas, gizzard and crop weight in relation to BW present an interesting observation, especially given that these differences did not translate to actual differences in BW. This suggests crop and gizzard muscle hypertrophy as well as pancreatic atrophy among foraging birds. The observed gizzard muscle hypertrophy is likely to have occurred in response to the need to accommodate large volumes of fibrous material. This would be accompanied by a concomitant increase in crop volume in order to increase its capacity for temporary storage of feed material before the gizzard empties into the proventriculus, after-which the crop receives hunger signals and conveys its contents to the gizzard. This can be traced back to diet nutrient density which influences the amount of feed that has to be passed through the gastrointestinal tract of the bird for a given quantity of energy and protein to be absorbed. The foraging birds mainly fed on the Katambora grass though with access to a wide range of insects. In contrast, housed birds had *ad libitum* access to a nutrient-dense diet which perhaps eliminated the need for the gizzard and crop to hold large quantities of feed at any one point in time. For that reason, minimal, if any, hypertrophy was observed. More importantly, the retention time for fibre-rich diets in the foraging birds would be expected to be significantly longer thus contributing to the observed modification of the gut organs.

Pancreatic atrophy can be caused by various factors. It has been demonstrated that pancreas functions are influenced by such factors as changes in major dietary components, patterns in food intake and trace element deficiencies (Pitchumoni and Scheele, 1993). It is possible that the foraging birds suffered protein-calorie deficiency as also evidenced by weight loss experienced by the birds. In a separate study, pancreatic atrophy was observed with birds experiencing selenium deficiency among other mineral nutrients (Avanzo *et al*., 2002) while other causes of pancreatic atrophy would include pancreatic duct obstruction leading to stunting. Such factors may need further investigation as they were out of the scope of the present study though possible contributions cannot be ruled out.

The observation that females yield more fat is consistent with earlier reports (Hrnčár *et al*., 2010). Females generally deposit more body fat compared to males across most species. It would be important to consider this when choosing sexes to rear in meat type chickens although the practicability of this act at farmer level would be questionable. The excessive fat in modern poultry strains has been one of the major problems facing the poultry industry (Zhou *et al*., 2006) and that might just be one of the strengths of the slow-growing strains.

The major goal is to increase the carcass yield and to reduce fatness, mainly the abdominal fat pad (Fouad and El-Senousey, 2014). In general, excessive fat deposition is an unfavourable trait for producers and consumers because it is considered to be wasted dietary energy and a waste product with low economic value, which also reduces the carcass yield and affects consumer acceptance (Emmerson, 1997). Furthermore, from a consumer point of view, excessive fat intake is a threat since it is associated with several health conditions including atherosclerosis which predisposes consumers to heart problems. The farmer has to strike a balance since some degree of fatness is essential. Whether in adipose tissue or muscle, fat plays an important role in meat quality and human health (Wood *et al*., 2008; Peng *et al*., 2015). The success of poultry meat production has been strongly related to improvements in growth and carcass yield, mainly by increasing breast proportion and reducing abdominal fat (Griffin, 1996).

There are other factors however, that contribute to fatness in chickens including diet composition. Low-protein diets have been reported to cause a significant increase in the abdominal fat content (Collin *et al.*, 2003). In a separate study, abdominal fat content was significantly lower in chickens fed diets containing sunflower oil (Fouad and El-Senousey, 2014). In general, body fat accumulation may be considered the net result of the balance among dietary absorbed fat, endogenous fat synthesis (lipogenesis) and fat catabolism via b-oxidation (lipolysis). Thus, if the amount of absorbed fat is the same, lower body fat deposition may be attributed to increased fat catabolism or diminished endogenous fatty acid synthesis or to both processes (Sanz *et al*., 2000). These rather extrinsic factors interact with the inherent ability of a given animal to metabolise fat and it is this interaction that determines the ultimate fat content. Sales (2014) observed lower fat concentrations in free-range chickens compared to those without access to a pasture.

7.5 Conclusions

The results indicate that males yield higher portion weights compared to females. Sex also plays a role in fat deposition with females yielding more fat. Strain influences yield with the

OV yielding the heaviest portions. Free-range birds experienced crop and gizzard hypertrophy and pancreas atrophy, an adaptive mechanism perhaps meant to cope with bulky fibrous diets.

7.6 References

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Chapter 8: General discussion, Conclusions and Recommendations

8.1 General discussion

Free-range poultry production has increased dramatically in recent years, along with the need to ensure bird welfare. The broad hypothesis tested was that free-range slow-growing chicken behavioural and physiological responses, tonic immobility, meat and fat yield are influenced by sex and strain of bird. These were evaluated in the context of their importance as welfare indicators in birds. This was premised on the ever-increasing importance attached to bird welfare, hence free-range and related poultry rearing systems across the world. Free-range systems make use of slow-growing strains which are more suitable for these production systems (Castellini *et al*., 2002; Gordon and Charles, 2002). The promotion of free-ranging and the use of adapted strains is argued along with bird welfare and consumer preference for safe and healthy poultry products. Slow-growing strains are preferred to imported chickens, because of their meat quality characteristics including; pigmentation, taste, flavour and leanness (Moreda *et al*., 2013). Free-range systems allow the expression of normal behaviours such as dust-bathing, foraging and exercising through allowing access to an outside area, thus theoretically improving bird welfare. In Southern Africa, popular slow-growing strains include NN, OV and PK chickens (Grobbelaar *et al*., 2010; Nthimo *et al*., 2004) and these strains are regarded as adapted to harsh conditions yet their productivity remains low. Despite their several advantages, free-range systems may expose birds to harsh, inherently variable, environmental conditions.

Chapter 3 hypothesized that strain and sex of bird influence the behaviour of free-range NN, OV and PK chickens. To that effect, foraging and other behavioural activities of these strains were monitored under cyclic ambient temperature and humidity in a generally hot free-range environment. This was conducted in the context that NN, OV and PK chickens are regarded as hardy and adapted to harsh rearing conditions. There is a paucity of information on the relative hardiness and no documented information on time budgets, under free-ranging conditions, for NN, OV and PK chickens. It was anticipated that NN would spend the most time on feeding and related behavioural activities owing to its reduced plumage cover and fairly light BW. The thermoregulatory ability of NN chickens at high temperature is thought to be superior to that of normally feathered birds (Yahav *et al.*, 1998; Eberhart and Washburn, 1993) due to reduced overall plumage cover (Rajkumar *et al.*, 2010; Fathi *et al.*, 2013). The study confirmed that strain influences time spent on foraging and related behavioural activities. Other important factors, in that regard, were; sex, time of day and THI. Generally, time spent foraging decreased with increasing THI. OV in particular, and females in general, spent more time foraging. Strain differences in response to heat stress (Atlan *et al.*, 2003) and free-ranging behaviour (Nielsen *et al.*, 2003) have been reported before. That females spent more time foraging than males was surprising and is not consistent with literature (Nthimo *et al.*, 2004). This was attributed to possible high metabolic demands expected in laying hens.

The hypothesis tested in Chapter 4 was that strain, sex and rearing system influence physiological responses in NN, OV and PK chickens. The 3 strains appeared to exhibit comparable thermal tolerance since they were able to maintain fairly constant core temperatures. The observation that there were no strain differences in BR is consistent with the study of Yahav *et al*. (1998). It was anticipated that the NN strain would better withstand the effects of high T_a and RH but no strain differences were observed in BR in this study. Birds exhibited signs of some degree of thermal stress though the intensive rearing system appeared to subject birds to greater heat loads. This study hinted that the variability in ambient temperature and humidity, due to the cyclicity of exposure to the same, are largely responsible for the stress observed under free-ranging conditions.

In Chapter 5, it was hypothesised that diet, strain, sex of bird and rearing system would influence gut and ileal histomorphology, hence general performance of slow-growing birds. Two experiments were conducted where, in Experiment 1, feeding provitamin A bio-fortified maize did not influence BW nor ileal villus morphology. Sex and strain of bird influenced BW and ileal villus morphological characteristics. In experiement 2, it was shown that strain and sex of bird affect BW and ileal villus morphology in NN, OV and PK chickens. Generally, housed birds out-performed free-range birds. This was attributed to inherent variability in environmental conditions experienced under free-range systems (Lin *et al*., 2016). Lighter and shorter intestines were observed in free-range birds. The explanation was that birds reduce gut size in order to reduce wastage of nutrients due to maintenance costs (Xu *et al*., 2015) in response to exposure to a hypo-protein diet (Incharoen *et al*., 2010). The reduction in intestinal weight was attributed to a reduction in ME thickness (Gunal *et al*., 2006).

In Chapter 6, the hypothesis tested was that strain and sex of bird have an effect on duration of TI, H/L ratio and organ weights of slow-growing chickens. Fearfulness as an indicator of stress, hence possible violation of bird welfare, was tested. It was anticipated that slow-growing chickens were suitable for outdoor conditions and would survive under high temperature and relative humidity with minimal stress. It was confirmed in this study that strain and sex of bird influence performance, fearfulness, organ weights, hence general welfare of NN, OV and PK chickens. A H/L ratio of 0.42 indicates minimum or acceptable stress in chickens (Gross and Siegel, 1983) and higher ratios were observed in this study indicating potentially detrimental stress levels. In this study, longer durations of TI were observed in free-range birds and this is consistent with earlier reports (Campo *et al*., 2008; Hrabcakova *et al*., 2012). The finding that sex of bird did not influence duration of TI is contrary to literature reports (Campo *et al*., 2000). In Chapter 7, it was hypothesised that free-range birds would yield heavier portions and less fat than confined birds, with differences among strains and between sexes. Contrary to previous observations (Chikumba and Chimonyo, 2014; Nthimo *et al*., 2004), strain did not influence BW in this study but sexual dimorphism was observed in BW. Free-range carcasses were less fatty than confined birds (Sales, 2014). Males yielded less fat compared to females in the current study, indicating effect of sex on fat yield in slow-growing chickens.

8.2 Conclusions

The NN strain spent the most time walking relative to the other strains while males spent more time walking than females. Foraging and drinking behaviours were more prominent in the morning while preening and dust-bathing occurred mostly around mid-day. There was negative correlation between time spent foraging and THI while time spent standing and preening increased with increasing THI. Strain and sex of bird influence both gut macro- and microstructure, particularly intestine length, weight, apical villus width and ileal aVSA, hence gut absorptive capacity in slow-growing chickens. Villus width, aVSA and ME thickness were higher in OV and PK than NN chickens. Ileal villi were taller, wider hence greater aVSA in males than females. Naked Neck chickens spent more time foraging than OV and PK. The highest BW were achieved with OV chickens. The NN, OV and PK strains appear to exhibit comparable thermal tolerance as they were able to maintain a fairly constant core temperature as reflected in the RT. The duration of TI was lower for PK compared to NN and OV birds showing possible strain differences in fearfulness. Females were more stressed than males.

Environmental temperature and relative humidity influence behavioural and physiological responses, hence performance of slow-growing chickens. The objectives of the study were met successfully and it can be concluded that NN, OV and PK chickens exhibit similar behavioural and physiological responses under similar rearing conditions. Ovambo and PK chickens, however, achieve better BW than NN which are marginally superior in terms of thermal tolerance.

8.3 Recommendations

In view of the increasing demand for free-range products, it is important to explore ways of improving productivity of slow-growing chickens. In addition to taking advantage of these strains' ability to withstand, or at least, tolerate harsh rearing conditions, farmers should make an effort to ensure comfortable rearing conditions. There is need for a balance between outdoor access and providing with shelter to enable birds to escape from harsh weather elements for birds reared in hot environments. The detrimental effects of heat stress could be reduced by providing shelter and water.

Further understanding of gut morphology, strain and sex differences in response to various factors need to be investigated in slow-growing chickens. A faster growing strain maybe included in the experiment to evaluate possible age-related changes in fine gut structure. It is important, in that regard, to perform measurements at an early growth stage to assess possible effects of age on the ability of slow-growing birds to respond to feeding interventions. Another dimension that would add value to this type of research would be to evaluate physiological responses in slow-growing chickens under artificially induced heat stress. An important aspect, however, would be to ensure that the exposure to high temperatures is mediated in such a way that birds experience cyclic exposure. This is important since birds in open free-range environments experience cyclic, rather than constant, exposure to inherently variable weather elements. In addition, fear-inducing agents could also be introduced in order to assess their effects on the welfare of birds, particularly because free-range birds encounter various agents that may cause anxiety and fear. A possible approach would be to subject birds to the presence of a common predator e.g. eagle or cat for a specified period followed by the determination of such responses as tonic immobility and changes in stress-related enzyme assays as welfare indicators.

One of the most fundamental challenges associated with free-range systems is the intimate interaction by birds with various biotic and abiotic factors including soil, fomites and droppings. These may pose serious health hazards and the effects of the same on bird welfare, with regards to freedom from disease, need to be examined.

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Appendix

Appendix 1: Ethical Approval

UNIVERSITY OF KWAZULU-NATAL™

INYUVESI YAKWAZULU-NATALI

8 January 2015

Reference: 039/15/Animal

Mr T Mutibvu
School of Agricultural, Earth &
Environmental Sciences
University of KwaZulu-Natal
PIETERMARITZBURG Campus

Dear Mr Mutibvu

Ethical Approval of Research Projects on Animals

I have pleasure in informing you that the Animal Research Ethics Committee has granted ethical approval for **2015** on the following project:

"Effect of feed restriction on growth, gut morphology & carcass characteristics of chickens."

Yours sincerely

THT Coetzer

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

Cc Acting Registrar – Mr B Poo
Research Office – Dr N Singh
Supervisor – Prof. M Chimonyo
Head of School – Prof. A Modi
SAEES – Mrs M Manjoo

Appendix 2: List of publications

1. Mutibvu T., Chimonyo M. and Halimani T. E. 2017. Effects of strain and sex on the behaviour of free-range slow-growing chickens raised in a hot environment. Journal of Applied Animal Research: DOI: 10.1080/09712119.2017.1287079.

2. Mutibvu T., Chimonyo M. and Halimani T. E. 2017. Tonic immobility, heterophil to lymphocyte ratio and organ weights in slow-growing chickens. Journal of Applied Poultry Research. DOI: 10.3382/japr/pfw066.

3. Mutibvu T., Chimonyo M. and Halimani T. E. Effect of strain and sex of bird on carcass and fat yield of slow-growing chickens. The Indian Journal of Animal Sciences. *In Press*