EFFECTS OF DIETARY SUPPLEMENTATION WITH *HIBISCUS SABDARIFFA* CALYCES MEAL IN BROILER AND EGG LAYING QUAIL (*CORTUNIX COTURNIX JAPONICA*)

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand in fulfilment of the requirements of the degree of

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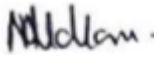
DECLARATION

I**,** Nomagugu Ndlovu, hereby declare that this thesis, hereby submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, is my original work. It has not been submitted before for an award of any other University. Where other sources have been used, they have been appropriately cited and acknowledged.

……  ……

(Signature of candidate)

21st day of June 2021 in Gweru, Zimbabwe.

DEDICATION

In memory of my late mother

Sitshengisiwe Ndlovu

1963-2017

PRESENTATIONS ARISING FROM THIS RESEARCH PROJECT

Conference and Seminar Presentations

i. **Ndlovu, N**., Erlwanger, K. H., and Chivandi, E., Dietary *Hibiscus sabdariffa* calyces meal: Effect on egg quality of Japanese quail, 10th Cross Faculty Symposium, 2-3 September 2019, University of the Witwatersrand, Johannesburg, South Africa.

ii. **Ndlovu, N**., Erlwanger, K. H., and Chivandi, E., Effect of dietary supplementation with *Hibiscus sabdariffa* calyces meal on meat quality of Japanese broiler quail (C*oturnix coturnix*), 2016 Autumn International Scientific Conference on Food Safety and Security (FSaS), 16-18 May 2016, University of Johannesburg, South Africa.

iii. **Ndlovu, N**., Erlwanger, K. H., and Chivandi, E., Lipid and protein content of Japanese broiler quail (*Coturnix coturnix*) fed with *Hibiscus sabdariffa* calyces meal, 7th Cross Faculty Symposium, 1-2 March 2016, University of the Witwatersrand, Johannesburg, South Africa

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MANUSCRIPTS ARISING FROM THIS RESEARCH PROJECT

i. **Ndlovu, N**., Erlwanger, K. H., and Chivandi, E. (2020). Supplemental *Hibiscus sabdariffa* calyces meal improves water holding capacity and decreases fat content of

Japanese quail meat without compromising meat yield and tenderness, Journal of Animal science and research. (Manuscript under review)

ABSTRACT

The greatest concern of poultry producers is to reduce feed costs while meeting consumer expectations of safe, wholesome products. Poultry producers use synthetic feed additives to promote growth and feed utilisation efficiency as well as to improve health of the birds. There is an urgent need of replacing "synthetics" with natural plant-derived additives which are deemed safer in poultry production. This study sought to determine the effects of supplemental *Hibiscus sabdariffa* calyces meal on growth performance, health as well as meat and egg quality of Japanese quail. In the broiler study, a standard Japanese quail finisher diet was supplemented with *H sabdariffa* calyces meal at 0%, 5% and 10%: diets 1, 2 and 3, respectively. Seventy-five, 5-week old Japanese quail were randomly allocated to and fed the finisher diets for 28 days. Body mass and feed intake were determined and on slaughter, carcass yield, haematocrit, serum malondialdehyde concentration and plasma uric acid, total bilirubin, total protein, aspartate transferase (AST), albumin and globulin were measured. Meat physico-chemical quality was determined. In the pullet study, a standard Japanese quail layer diet was supplemented with *H. sabdariffa* calyces meal at 0%, 5% and 10%: diet1, 2 and 3, respectively. Ninety, 5-week old Japanese quail hens were randomly allocated to and fed the layer diets for 56 days. Body mass, feed intake and egg quality (egg mass, width and length, egg shell mass and thickness, yolk mass, height and diameter; albumen mass, length width and height, yolk and albumen proximate compositon as well as yolk fat content and fatty acid profiles) were determined and on slaughter, carcass yield, haematocrit, serum malondialdehyde concentration and plasma uric acid, total bilirubin, total protein, AST, albumin and globulin were measured. Meat physico-chemical quality was determined. There were no significant differences ($P > 0.05$) in the body weight gain of male and female Japanese quail kept for meat. Dietary *H. sabdariffa* calyces meal had no effect ($P > 0.05$) on the trial feed conversion ratio of female Japanese quail, but it significantly increased ($P< 0.05$) that of the male counterparts. Dietary *H. sabdariffa* calyces meal significantly reduced ($P = 0.0092$) the fat content of the thigh meat and increased ($P < 0.05$) the protein content of both breast and thigh meat of Japanese male quail. Supplemental *H. sabdariffa* calyces meal delayed the onset of laying and reduced ($P < 0.0001$) the number of eggs produced by Japanese quail. Supplemental *H. sabdariffa* calyces meal significantly

reduced ($P < 0.05$) the shell thickness of Japanese quail eggs and increased ($P < 0.05$) the yolk fat and particularly the saturated fat content. There were no significant differences ($P > 0.05$) in the feed conversion ratio and total body weight gain of layer Japanese quail. Dietary *H. sabdariffa* calyces meal reduced the meat's fat content thus can potentially be exploited to produce lean meat with better keeping quality. Supplemental *H. sabdariffa* calyces meal in layers may result in losses to the farmers and can compromise consumer health. Future studies should consider measuring bioavailability of the phytochemicals present in *H. sabdariffa* calyces meal.

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NOMENCLATURE

ACC: Acetyl-CoA Carboxylase

ACE: Angiotensin converting enzyme

ADG: Average daily gain

ALP: Alkaline phosphatase

ALT: Alanine transferase

ANOVA: Analysis of variance

AOX: Antioxidants

AST: Aspartate transferase

BHT: Butyl hydroxyquinone

CAT: Catalase

CHD: Coronary Heart Diseases

CP: Crude protein

CRC: Colorectal Cancer

CVD: Cardiovascular diseases

d-ALA-D: d-aminolevuline dehydratase

DBWG: Daily body weight gain

DHA: Docohexaenoic acid

DM: Dry matter

DNA: Deoxyribonucleic acid

EDNO: Endothelium derived nitric oxide

EE: Ether extract

eNOS: Endothelial nitric oxide species

EQ: Ethoxyquinone

FAMEs: Fatty acid methyl esters

FCR: Feed conversion ratio

FI: Feed intake

FRAP: Ferric reducing antioxidant power

GC: Gas chromatography

GIT: Gastrointestinal tract

GPx: Glutathione peroxidase

HCA: Hydroxy citric acid

HIV: Human Immunodeficiency Virus

Hs: *Hibiscus sabdariffa*

HSC: *Hibiscus sabdariffa* calyces

HSCM: *Hibiscus sabdariffa* calyces meal

HSE: *Hibiscus sabdariffa* extract

IGT: Impaired glucose tolerance

LDL: Low Density lipoproteins

MDA: Malonaldehyde

MUFA: Mono-unsaturated fatty acid

NADPH: Nicotinamide adenine dinucleotide phosphatase hydrogen

NOx: NADPH oxidases

NO: Nitric Oxide

PUFA: Poly-unsaturated fatty acids

ROS: Reactive Oxygen species

RNA: Ribonucleic acid

SOD: Superoxide Dismutase

SD: Standard deviation

SFA: Saturated fatty acids

SQFD: Standard quail finisher diet

SQLD: Standard quail layer diet

TB: Tuberculosis

TBARs: Thiobarbituric reactive substances

TBHQ: *Tert*-Butyl hydroxyquinone

TNF α: Tumour Necrosis factor

VLDL: Very low density lipoproteins

WHC: Water holding capacity

1. CHAPTER ONE - INTRODUCTION AND JUSTIFICATION

1.0 Thesis overview

The increasing demand for poultry and livestock products places a high demand on feed resources. High feed cost due to competition for feed resources between humans and poultry production, disease outbreaks and oxidative stress which affects bird welfare and product quality constitute major challenges for the poultry industry. To enhance productivity, supplements such as antibiotics, prebiotics and synthetic antioxidants are added in poultry feed to promote growth performance, feed utilisation efficiency and mitigate stress. However, the use of antibiotics as growth promoters is associated with microbial drug resistance which has become a major public health concern. Residues of synthetic antioxidants in poultry products elicit adverse side effects in consumers. Plants contain phytochemicals with health beneficial bioactivities such as antioxidant, antibacterial, antifungal, growth stimulation, and immune modulation. These plant-derived substances can be safer alternatives to antibiotics and synthetic antioxidants in poultry production. Therefore, the potential of deploying phytogenics to enhance cleaner poultry production and safeguard consumer health needs to be explored. Research has shown that *Hibiscus sabdariffa* possesses nutritional and antioxidant activities. I therefore evaluated the potential benefits of supplementing Japanese quail diets with *H. sabdariffa* calyces meal ascertaining its impact on growth performance, feed utilisation efficiency, health and product (meat and egg) quality. Chapter 1 gives a background of the importance of poultry (chicken) production and the use of feed additives including antibiotics and synthetic antioxidants to enhance productivity and product quality. The capacity of Japanese quail as an animal-derived source of protein for human consumption is highlighted. Health challenges associated with use of antibiotics and synthetic antioxidant in poultry production are discussed. A description and justification for the need to evaluate potential plant-derived products (phytochemicals, extracts and powders) as replacements of synthetic compounds as feed additives in poultry feeds is articulated and a justification for determining the potential benefits of supplementing Japanese quail diets with *Hibiscus sabdariffa* calyces meal is also provided. The chapter concludes by stating the study aim, objectives and hypotheses.

Chapter 2 is an evaluation of literature pertinent to the thesis. Trends in poultry (chicken) meat and egg production as well as per capita consumption in sub-Saharan Africa are discussed. The impact of high feed cost and use of feed additives (synthetics and antibiotics) in the poultry industry are discussed. A case is made for the potential use of phytogenics in poultry production and the mechanism through which they can positively impact poultry production are explored. A focused narrative on the potential of *H. sabdariffa* as a natural growth promoter which can be exploited in poultry, including quail production is given. This narrative reviews the phytochemical composition and biological activities of interest of *H. sabdariffa*. A discourse on previous research outcomes wherein nutritional benefits *H. sabdariffa* were evaluated in in humans, poultry and livestock is also given. Chapter 3 is the first experimental chapter. The effect of supplementing a standard Japanese quail finisher diet with *H. sabdariffa* calyces meal on growth performance, feed utilisation efficiency, gastrointestinal tract (GIT) organ morphometry, metabolic substrate (circulating and stored) content, haematocrit and erythrocyte membrane fragility as well as on liver and kidney health of broiler Japanese quail is presented.

Chapter 4 presents the outcomes of supplementing a standard quail finisher diet with *H sabdariffa* calyces meal on the physico-chemical attributes of Japanese quail meat. Chapter 5 presents the effects of supplemental *H. sabdariffa* calyces meal on egg production and quality by Japanese quail. Chapter 6 presents an evaluation of the effects of supplementing standard quail layer diet with *H. sabdariffa* calyces meal on growth performance, feed utilisation efficiency, GIT organ gross measurements, metabolic substrate (circulating and stored) content, haematocrit and erythrocyte membrane fragility as well as on liver and kidney health of Japanese quail. Chapter 7 summarises the major findings of the study and provides caveats of the study and while making suggestions for futher extension of findings from the current study. A bibliography of the references cited in the thesis is then provided. Additionally, some appendices have been included, namely appendix A which is a letter of identification of the *Hibiscus sabdariffa* plant and appendix B, a copy of the clearance certificate (from the University of the Witwatersrand animal research ethics committee) for this study.

1.1 Introduction

A majority of consumers depend on animal products as the major source of protein in their diets (Godfray, 2019; Henchion et al., 2017). Animal proteins contain non-essential and essential amino acids in adequate quantities for human requirements compared to foods of plant origin (Godfray, 2019). In addition to supplying dietary protein, poultry and livestock derived products are also important sources of vitamins B_{12} and D, zinc, haem iron and docohexaenoic acid for humans (Godfray 2019; Milicevic et al., 2014). Despite being a source of important nutrients, the consumption of certain meat types over long periods can result in metabolic diseases develoment. The consumption of processed red meat has been linked with a high likelihood of developing cardiovascular pathology, diabetes mellitus and neoplasia (Wolk, 2017). Unlike processed red meats, research evidence demonstrates that poultry meat and fish can be consumed but with a reduced risk of developing metabolic diseases (Milicevic et al., 2014; Petracci et al., 2013). Consumers now prefer poultry and other lean meats due to their (meats) healthier nutrient profile (Milicevic et al., 2014). Meat and eggs are the main products derived from poultry for human consumption and their consumption helps mitigate protein deficiency and help fortify with vitamins, essential amino acids and essential fatty acids in diets of resource-poor households (Schonfeldt et al., 2013, Kwasek et al., 2020). Poultry meat, particularly broiler chicken meat contains comparatively less lipid, saturated fatty acid and cholesterol (Marangoni et al., 2015, Schonfeldt et al., 2013) making it a healthier product for human consumption. Due to its relatively lower price per unit weight coupled with desirable nutrient profile, the demand for poultry meat is increasing significantly (Godfray, 2019; Henchion et al., 2017). The increase in the number of people worlwide, expansion in urban settlement as well as increasing incomes is further fuelling increase in demand for poultry meat and eggs in sub-Saharan Africa (Thornton, 2010). In addition to being a major source of essential nutrients for humans, the poultry industry is also a major source of employment for many in developing (Birol et al., 2010) as well as in developed countries. By products from poultry production such as manure, feathers, egg shells and blood are also valuable additions to the poultry production value chain (Mishra et al., 2015; Jayathilakan et al., 2012).

1.2 Problem Statement

Worldwide, people are increasingly adopting an energy dense diet particularly rich in animal protein and fat (Nardoia et al., 2015). The consumption of chicken meat and eggs, even among the poorer communities, is increasing (Nardoia et al., 2015). In South Africa, per capita consumption of chicken meat increased from 22kg in 2000 to 40 kg by 2016 (Esterhuizen, 2016). Chicken is relatively cheaper compared to beef, fish and pork making it the more affordable animal-derived protein source in human diets (Esterhuizen, 2016). Despite its desirable lipid profile compared to red meats, consumption of broiler chicken breast over a long time can potentially increase the risk of development of metabolic diseases (Milicevic et al., 2014) due to its palmitic acid content. The high polyunsaturated fatty acid content of chicken meat also presents processing and storage challenges (Selani et al., 2011) that result in lipid peroxidation which compromises the quality of meat , its acceptability of the meat and its products (Nardoia et al., 2015). In order to uphold quality and improve the keeping properties of meat, synthetic preservatives are commonly used (Qwele et al., 2013). Examples of these synthetic preservatives are butylated hydroxytoluene (BHT), Ethoxyquinone and tertiary butyl hydroquinone (TBHQ). Synthetic preservative use is not without challenges as they can increase cost and their residues in the meat and/or eggs elicit deleterious side effects in consumers and also pollute the environment (Ruiz-Capillas and Jimenez Colmenero, 2008).Therefore, due to the public health and environmental costs associated with the use of these synthetic preservatives, (prebiotics and antibiotic based growth promoters) consumers have and are advocating for the replacement of these compounds from the food production chain with natural efficacious non-toxic substitutes (Zhang et al., 2010). In addition to causing costly antibiotic resistance, the use of antibiotics as growth promoters to enhance feed utilisation efficiency in poultry production (Salim et al., 2018; Dhama et al., 2014) results in antibiotic residues in the meat which adversely affect consumer health (Alloui et al., 2014). Plant-derived substances (extracts, phytochemicals and powders) have been and continue to be used as ethnomedicines due to the health beneficial biological activities (antioxidant, anti-inflammatory, antibacterial, antifungal, antiprotozoal, antidiabetic, hepato- and reno-protective) which they possess (Zhang et al., 2010).

Antibiotics and prebiotics incorporated in poultry feeds promote the proliferation of desirable GIT microbiota at the expense of the undesirable types (Alloui et al., 2014) and alter the micro-architecture of the small and large intestinal mucosa resulting in enhanced nutrient digestion and absorption capacity (Hashemi and Davodi, 2010; Dhama et al., 2014). These antibiotic-induced outcomes translate to increased growth performance and boost feed utilisation efficiency. Due to their health beneficial biological activities which mimic antibiotic effects, phytochemicals and plant extracts can potentially replace antibiotics and synthetic preservatives in poultry and livestock production (Dhama et al., 2014). *Hibiscus sabdariffa* Linn is one among plants with many phytochemicals that exhibit health beneficial biological activities that can be tapped into in ethnomedicine and ethnoveterinary medicine (Mohd-esa et al., 2010).

Hibiscus sabdariffa, a herbal shrub, commonly known as roselle, has antibacterial, antifungal, antiparasitic, anti-obesity, antianaemic and antioxidant activities (Da-Costa Rocha et al., 2014). Its antioxidant activity is higher compared to that of BHT a commercial synthetic preservative (Mohd- esa et al., 2010). It is reported to be indigenous to tropical Africa and is grown in Nigeria (Sayago-Ayerdi et al., 2007). *Hibiscus sabdariffa* calyces are used to prepare jams, jellies and herbal beverages (Rao, 1996; Abu-Tarboush et al., 1997). Its calyces are also used as an ethnomedicine for treating colds and coughs, diarrhoea and for lowering cholesterol (Wahabi et al., 2010; Lans, 2006). Extracts of *H. sabdariffa* contain polyphenolics including anthocyanins and flavonoids (Chen et al., 2004). Its dried calyces contain the flavonoids hibiscetine and sabdaretine (Langenhoven et al., 2001) which exhibit neuroprotective properties (Abat et al., 2017). *Hibiscus sabdariffa* calyces contain (+) - hydroxyl citric acid (Yamada et al., 2007; Ubnusad and Thomas, 2003; Jena et al., 2002). The hydrocitric acid inhibits carbohydrate-induced lipogenesis in rats (Carvajal- Zarrabal et al., 2009) as well as citrate lyase hence it has potential to be used as an antiobesity agent (Herranz-Lopez et al., 2012). *H. sabdariffa* calyx extract in drinking water enhanced weight gain and increased feed utilisation efficiency of broiler chickens (Unigwe, 2011). There is, however, insufficient literature on the effects of dietary supplementation of Japanese quail standard diets with *H. sabdariffa* calyces meal on growth performance and feed utilisation

efficiency, GIT viscera morphometry, metabolic substrate content, liver and kidney health, meat and egg quality.

1.3 Justification of the study

The public health challenges associated with antibiotic use at sub-therapeutic concentrations and synthetic preservatives in poultry production are public knowledge (Dhama et al., 2014). Their withdrawal from poultry feeds results in compromised growth performance, feed utilisation efficiency and product quality on the backdrop of increased demand for poultry products (Allen et al., 2013). In order to maintain productive performance as well as produce residue free poultry products, there is need to find alternative growth promoters that do not compromise consumer health (Alloui et al., 2014) but at the same time maintaining and enhancing productivity (Cowan et al., 1999). Commercial broiler and pullet chicken production is beset with several challenges: the need to produce leaner meat, high feed cost, infectious and non-infectious diseases and stress in the birds. In broiler chicken production stress decreases growth performance by compromising feed intake and feed utilisation efficiency (Unigwe et al., 2011). In egg laying chicken (pullets), stress negatively affects egg production and eggshell quality (Mashaly et al., 2004). Stress-induced reduced feed intake results in subnormal intake of key micronutrients such as vitamin E and C as well as calcium (Roland et al., 1996). Vitamins E and C constitute the body's system defence against damaging oxidative lipid peroxidation (Jena et al., 2013) while calcium is the principal element in bone and eggshells (Ketta and Tumova, 2016). If not efficiently and effectively managed, stress results in poor poultry growth performance and ill-health due to the deleterious effects of free radicals from lipid peroxidation (Mishra and Jha, 2019). In laying chicken, stress-induced calcium deficiency causes poor bone strength due to the mobilisation of bone calcium and magnesium (Roland et al., 1996) as well as poor eggshell quality (Adi et al., 2014) that manifest with bone fractures and increased egg breakages leading to losses. In addition to high feed cost and stress, deterioration of meat quality in storage is another major challenge faced by the poultry industry. The peroxidation of polyunsaturated fat acids in meat due to their oxidative instability generates free radicals which cause meat to deteriorate and become less acceptable to consumers (Mishra et al., 2019).

The poultry industry uses antibiotic growth promoters to imcrease/improve growth performance and to control gut microbiota and parasite-induced stress in live birds. Synthetic antioxidants are also incorporated in poultry feeds to prevent deterioration of the feed as well as to build the birds' systemic antioxidant pool which is vital for mitigating stress in live birds (Sahin et al., 2003; Lin et al., 2014) and enhancing shelf life of the meat during storage (Mishra and Jha, 2019). In the discourse earlier on, it was clearly shown that use of antibiotics and synthetic antioxidants in the poultry/food production chain has negative effects on consumer and bird health, poultry product quality and the environment (Alloui et al., 2014; Dhama et al., 2014). Due to the negative effects of antibiotic and other synthetic feeds additives, and the emergence of more enlightened and empowered consumer population, researchers have and are investing resources in the search and development of natural products that can be used in the poultry production chain. Consumer preference for organically produced poultry products (Paravar et al., 2013) is the major driver for the food industry, including the poultry production industry, to consider use of natural feed supplements to enhance food product quality (Velasco and Williams, 2011). These natural feed supplements are also targeted at increasing the oxidative stability of poultry meat (Paravar et al., 2013). The potential natural feed supplements are largely derived from plants in the form of phytogenic products. The potential of such products has been evaluated but there is need for more research in order to broaden the product range.

Plants and herbs rich in polyphenols when incorporated in poultry feed can save production costs for farmers by improving feed conversion ratio and minimising effects of oxidative stress that normally affect bird health (Gebremedin et al., 2017). Apart from an interest in the nutritional and health contribution of poultry products, consumers are increasingly concerned about the welfare of poultry as they are reared (Lipinski et al., 2017). Therefore, it is important that health and welfare issues of the birds are considered in studies.

Majority of the studies evaluating the potential phytogenics to replace prebiotics, antibiotics and synthetic antioxidants in poultry have largely focused on chicken despite the potential of Japanese quail to complement chicken production in the supply of meat and eggs. It is

important to note that there is a growing consumer interest and preference for Japanese quail meat and eggs (Nasr et al., 2017) which is driven by healthier nutrient profile of quail meat when compared to broiler chicken meat (Nasr et al., 2017; Aminzade et al., 2012). There is a dire need to evaluate and develop phytogenic feed additives that can be used to promote and enhance cleaner Japanese quail production without negatively impacting on growth performance, feed utilisation efficiency, bird health and product quality. Candidate phytogenics have to possess antibiotic, antifungal, antioxidant, growth stimulation, immune-modulation and other health beneficial biological activities (Gebrehmedim et al., 2017; Lipinski et al., 2017) that can be harnessed to enhance the production and product quality of Japanese quail. Therefore findings of this study will reveal the potential and extent to which *H. sabdariffa* can be used as a safe alternative in promoting growth, health and productivity of Japanese quail kept for meat and eggs.

1.4 Aim of the study

This study evaluated whether dietary supplementation with *H. sabdariffa* calyces meal could have positive outcomes on growth performance, feed utilisation efficiency, health, meat and egg quality of Japanese quail.

1.4.1 Specific objectives for the study

The specific objectives of the study were to ascertain, in Japanese quail, the effects of supplemental *H. sabdariffa* calyces meal on:

a. growth performance [(body mass and long bone indices (mass, length, mass: length ratio)], feed intake and feed conversion efficiency as well as gastrointestinal tract morphometry - masses and lengths (where appropriate).
b. meat quality: texture, pH, colour, water holding capacity, fatty acid and proximate content of Japanese quail meat.
c. egg quality and composition: egg mass, length and width; albumen mass, length, height and width; yolk mass, diameter and height; shell mass and thickness, proximate and fatty acid content and yolk fatty acid profile of Japanese quail eggs.
d. general health as determined by:

i. erythrocyte osmotic fragility and packed cell volume.
ii. serum surrogate markers of kidney (uric acid) and of liver function (AST, total bilirubin, total protein, albumin, globulin) function and general clinical biochemistry and serum malonaldehyde concentration, a surrogate marker for oxidative stress.

1.5.1 Hypothesis: growth performance, health and meat study

H_0: Dietary supplementation with *H. sabdariffa* calyces meal does not affect the growth performance (as measured by body mass and long bone indices) and feed utilisation efficiency, health and meat quality of Japanese quail.

H_1: Dietary supplementation with *H. sabdariffa* calyces meal promotes the growth performance (as measured by body mass and long bone indices) and feed utilisation efficiency, health and meat quality of Japanese quail.

1.5.2 Hypothesis: egg production and quality and health study in laying quail

H_0: Dietary supplementation with *H. sabdariffa* calyces meal has no effect on egg production, egg quality, health and growth performance, feed utilisation efficiency of laying Japanese quail hens

H_1: Dietary supplementation with *H. sabdariffa* calyces meal promotes egg production, egg quality, health and growth performance (as measured by body mass and long bone indices) and feed utilisation efficiency of laying Japanese quail hens.

In the next chapter, literature on trends in poultry production, challenges besetting poultry producers and processors in the use of synthetic feed additives. The chapter also interrogates the potential of phytogenics as possible candidates in "greener" poultry production with a focused discussion on the possible use of *H. sabdariffa* calyces meal as a supplement in Japanese quail feeds.

2. CHAPTER TWO - LITERATURE REVIEW

2.0 Introduction

Meat and its products are a major component of the diets of many people worldwide. A standard meal for many families in Africa (both urban and rural communities) constitutes at least one type of meat (Chakona and Shackleton, 2019). The most common type of meat is beef, while chicken and egg consumption surpasses that of all the other poultry (Henchion et al., 2017). Although chicken consumption never competed closely with that of beef, lately consumers have since shifted their interests towards chicken (Godfray, 2019) mainly because chicken is easier to produce and cheaper compared to beef (Esterhuizen, 2016) resulting in an increase of poultry products consumption.

2.1 Global poultry products demand and consumption

Worldwide, poultry meat and eggs production as well as consumption, especially that of poultry meat, have increased uniformly over the years in a pattern that is expected to continue into the foreseeable future (Nardoia et al., 2015). Per capita consumption of meat increased from 23kg in 2011 to 42kg in 2013 (Sansa et al., 2015) with poultry meat accounting for 23.42% of the increase (Ritchie, 2017). Poultry meat and products are characterised with a low fat content and a relatively high concentration of polyunsaturated fatty acids (Bourre, 2005) making them more desirable to consumers. Consumption of meat with a high fat content is linked to the development of metabolic abnormalities such as obesity and development of cancers (Wolk, 2017). In addition, poultry meat is cheaper due to its lower price per unit mass when compared to other meats (Esterhuizen, 2016) hence its high preference in food preparations and dishes (Nardoia et al., 2015). The rapid increase in the demand of poultry meat and products in developing countries is driven by an increase in urbanisation, higher household incomes and an increase in human population (Nardoia et al., 2015; Chang, 2007). The significant rise in the production and consumption of poultry in developing countries results in competition in the international trading of all meat products, including feed and other inputs (Chang, 2007; Landes et al., 2004). There are benefits associated with the consumption of poultry products.

2.1.1 Poultry products: the benefits

Beef unlike poultry meat has more saturated fatty acids (Chang, 2007). Therefore, the consumption of beef, when compared to poultry meat, increases the risk of developing metabolic such as type non-insulin dependent diabetes and cancer (Wolk, 2017). Furthermore, when compared to beef, chicken meat is more tender (Nardoia et al., 2015. It is suitable for quick and easy home-cooking making it a good time saver especially in modern societies where less time is reserved for meal preparation at home (Petracci et al., 2015). Eggs are low-cost, nutrient-rich including essential amino acids (Griffin et al., 2016) vitamins and iron (Lopez Sobaler et al., 2017) and the xanthophyll carotenoids lutein and zeaxanthin (Abdel- Aal et al., 2013; Johnson, 2014). These carotenoids are known to help improve visual and cognitive function (Johnson, 2014). Additionally, it has been shown that the consumption of poultry eggs increases the bioavailability of co-consumed carotenoids (Kim et al., 2015) and vitamin E (Kim et al., 2016). Vitamin E is used by the body as a natural antioxidant (Kim et al., 2016). The consumption of poultry products is not confined to humans. By products from poultry, for example, blood meal and feathers are processed and then used as feed supplements by the livestock feed industry (Alao et al., 2017). Poultry production is not without challenges though.

2.2 Challenges in poultry production

Among the challenges that beset poultry production high feed cost and disease outbreaks are among the major (Hafez and Attia, 2020). Feed cost represents the major part of poultry production cost accounting for 60% to 80% of the overall cost of production (Thirumalaisamy et al., 2019). Poultry feeds contain an average of 53% cereal meal and 37% legume seed meal (Attia et al., 2013) which are also used for human food preparation (Iji et al., 2017). This results in competition for feed/food resources which result in high poultry feed cost. In a bid to contain the negative impact of high feed cost on the viability of poultry enterprises, producers supplement poultry feed with synthetic (antibiotics and preservatives) growth promoters (Alloui et al., 2014). However, the use of antibiotics at sub-therapeutic levels and synthetic antioxidants has been shown to elicit antibiotic resistance (Dhama et al., 2014, Zhang et al., 2010) and to result in antibiotic and synthetic antioxidant residues in

poultry meat and eggs that compromise consumer health (Salim et al., 2018). Besides high feeding cost, poultry production also faces the burden of diseases.

Diseases in poultry can be of an infectious or non-infectious nature. Infectious poultry diseases include respiratory (e.g. infectious bronchitis, avian flue) and non-respiratory bacterial (e.g. fowl cholera, necrotic enteritis, staphylococcus, botulism) diseases (Nawab et al., 2018), while non-infectious conditions include disorders of the egg tract, vices, nutritional disorders and poisoning (Fitzpatrick and Morton, 2010).

In addition to the burden of disease, the intensive nature of poultry production places a tax on the birds' metabolic processes which elicits oxidative stress. Diseases in poultry result in reduced productivity (less eggs, reduced meat, reduction in growth rate, poor FCR, poor product quality), loss of money due to deaths, medication and low performance (Mendes et al., 2014).

2.3 Poultry production and oxidative stress

Oxidative stress occurs when body's free radical quenching system (antioxidant pool) is overwhelmed by the production of damaging reactive species largely from lipid peroxidation (He et al., 2017). Reactive oxygen species, when in excessive amounts, override the body's antioxidant system and destroy cell proteins, cellular carbohydrate moieties, plasma membranes and DNA molecules (Rehman et al., 2018). The oxidative stress mediated changes in biological molecules causes structural changes to these biological molecules making them fail to execute functions resulting in cell death (Jena et al., 2013). The occurrence of oxidative stress in poultry and other livestock affects their health and compromises productive performance and product quality (Jena et al., 2013; Mishra and Jha, 2019). Commercial poultry production is essentially intensive and if the housing of birds is poorly ventilated it introduces the threat of pathogens (Rehman et al., 2018) and increases metabolic demand/activity. An increased metabolic activity triggers the production of reactive oxygen species (Rehman et al., 2018) which damage biological molecules thus compromising poultry growth performance and feed intake and utilisation (Mishra and Jha,

2019). In addition to negatively affecting poultry production performance, lipid peroxidation also affects poultry feed and meat shelf life.

2.4 Lipid oxidation: effects on poultry feed and meat

Lipids in feed ingredients as well as in meat (in storage) can undergo peroxidation which causes the deterioration of the feed and or meat. Exogenous synthetic antioxidants are routinely incorporated in commercial poultry and livestock feeds to help preserve the sensory qualities of the feed and guard against the destruction of critical micronutrients particularly vitamins and dietary pigments with nutraceutical value (Salim et al., 2018). The production of unpleasant smells and flavours due to lipid peroxidation reduces feed intake which compromises growth performance and product yield (Estevez, 2015). In meat lipid oxidation limits quality and acceptability (Zamora et al., 2001) by compromising shelf life. Oxidation of lipids leads to reduction in the quality of sensory attributes (colour, water holding capacity, flavour and texture) as well as the development of chemicals which may be toxic (Estevez, 2015; Amaral et al., 2018). In addition to reducing shelf life of meat, lipid peroxidation also causes loss of nutritive and economic value as well toxicities in consumers (Amaral et al., 2018). In order to maintain the quality and shelf life of feeds, synthetic antioxidants are incorporated in commercial feed to prevent lipid peroxidation and oxidative rancidity (Salim et al., 2018). These synthetic antioxidants in feeds are incorporated and accumulated *in vivo* in poultry meat and eggs wherein they also exert antioxidant activity and in the process reduce lipid peroxidation of poultry meat in storage contributing to increased shelf life (Estevez, 2015). In addition to synthetic antioxidants, growth promoters are also incorporated in poultry feeds.

2.5 Growth promoters in poultry production

In commercial poultry feed production, the incorporation of supplements in feeds with no direct nutritional value is common practice. These supplements enhance growth performance and feed utilisation efficiency by suppressing the proliferation of undesirable gut microbiota hence reduce the competition for nutrients between the host and undesirable gut microbiota (Li et al., 2014). Additionally, they also stimulate the proliferation of beneficial microbes (Li

et al., 2014). In addition to availing more nutrients to the host, these growth promoters also mitigate against the gut microbiota induced GIT mucosa irritation resulting in enhanced nutrient absorption (Dhama et al., 2014).

2.5.1 Antibiotics as growth promoters in poultry production

The poultry industry has for over half a decade used antibiotics as feed additives in poultry feeds (Alloui et al., 2014). These antibiotics are used as therapeutic and prophylactics and as growth promoters (Li et al.,2014). Bacitracin, penicillin, doxyclin, flavomycin, erythromycin and availmycin are commonly used (Chowdhury et al., 2009; Hassan et al., 2010). These antibiotics prevent the exponential multiplication of pathogenic bacteria in the GIT thus help reduce incidences of non-specific diarrhoea and enteritis in poultry (Huyghebaert et al., 2011). These effects result in improved GIT health and reduced competition for nutrients between the host (bird) and GIT resident bacteria (Dhama et al., 2014). These antibiotics, from a mechanistic point of view, regulate and maintain the optimal balance between gram-negative and gram-positive bacteria in the avian GIT (Dhama et al., 2014). They also selectively suppress the proliferation of undesirable gut bacteria and induce growth and proliferation of desirable gut bacteria (Li et al., 2014). However, the use of antibiotics in the food production chain has and continues to raise concerns (Katz and Baltz, 2016). Their prolonged and continued use in the food production chain has led to the genesis of bacteria resistant to antibiotics which has and continues to have deleterious effects on public health delivery (Katz and Baltz, 2016; Dhama et al., 2014). Additionally, the antibiotic residues in eggs, meat and milk have been shown to cause toxicities in consumers of the poultry and livestock products (Salim et al., 2018). Over and above causing toxicities in consumers of poultry and livestock products, the antibiotic residues in these products alter microbiota in the human GIT resulting in the genesis of antibiotic resistant bacterial strains which negatively impact the brain-gut axis (Brenes et al., 2016). The antibiotic induced negative impact on the brain-gut axis compromises the regulation of several physiological functions including maintaining homeostasis as well as the modulation of satiety and hunger (Brenes et al., 2016). The emergence of antibiotic resistant bacteria, partly through reckless use of antibiotics in poultry and livestock production, has and continues to place a heavy burden on

public health delivery system due to failure of antibiotic therapy for clinical (Lipinski et al., 2017) and veterinary purposes (Dhama et al., 2014). Despite the negative effects of antibiotic use as growth promoters in poultry and livestock production, their exclusion compromises poultry productive performance in addition to increasing morbidity and mortality in flocks (Lilehoj et al., 2018). There are some newer generation growth/productivity promotors such a zinc bacitracin however being synthetic in origin there are still consumer concerns about their use (Dhama et al., 2014). The health challenges and the environmental pollution associated with the unscrupulous use of antibiotics in the food production chain has helped create a niche research area focusing on finding efficacious natural alternatives that do not elicit drug (antibiotic) resistance, cause toxicities and pollute the environment (Lilehoj et al., 2018). Most of this research has and continues to focus on plant-derived extracts and or phytogenic compounds that have a preponderance of polyphenolics (Mahfuz et al., 2017).

2.5.2 Polyphenols in poultry production

Allium sativum, Curcuma longa, Thymus vulgaris, and Zingiber officinale are herbal plants whose products have been incorporated in poultry feeds. Their inclusion in poultry feeds has been demonstrated to enhance growth performance and egg production in broiler and pullet chicken, respectively (Guo et al., 2004; Sunder et al., 2013). These herbal plants and products derived from them contain polyphenolic compounds inclusive of flavonoids, tannins, saponins and alkaloids (Lilehoj et al., 2018; Mahfuz et al., 2017). It is argued that the enhanced growth performance and egg production by broiler and pullet chicken observed when fed containing these herbal supplements is mediated by the polyphenolic compounds in the products (Yang et al., 2015). In addition to polyphenolics, such herbal plants, whose products are used as supplements in poultry feeds, also contain other phytochemicals with health beneficial and growth promoting biological activities (Alloui et al., 2014; Mahfuz et al., 2017). Among these beneficial phytochemicals are essential oils, alkaloids, flavonoids, phytosterols and triterpene terpenoids (Lilehoj et al., Dhama et al., 2014).

Polyphenols, through their pro-oxidant activity which makes them antimicrobial (Mahfuz et al., 2017) influence the gut health significantly (Exterberria et al., 2013; Brenes et al., 2016) by killing bacteria (Gordon et al., 2010) and inhibiting growth of bacteria (Exterberria et al.,

2013). In addition, polyphenolic compounds decrease the attachment of pathogenic *E coli* and *Clostridium* species onto the GIT mucosa that helps reduce infection (Lilehoj et al., 2018). These polyphenolic-mediated effects in the gut result in improved nutrient absorption that translates to improved poultry and livestock growth performance (Brenes et al., 2016; Duenas et al., 2015;Viveros et al., 2011). Anthocyanins, another class of polyphenols plentiful in many plants, have bacteriostatic and bactericidal effects against several bacteria species including the *Bacillus, Klebsiella*, and *Helicobacter pylori* (Yang et al., 2015).

Quercetin, a polyphenol, mainly present in fruits and vegetables, has hydroxyl groups which allow it to be incorporated in bacterial lipid membranes which increases permeability making them susceptible to antibacterial compounds (Paszkiewicz et al., 2012; Chiva-Blanch and Visoli, 2012). In addition to having immune modulating effects (Alloui et al., 2014), polyphenols have been shown to promote proliferation in the gut, of beneficial bacteria and the stabilisation gut microflora resulting in improved host gut health (Exteberria et al., 2013) which translates to improved nutrient uptake (Kamboh et al., 2015). Essential oils containing phenolic compounds mediate significant increases in the villi height in broiler chicken when incorporated in feed as supplements (Hong et al., 2012). Similarly, flavonoids, hesperin and genistein from *Gingko biloba* leaves enlarged the absorption area in the ileum of broiler chicken exposed to lipopolysaccharide-induced stress (Zhang et al., 2013). These findings demonstrate that use of plant-derived products containing polyphenolic compounds as feed supplements positively impacts nutrient absorption by increasing the absorptive surface area. Dietary supplementation with cranberry extract, rich in flavonols, anthocyanins and phenolic acids, reduced the enterococcus bacteria species in the gut of broiler chicken (Leusink et al., 2010).

Lewis et al. (2003) showed that garlic powder in feed may stimulate growth and increase feed intake in chickens. The nutritious *Moringa oleifera* leaves possesses phytochemicals, for example, flavonoids, alkaloids and phytosterols (Saini et al., 2016) are used as a supplement in poultry feeds. When used as a dietary supplement *M. oleifera* leaf meal improved the health status and productive performance (increased body weight and body

weight gain) of broiler chicken (Mahfuz and Piao, 2019). In layer chickens, dietary *M. oleifera* leaf meal was shown to mediate increased body weight gain to improve feed utilisation efficiency coupled with increased egg production (Voemesse et al., 2019; Abouz-Elezz et al., 2011). These *M. oleifera* leaf meal derived benefits are attributed to the phytochemicals in the leaf meal which possess health beneficial (antibacterial, antifungal, antioxidant) activities that improve gut health, nutrient digestion and absorption and also mitigate stress (Mahfuz and Piao, 2019). In addition to *M. oleifera* leaf meal, *H. sabdariffa* calyces meal has been extensively used as a feed additive as well as an ethnomedicine.

The use of *H. sabdariffa* calyx extract, a rich source of poylphenolics, as a supplement in the drinking water for broiler chicken drinking resulted in increased weight gain and feed conversion efficiency (Unigwe, 2011). These *H. sabdariffa* growth promoting effects were ascribed to phytochemicals found in the plant extract (Alloui et al., 2014).

Naturally-derived probiotics and prebiotics can be used to enhance poultry growth performance and feed utilisation efficiency.

2.5.3 Prebiotics and probiotics as feed additives

Prebiotics are indigestible feed ingredients that alter the make-up and metabolism of gut microbiota selectively (Yadav and Jha, 2019). They are used to favourably alter the poultry GIT environment in order to boost productive performance (Costa et al., 2013). Probiotics stabilise gut pH which helps improve health and immune status of the birds (Dhama et al., 2014). Prebiotics, largely galactose, fructose and mannose oligosaccharide derivatives (Dhama et al., 2014), are added as non-digestible supplements to poultry feeds (Salim et al., 2018) exert beneficial effects on the host by selectively promoting the proliferation of desirable but harmless GIT bacteria (Dhama et al., 2014). In addition, they provide substrates for bacterial fermentation in colon/caecum which produces vitamins and antioxidants that are utilised by the host (Dhama et al., 2014). The probiotics also stimulate the absorption of minerals in the intestines resulting in increased feed utilisation efficiency (Dhama et al., 2014).

Having earlier considered the pros and cons of use of mainly synthetic feed additives, there is need to review the use of phytogenic feed additives in poultry production.

2.6 Hibiscus: Description and varieties

Hibiscus has more than three hundred species distributed in tropical and subtropical regions around the world and are used as ornamental plants (Sayargo Ayerdi et al., 2007). Among numerous varieties of Hibiscus, *Hibiscus altissima* and *Hibiscus sabdariffa* are the commonest and better introduced. *Hibiscus altissima* is branchless plant with yellow flowers and red or green colored calyces. Though this species is not used for food, this plant is more economically important than *Hibiscus sabdariffa* because of its high fiber content (Rao, 1996; Abu-Tarboush et al., 1997). The other distinct type *Hibiscus sabdariffa* or "Roselle" grows in a bush with many branches. The Roselle flowers are axillaries or in terminal racemes, the petals are white with reddish center at the base of the stamina column and this species is widely used as food (Singh et al., 2017). In this study, I used the red calyces *H. sabdariffa* variety.

2.7 *Hibiscus sabdariffa:* nutrient and phytochemical content

H. sabdariffa calyces and leaves contain protein, fat, carbohydrates, fibre, vitamin C, β-carotene, calcium and iron while its seed is a rich source of oil, protein, carbohydrates and fibre (Ismail et al., 2008). Palmitic and stearic acids which make up 28% (Kilic et al., 2011) of the *H. sabdariffa* seed oil are the major saturated fatty acids in the seed oil while linoleic acid and oleic constitute the oil's main unsaturated fatty acids (Nzikou et al., 2011). Stearic and oleic acid reduce the risk of cardiovascular risk by reducing cholesterol in the blood (Johnson and Bradford, 2014). *Hibiscus sabdariffa* therefore , apart from being rich in oleic and stearic acid, can also be exploited for its rich ascorbic acid and thiamine content. Ascorbic acid (vitamin C) is a vital nutrient with a critical role in collagen formation, absorption of iron and is also required for normal immune function and response (Chambial et al., 2013). Thiamine is a critical precursor of the co-enzyme thiamine pyrophosphate which required for the oxidative decarboxylation of pyruvate in energy generation (Fattal-

Valevski, 2011). Thus *H. sabdariffa* over and above being a potential source of macronutrients, can also supply micronutrients with critical metabolic functions.

Hibiscus sabdariffa contains several phytochemicals: anthocyanidins, flavonoid-based polyphenols, for example, hibiscitrin, sabdaritrin, gossypitrin, gossypetin glucosides, quercetin and luteolin (McKay, 2009). It also has chlorogenic acid, pelargonidic acid, eugenol, quercetin, luteolin and ergosterol (Williamson et al., 2009). These phytochemicals in *H. sabdariffa* impart its meals and extracts, health beneficial biological activities, including among many, antimicrobial, antioxidant, hepatoprotective, nephroprotective, hypocholesterolemic and antianaemic (Da-Costa-Rocha et al., 2010) which can be exploited in poultry production.

Extracts from *H. sabdariffa* exhibit strong antioxidant activity mediated by hibiscetine, gossypetin and sabdaretine in the extract (Williamson et al., 2009). These phytochemicals possess significant free radical scavenging activity (Izquierdo-Vega et al., 2020). In addition to growth promoting, bactericidal and antioxidant activities, extract derived from *H. sabdariffa* have hepato- and reno-protective properties (Izquierdo-Vega et al., 2020; Abat et al., 2017). In addition to reducing serum triglycerides, total cholesterol and LDL in rats, aqueous *H. sabdariffa* extracts were shown to reduce lipid peroxidation but increased catalase and glutathione activities in rats with diabetes (Lee et al., 2009). Taken together, the host of health beneficial biological activities in *H. sabdariffa* calyces/leaf meals and or its extracts can be exploited in the poultry including quail production.

2.8 *Hibiscus sabdariffa* in poultry production

Hibiscus sabdariffa calyces meal and or extracts have been used as dietary supplements in chicken production. The addition of *H. sabdariffa* calyces meal and antibiotics in the drinking water of broiler chicken resulted in improved growth performance and meat quality (Usman et al., 2016). *H. sabdariffa* calyces extracts as dietary supplements have been shown to improve digestibility of broiler chicken diets (El- Husseiny et al., 2002). Importantly, they have been found to improve the average daily weight gain of chickens (Unigwe, 2011).

Afolabi et al., (2008) showed that *H. sabdariffa* calyces extract is bactericidal to *Streptococcus mutans*. It has also been shown that *H. sabdariffa* calyces extract inhibits Campylobacter species that cause deterioration of poultry meat, beef and pork (Yin and Chao, 2008). There is evidence of the use and positive effects of *H. sabdariffa* in chicken production (El- Husseiny et al., 2002; Unigwe, 2011; Usman et al., 2016), as well as in livestock production (Elamin et al., 2012; Mukhtar, 2007; Plotto, 2004), and human health (Frank et al., 2012; Mohd– Esa et al., 2010). However, there is lack of information on its use in the promotion of the production of Japanese quail. Based on the health beneficial biological activities driven by phytochemicals present in *H. sabdariffa* as well as the positive outcomes associated with its use in chicken production as a supplement in feed and or drinking water, it can be postulated that *H. sabdariffa* calyces meal is likely to have a positive impact on Japanese quail.

The next chapter reports and discusses the effects of supplemental *H. sabdariffa* calyces meal on the growth performance, feed utilisation efficiency and health of Japanese quail.

3. CHAPTER THREE - EFFECT OF *HIBISCUS SABDARIFFA* CALYCES MEAL ON GROWTH PERFORMANCE, GASTROINTESTINAL TRACT VISCERA MACROMORPHOMETRY AND GENERAL HEALTH OF JAPANESE BROILER QUAIL

3.0 Introduction

There is an increased interest in Japanese quail meat worldwide (Mizutani et al., 2010). The observed increase is premised on several advantages of quail meat compared to other poultry meat types like chicken. Japanese quail are small, hardy birds, requiring less space for production compared to chicken and other poultry (Alumuoye et al., 2015). Furthermore, Japanese quail have a shorter generational period and produce more eggs compared to chicken (Onyewuchi et al., 2013). Despite their advantages over chicken, like other poultry species, the production of Japanese quail is faced with challenges such as high feed cost and oxidative stress caused by the production environment (Mishra et al., 2019).

Production of poultry owes 60-80% of its overall cost to feed (Thirumalaisamy et al., 2019). In order to mitigate this challenge, farmers supplement poultry feed with synthetic growth promoters to improve the birds' feed utilisation efficiency and enhance growth (Alloui et al., 2014). However, the use of synthetic growth promoters is limited in many countries owing to their residual effects on animal products which can be carried over to humans upon consumption resulting in ill health and diseases, for example, cancer (Blasczyck et al., 2013). Therefore, it is imperative to find natural and safe growth, feed utilisation and health promoting alternatives for the poultry industry in order to ensure bird and consumer safety (Mishra et al., 2019)) as well as to entrench a culture of environmentally friendly poultry production (Salim et al., 2018).

Plant-derived materials, for example, leaf meals and extracts have been shown to have growth promoting and health beneficial biological activities. Among the many plants, *H. sabdariffa* has been shown to contain many phytochemicals, among many flavonoids and polyphenols (Sayago-Ayerdi et al., 2007) with antimicrobial (Nwaiwu et al., 2012), hepatoprotective (Lee et al., 2012), antihyperlipidaemic (Chen et al., 2004), antioxidant (Mohd-Esa et al., 2010) and growth promoting effects (Unigwe et al., 2011). Studies in poultry production have focused more on productive performance and product (meat and eggs) quality but with little and/or no attention to bird health and welfare. This study therefore evaluated the potential of supplementing broiler Japanese quail diets with *H. sabdariffa* calyces meal on growth performance, feed utilisation efficiency, gastrointestinal

tract (GIT) and accessory organs macro-morphometry and the general health of broiler Japanese quail.

3.1 Study objectives

The specific objectives of the study were to determine, in Japanese quail, the effects of dietary supplementation with *H. sabdariffa* calyces meal on:

a. growth performance (body weight and long bone indices), feed intake and feed utilisation efficiency, gastrointestinal macro morphometry of the quail (masses and lengths where appropriate),
b. general health as determined by the following:
    i. erythrocyte osmotic fragility and packed cell volume
    ii. serum surrogate markers of kidney (serum uric acid level) function and surrogate markers of liver (serum AST, total bilirubin total protein, albumin, globulin levels) function, general clinical biochemistry (calcium, phosphorus) and serum malonaldehyde concentration.

3.2 Study hypothesis

H_0: Dietary supplementation with *H. sabdariffa* calyces meal does not affect the growth performance (body mass and tibiae and femora based growth indices), gastro-intestinal tract viscera macromorphometry, erythrocyte osmotic fragility, packed cell volume, serum proxy measurements of kidney and liver function, general clinical biochemistry and serum antioxidant marker (malonaldehyde concentration) of Japanese quail.

H_1: Dietary supplementation with *H. sabdariffa* calyces meal promotes the growth performance (body mass and tibiae and femora based growth indices), gastro-intestinal tract viscera macromorphometry, erythrocyte osmotic fragility, packed cell volume, serum proxy measurements of kidney and liver function, general clinical biochemistry and serum antioxidant marker (malonaldehyde concentration) of Japanese quail.

3.3 Materials and Methods

3.3.1 *Hibiscus sabdariffa* calyces: source and preparation

The *H. sabdariffa* calyces used in this study was surplus from a study done in the School of Physiology, University of the Witwatersrand. The dried *H. sabdariffa* calyces with the red calyces bought from Sokoto Central Market in North West Nigeria and identified in the department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto by Mr Halilu E Mshelia (voucher PCG/UDUS/Malv/0001) were imported into Johannesburg, South Africa. The calyces were crushed into a fine powder using a laboratory blender (Waring®, Lasec SA, Johannesburg, South Africa).

3.3.2 Determination of the proximate content of *H. sabdariffa* calyces meal

The proximate content of the *H. sabdariffa* calyces meal was determined at the Agricultural Research Council's Irene Analytical Services Laboratories, South Africa. Fat from the *H. sabdariffa* calyces meal was extracted in diethyl ether according to method 920.39 of the Association of Analytical Chemists (AOAC, 2000). The dry matter, ash and crude protein content were determined as described by the Association of Analytical Chemists (AOAC, 2000: Methods 934.01 and 930.15, 942.05 and 954.01, respectively). Each assay was done in duplicate.

3.3.3 Determination of the total phenolic content of *H. sabdariffa* calyces meal

One hundred grams of powdered calyces of *H. sabdariffa* calyces were macerated in either distilled water or a 50:50 (v/v) methanol and ethanol mixture as a solvent (Chandran et al., 2012). The meal-solvent mixture was extracted for 24 hours in a conical flask on a shaker. The mixture was then immediately filtered and the filtrate concentrated by rota-evaporation at 40ºC for 45 minutes. The resulting solution was dried in an oven for 24 hours and the extracts kept at 4ºC until use. The total phenolic content in both the aqueous and methanol-ethanol extracts was determined using Folin Ciocalteau reagent as described by

Parimelazhanag (2016). Briefly, 5 aliquots of the standard working solution (0.2, 0.4, 0.6, 0.8 and 1 mL) were pipetted into a series of test tubes, after which 50µl of the crude extracts were added to each of the 5 test tubes. To each tube, 0.5ml of Folin Ciocalteau reagent were added and the mixture in each tube and topped with distilled water to get to1 ml. The mixture was held for 5 minutes at room temperature after which 2.5ml of 5% sodium carbonate was added to it and incubated in darkness for 40 minutes at room temperature. Following incubation, the absorbencies for each mixture from the 5 test tubes were read at 725nm using a UV/visible spectrophotometer (Ultro-spec 11, LKB Biochrom, Cambridge, England). Gallic acid was used as a standard and the total phenolic content of *H. sabdariffa* calyces extract was expressed as mg/g Gallic acid equivalent (mgGAE/g). The assay was done in duplicate for each extract.

3.3.4 Feed Ingredients and formulation of diets

Yellow maize, soyabean meal, wheat bran and methionine were purchased from Opti-feeds Private Limited, Lichtenberg, South Africa. The vitamin-mineral premix was purchased from Trow Nutrition, Johannesburg, South Africa. The standard quail finisher diet was formulated at the Central Animal Services (CAS) animal research facility, University of the Witwatersrand, to meet the minimum nutritional requirements of finishing male and female Japanese quail according to National Research Council (NRC, 2001) recommendations. The three dietary treatments were made by supplementing the standard Japanese quail finisher diet (SQFD) with *H. sabdariffa* calyces meal at 0%, 5% and 10% (w/w) for diets 1 up to 3, respectively. The ingredients, proximate, calcium and phosphorus content of the diets used in this study are presented in Table 3.1.

Table 3.1: Ingredient and chemical composition of control and test diets

Ingredients (g/kg)	Diet 1	Diet 2	Diet 3
Maize meal	627.27	627.27	627.27
Soya bean meal	315.46	315.46	315.46
Wheat bran	20.00	20.00	20.00
DCP/Limestone	12.80	12.80	12.80
Vit/Min premix	3.00	3.00	3.00
Salt	3.00	3.00	3.00
Methionine	4.18	4.18	4.18
Vegetable Oil	12.00	12.00	12.00
H. sabdariffa calyces meal	0.00	50.00	100.00
Total	997.70	1047.71	1097.71
Chemical composition			
Dry matter (%)	90.48	90.22	90.12
CP (% DM)	26.57	23.15	22.90
EE (% DM)	7.18	5.50	3.56
Ash (% DM)	9.51	8.12	8.24
Ca (%DM)	1.69	1.13	1.17
P (% DM)	0.48	0.42	0.40
Crude fibre (%)	4.72	5.35	5.54
Energy (MJ/Kg)			
Gross energy	17.69	17.33	17.08

DCP, dicalcium phosphate; DM, dry matter; CP, crude protein; EE, ether extract; Ca, calcium; P, phosphorus; vitamin-mineral premix: each kg contained vitamin A 4000 000 IU, vitamin D_3 600 000IU, vitamin E 8000IU, vitamin K_3 0.258g, vitamin B_1 0.6g, vitamin B_2 1.6g, niacin 11.94g, calcium pantothenate 3.92g, vitamin B_{12} 0.1g, vitamin B_6 0.98, choline 72.73g, folic acid 0.288g, biotin 0.0008g, MnSO4 9.92g, Zn 6.3g, Cu 0.252g, KI 0.2g, Co 0.0042g, Fe 2.1g, Se 0.0036g

3.3.5 Ethical approval and study site

This study, approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (Ethics clearance number: 2014/46/D), was conducted at the Central Animal Services research facility of the University of the Witwatersrand, Johannesburg.

3.3.6 Quail and quail management

The seventy-five 35-day old Japanese quail (30 males; 45 females) used in this study were purchased from South Africa Quail Breeders (East London, South Africa). The birds were individually housed in wire mesh cages (60 x 60 x 80cm) in a well-ventilated temperature and lighting controlled room. The floor of each cage had a cardboard fixed onto which dry and clean straw was laid as bedding. Bedding was changed once every week. A small box with an opening was placed inside each cage as enrichment to the environment for each bird. The boxes provided a hiding place and perch for the birds. The photoperiod was set at 12 hours with lights on between 0600h and 1800h. The environmental temperature of the room was maintained at 21±2°C. Habituation of the birds was done over 2 days before starting the experiment in order to familiarise with the environment. During habituation, the birds were dewormed with piperizine [Kyron Laboratories (PVT) Ltd, Johannesburg, South Africa] at 90 mg/litre of their drinking water before commencement of the experiment. The Japanese quail had *ad libitum* access to feed and clean water to drink during the feeding trial.

3.3.7 Experimental design

Seventy-five (male: 30; female: 45) 35-day old Japanese quail, each individually housed, were in a completely randomised design, randomly allocated to and fed finisher diets supplemented with *H. sabdariffa* calyces meal at 0%, 5% and 10%, respectively, and fed for 28 days. Each diet was replicated 10 times (n = 10) and 15 times (n = 15) for the male and female quail, respectively.

3.3.8 Measurements and computations

The induction body weight of each bird was measured on an electronic scale (Snowrex EQ-1200, Clover Scales Pty Ltd, Johannesburg, South Africa) and thereafter the body weight of

each bird was measured twice a week for the duration of the experimental period. The total body weight gain (BWG) and average daily gain (ADG) of each bird were computed using formulae:

i. $BWG = Terminal\ weight\ - Induction\ weight$

ii. $ADG = BWG \div Number\ of\ days\ of\ feeding\ trial$

Feed intake of each bird was determined twice a week and calculated using the formula:

$Feed\ intake = Feed\ offered\ (g) - residual\ feed\ (g)$

The feed conversion ratio (FCR) of each bird was computed using the formula:

$FCR = Feed\ intake(g) \div BWG$ (Onu et al., 2004).

3.4 Terminal procedures and measurements

After 28 days of feeding, the quails were weighed and fasted for 4 hours, after which they were slaughtered humanely using a guillotine (Harvard apparatus, Holliston, Massachusetts, United States). Blood was collected into plain and heparinised blood collection tubes. After bleeding out, the quail carcasses were manually de-feathered and the carcasses dissected carefully to remove GIT viscera for determination of morphometry. Blood in the plain blood tubes was centrifuged at 35 217g for 15 minutes (Thermo Sorvall® RT 600B centrifuge; Du Pont Instruments, Wilmington, CT, USA) and serum harvested. The harvested samples of serum were quickly frozen at -20^{o}C for determination of serumproxy measurements of general health and antioxidant status. Blood in the heparin coated blood tubes was used to determine haematocrit and erythrocyte osmotic fragility.

3.4.1 Determination of erythrocyte osmotic fragility

Erythrocyte osmotic fragility was measured as described by Baker and Silverton (1980). Briefly, 50µl of whole blood was added to tubes containing 5ml of serially increasing concentrations (0 – 0.9%) of phosphate buffered saline (PBS) with a pH of 7. The mixtures were incubated at room temperature for 30mins. Immediately thereafter they were centrifuged (Sorvall RT 6000B, Du Pont, UK) at 699g for 5 minutes. The supernatants were

carefully decanted into cuvettes and their absorbance read at 540nm on a spectrophotometer (Ultro-spec 11, LKB Biochrom, Cambridge, England). Distilled water was used as the blank. The haemoglobin released (%) in each PBS concentration was calculated for each bird and fragiligrams were plotted.

3.4.2 Determination of haematocrit

The haematocrit was determined by centrifuging heparinised blood in microcapillary tubes (sealed at one end with sealant) at 13 700g force for 2mins in an IDEXX Staspin VT centrifuge (IDEXX Laboratories Inc. USA). The haematocrit (%) were read on a haematocrit reader (IDEXX Laboratories Inc. USA).

3.5 Determination of long bone parameters

Tibia and femur bones from the right leg were carefully removed, cleaned of all soft tissue and placed in an oven to dry at 50^0C for 7 days till constant mass was reached. Bone dry mass was measured using a balance (Presica 310M, Laser, Johannesburg, South Africa) and the length of the bone was measured as the distance from the upper proximal point of the bone to the lower distal part of the bone of interest using a Vernier calipers (Hi-Impact, Dejuca, South Africa).

The mass: length ratio of bone was computed using the formula:

Bone mass: length ratio = mass/ length of bone (Seedor et al., 1991, Almeida et al., 2008)

3.6 Determination of general health profile

The quail's serum glucose, uric acid, calcium, total protein, albumin, globulin and aspartate transferase (AST) activity were determined using a colorimetric-based calibrated Clinical Chemistry analyser (IDEXX Laboratories Inc., Westbrook, ME, USA) as per the manufacturer's instructions. Briefly, each serum sample was thawed and allowed to warm to room temperature and inverted gently to ensure homogeneity of the mixture. The analyser

automatically sucked 150 µl of the serum and loaded 10 µl of serum onto each of the preloaded cassettes. The samples were analysed and printouts of the results provided.

3.6.1 Determination of oxidative stress status

A quantichrom thiobarbituric acid reacting substances (TBARS) assay Kit (DTBA-100, Bioassay systems, USA) was used to determine the serum malonaldehyde concentration using serum samples according to the manufacturer's protocol with minor modifications. Briefly, 200µL of ice-cold trichloroacetic acid (TCA) was added to 100µL of each of the serum sample and incubated on ice for 5minutes. The mixture was centrifuged at 19 480g for 7mins in a centrifuge (Eppendorf centrifuge, 5415 R, USA). Thereafter, 200µL of the supernatant was transferred to a separate labelled tube onto which 200µL of thiobarbituric acid (TBA) was added and the mixtures were incubated in a water bath at 100ºC for 60mins. The reaction mixtures were then cooled to room temperature and 100µL of the mixtures loaded in duplicate to wells of a clear flat bottom 96 well plate and the absorbance read at 540nm on a spectrophotometer (Multiskan Ascent, Lab systems, Germany).

3.7 Statistical analysis

The data were analysed separately for each gender. Data are expressed as mean ±SD. Graph Pad Prism Version 5 (Graph-pad Software Inc., San Diego, USA) statistical package was used to analyse the data. The General Linear Model (Proc GLM) of the Statistical Analysis System (SAS, 2003) was used to analyse data on weekly body weight using a repeated measures ANOVA. The model used for data analysis was:

$Yijk = \mu + Ti + Wj + TWij + eijk;$

where

Yijk = is the kth observation (body weight) of the ith dietary treatment of the j

th week

µ = is the overall mean

Ti = is the fixed effect of the ith dietary treatment (i = 1, 2…5)

Wj = is the effect of the jth week of measurement (j = 1, 2… 3…..9)

TWij = is the interaction between dietary treatment and week

eijk = is the random residual error

Data on growth performance (body weight based)

A one-way ANOVA was used to analyse other parametric data. The Bonferroni *post hoc* test was used to compare treatment means. The level of significance was set at $P < 0.05$. The statistical model used was:

$Y_{ij} = \mu + T_i + e_{ij}$;

where Y_{ij} = dependent variable of interest (growth performance indices (body weight, body weight gain, average daily gain, feed intake, and feed conversion efficiency), haematocrit, GIT viscera masses and length and serum markers of health

μ = is the overall mean common to all observations

T_i = is the fixed effect of the i^{th} dietary treatment (i = 1, 2, 3)

e_{ij} = is the random residual error.

3.8 Results

Hibiscus sabdariffa calyces meal: proximate and fibre content

The proximate and fibre content of the *Hibiscus sabdariffa* calyces meal is presented in Table 3.2.

Table 3.2: Proximate and fibre content of *Hibiscus sabdariffa* calyces meal

Parameter	Content
Dry matter (%)	89.82±0.09
Ash (% DM)	12.00±0.05
Protein (% DM)	4.70±0.14
Fat (% DM)	0.66±0.03
Crude fibre (% DM)	13.27±0.01
Neutral detergent fibre (% DM)	20.59±0.58
Acid detergent fibre (% DM)	15.82±0.02

DM - dry matter. n = 2.

3.8.2 *Hibiscus sabdariffa* calyces meal: phenolics content

Table 3.3 shows the total phenolic content of *H. sabdariffa* calyces meal aqueous and methanol: ethanol extracts. The methanol: ethanol extract had significantly higher (P = 0.003) total phenolics content (25.94mgGAE/g) compared to aqueous extract (23.75mgGAE/g).

Table 3.3: Total phenolics content in aqueous and methanol: ethanol extracts of *H. sabdariffa* calyces

	Aqueous extract	Methanol: Ethanol extract	P-value
Total phenolics (mgGAE/g)	23.75 ± 0.15^{b}	25.94 ± 0.57^{a}	0.003

[a, b] Within row means with different superscripts differ significantly at P < 0.05. mgGAE/g – milligram gallic acid equivalent per gram, Data are expressed as mean± SD.

3.8.3 Growth performance

Tables 3.4 and 3.5 show the weekly and total trial growth performance of male and female Japanese quail respectively. There were no significant differences in the induction body weight and dietary *H. sabdariffa* calyces meal did not significantly affect (P = 0.3691) the terminal body weight (TBW) of male Japanese quail. In week 1 of the feeding trial the BWG of male Japanese quail across dietary treatments was not affected (P = 0.6136) by supplemental *H. sabdariffa* calyces meal. Supplemental *H. sabdariffa* calyces meal at 5% significantly reduced (P = 0.0350) the BWG of male Japanese quail in week 2 but it (*H. sabdariffa* calyces meal) did not affect (P > 0.05) the BWG of male Japanese quail in weeks 3 and 4 of the experimental period. Supplemental *H. sabdariffa* calyces meal did not affect (P = 0.3588) the total BWG of male Japanese quail across dietary treatments. There were no significant differences in the ADG (P = 0.0741) of male Japanese quail in the first week of the experimental period. Supplemental *H. sabdariffa* calyces meal at both 5 and 10% reduced (P < 0.0001) the ADG of male Japanese quail in week 2 of the experiment. The ADG of male Japanese quail was significantly reduced (P = 0.0231) in week 3. There were no significant differences (P = 0.4001) in the ADG of the trial period across dietary treatments.

Supplemental *H. sabdariffa* calyces meal (5% and 10%) significantly increased (P = 0.0065) the feed intake of male Japanese quail in week 1 of the experimental period but did not affect (P = 0.0646) feed intake of the male Japanese quail during week 2 of the feeding trial. However, it significantly increased (P < 0.05) the feed intake of male Japanese quail in week 3 and 4 of the experimental period as well as the total feed intake of the male Japanese quail over the trial period. Supplemental *H. sabdariffa* calyces meal at 5% significantly increased (P =0.0034) FCR of male Japanese quail in week 1 of the experiment. The FCR of male Japanese quail fed diet 3 was significantly higher (P = 0.0020) in week 2 compared to that of male Japanese quail fed diets 1 and 2. At both 5% and 10% supplemental *H. sabdariffa* calyces meal increased (P < 0.0001) the FCR of the male Japanese quail in week 3 of the feeding trial but had no effect (P = 0.0894) the quail's FCR in week 4. However, supplemental *H. sabdariffa* calyces meal significantly increased the trial FCR of the male Japanese quail.

There were no significant differences in the induction body weight and dietary *H. sabdariffa* calyces meal did not significantly affect ($P > 0.05$) the terminal body weight (TBW) of female Japanese quail. Supplemental *H. sabdariffa* calyces meal did not affect ($P > 0.05$) the BWG (weekly and trial) of female Japanese quail. There were no significant differences ($P > 0.05$) in the ADG (week 1-3) of female Japanese quail. Supplemental *H. sabdariffa* calyces meal at 10% significantly increased ($P = 0.0259$) the ADG of female Japanese quail during week 4 of the feeding trial. Supplemental *H. sabdariffa* calyces meal (5 and 10%) significantly increased ($P < 0.0001$) the ADG of the feeding trial in female Japanese quail. Supplemental *H. sabdariffa* calyces meal did not significantly affect ($P > 0.05$) the feed intake (weekly) of female Japanese quail. However, the total feed intake was significantly increased ($P < 0.0001$) for female Japanese quail fed diets 2 and 3. There were no significant differences ($P > 0.05$) in the FCR of female Japanese quail in week 1 and 2 of the experiment. Supplemental *H. sabdariffa* calyces meal (5% and 10%) significantly reduced ($P < 0.05$) the FCR of female Japanese quail in weeks 3 and 4 of the feeding trial. The trial FCR of female Japanese quail was the same ($P = 0.0955$) across dietary treatments.

Table 3.4: Effect of supplemental *H. sabdariffa* calyces meal on growth performance, feed intake and utilisation efficiency of male Japanese quail

		Dietary treatments			
Parameter	**Week**	**Diet 1**	**Diet 2**	**Diet 3**	**P-value**
Induction body weight (g)		139.00±17.83	139.42±22.55	130.18±16.90	0.4736
Terminal body weight (g)		162.27±9.84	165.67±14.43	158.29±9.28	0.3691
Weekly BWG (g)	1	22.25±9.75	19.73±8.25	23.88±3.55	0.6136
	2	14.95±5.74^{a}	7.36±2.91^{b}	12.23±7.19^{a,b}	0.0350
	3	6.62±4.13	3.04±0.42	2.62±1.23	0.1302
	4	6.33±3.59	5.11±4.58	6.33±3.59	0.8626
Total BWG (g)		**46.09±17.80**	**33.77±17.40**	**40.21±7.55**	**0.3588**
Weekly ADG (g/day)	1	2.48±1.22	2.70±1.16	3.83±0.97	0.0741
	2	2.61±0.05^{a}	0.82±0.59^{c}	1.74±1.03^{b}	< 0.0001
	3	1.16±0.71^{a}	0.46±0.06^{b}	0.83±0.47^{a,b}	0.0231
	4	1.11±0.53	0.89±0.64	0.90±0.51	0.9037
ADG (Trial) (g/day)		**1.84±0.82**	**1.24±0.10**	**1.83±1.40**	**0.4001**
Weekly FI (g)	1	133.27±9.26^{b}	144.27±11.83^{a}	147.34±4.87^{a}	0.0065
	2	146.23±7.83	156.12±13.66	157.71±6.29	0.0646
	3	153.01±10.29^{b}	163.03±13.41^{a,b}	169.04±9.96^{a}	0.0171
	4	147.53±11.29^{a,b}	142.41±14.67^{b}	161.18±8.46^{a}	0.0043
Total FI (g)		**580.03±28.40^{b}**	**605.82±36.98^{a,b}**	**635.27±19.09^{a}**	**0.0014**
Weekly FCR	1	9.61±4.27^{a,b}	12.87±2.09^{a}	6.27±1.23^{b}	0.0034
	2	11.24±5.94^{b}	22.41±5.42^{a,b}	36.70±6.16^{a}	0.0020
	3	17.94±6.67^{b}	5.17±0.50^{c}	47.19±7.33^{a}	< 0.0001
	4	7.18±2.09	25.32±11.75	31.17±16.39	0.0894
FCR (Trial)		**10.33±2.01^{c}**	**17.79±3.79^{b}**	**22.76±3.82^{a}**	**< 0.0001**

$^{a, b,c}$ Within row means with different superscripts differ significantly at $P < 0.05$. No significant differences on induction weight, terminal weight body weight gain. *H. sabdariffa* calyces meal at 5% significantly reduced the BWG of Japanese quail in week 2. *H. sabdariffa* calyces meal at 10% increased the weekly FI and FCR of Japanese quail. Diet 1: standard quail finisher diet, Diet 2: standard quail finisher diet + 5% *H. sabdariffa* calyces meal (w/w); Diet 3: standard quail finisher diet + 10% *H. sabdariffa* calyces meal (w/w). ADG - Average daily gain; FCR - Feed conversion; Data expressed as mean±SD; n = 9 for Diet 1, n = 10: Diets 2 and 3.

Table 3.5: Effect of supplemental *H. sabdariffa* calyces meal on growth performance, feed intake and utilisation efficiency of female Japanese quail

Parameter	Week	Dietary treatments			P-value
		Diet 1	Diet 2	Diet 3	
Induction body weight (g)		118.33±14.10	115.121±3.99	117.67±13.16	0.8053
Terminal body weight (g)		180.49±19.08	179.21±15.76	195.08±16.32	0.0280
Weekly BWG (g)	1	26.69±12.16	27.87±14.97	22.55±10.12	0.5908
	2	21.77±10.69	19.44±12.17	27.26±8.63	0.1474
	3	13.53±11.67	11.29±6.92	13.57±7.54	0.7526
	4	6.41±3.31	11.38±7.16	14.04±8.01	0.0708
Total BWG (g)		**61.92±17.39**	**62.68±20.59**	**72.56±17.03**	**0.2236**
Weekly ADG (g/day)	1	4.15±1.29	4.33±0.79	3.74±0.77	0.3816
	2	3.29±1.40	3.29±1.32	3.62±0.67	0.6939
	3	1.73±0.81	1.926±0.87	2.07±0.58	0.5678
	4	0.90±0.52^{b}	1.77±0.60^{a,b}	2.13±1.14^{a}	0.0259
ADG (Trial) (g/day)		**2.42±1.47^{b}**	**2.83±1.21^{a}**	**2.89±0.91^{a}**	**< 0.0001**
Weekly FI (g)	1	127.46±24.82	137.43±19.82	139.05±31.79	0.4294
	2	139.91±22.13	143.87±23.28	141.26±30.62	0.9156
	3	125.43±36.23	131.15±37.24	138.69±43.81	0.6548
	4	134.83±20.09	129.59±20.06	136.26±21.01	0.6568
Total FI (g)		**400.17±61.09^{b}**	**542.04±92.14^{a}**	**555.22±121.02^{a}**	< 0.0001
Weekly FCR	1	5.31±1.89	6.27±2.98	6.07±1.94	0.6515
	2	7.02±2.0	6.67±2.79	6.32±1.08	0.8213
	3	13.40±5.23^{a}	7.62±2.06^{b}	7.93±2.23^{b}	0.0110
	4	15.00±4.11^{a}	7.54±1.95^{b}	7.65±3.53^{b}	0.0010
FCR (Trial)		**6.60±1.62**	**7.9±2.50**	**7.96±1.99**	**0.0955**

$^{a, b,c}$ Within row means with different superscripts differ significantly at $P < 0.05$. No significant differences on the induction weight, terminal weight, weekly body weight gain and weekly feed intake. *H. sabdariffa* calyces significantly reduced the FCR in weeks 3 and 4. Diet 1: standard quail finisher diet, Diet 2: standard quail finisher diet + 5% *H. sabdariffa* calyces meal (w/w); Diet 3: standard quail finisher diet + 10% *H. sabdariffa* calyces meal (w/w). ADG -Average daily gain; FCR - Feed conversion. Data are expressed as mean ± SD. n=15 for Diet 1 and 3 and n=14 for diet 2.

3.8.4. Gastrointestinal tract viscera morphometry

The effects of dietary *H. sabdariffa* calyces meal on GIT viscera morphometry of male and female Japanese quail are shown in Tables 3.6 and 3.7, respectively. The small and large intestines, proventriculi, ventriculi, livers and pancreata masses of male and female Japanese quail relative to their body weight were not significantly affected by supplemental *H. sabdariffa* calyces meal ($P > 0.05$). At 5% dietary inclusion level, supplemental *H. sabdariffa* calyces meal significantly reduced the mass (relative to body weight) of the caecum in male Japanese quail ($P = 0.0322$). Dietary *H. sabdariffa* calyces meal did not affect ($P > 0.05$) the lengths of small and large intestines of male and female Japanese quail relative to their body masses.

Table 3.6: Effects of supplemental *H. sabdariffa* calyces meal on the gastrointestinal tract viscera morphometry of male Japanese quail

Organ	Diet 1	Diet 2	Diet 3	P-value
Relative to body mass				
Proventriculus (%)	0.38±007	0.39±0.05	0.39±0.06	0.9326
Ventriculus (%)	1.80±0.17	2.00±0.45	1.91±0.28	0.4305
Small intestines (%)	2.11±0.19	2.12±0.47	2.23±019	0.6675
Small intestines (mm/g)	3.57±0.31	3.61±0.39	3.75±0.21	0.4560
Large intestines (%)	0.22±0.04	0.23±0.05	0.25±0.05	0.4484
Large intestines (mm/g)	0.39±0.06	0.40±0.04	0.41±0.05	0.1524
Liver (%)	1.67±0.35	1.72±0.37	1.79±0.18	0.6748
Pancreas (%)	0.22±0.07	0.41±0.56	0.24±0.03	0.3854
Caecum (%)	$0.36\pm0.05^{a,b}$	0.30 ± 0.06^{b}	0.43 ± 0.16^{a}	0.0322

[a, b] Within row means with different superscripts differ significantly at $P < 0.05$. No significant differences on GIT viscera masses and lengths except for the caecum of quail fed diet 3 which was significantly heavier than that of quail fed diet 2. Diet 1: standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean ± SD. n=14 for diet 1 and diet 2; n=15 for diet 3.

Table 3.7: Effect of supplemental *H. sabdariffa* calyces meal on the gastrointestinal tract viscera morphometry of Japanese female broiler quail

Organ	Diet 1	Diet 2	Diet 3	P-value
Relative to body mass				
Proventriculus (%)	0.38±0.04	0.39±0.06	0.42±0.06	0.1290
Ventriculus (%)	1.94±0.28	2.05±0.30	2.23±0.36	0.2180
Small intestines (%)	4.21±0.85	2.20±0.78	2.69±0.62	0.0512
Small intestines (mm/g)	3.31±0.38	3.40±0.34	3.53±0.55	0.7841
Large intestines (%)	0.24±0.10	0.27±0.08	0.27±0.06	0.8549
Large intestines (mm/g)	0.35±0.05	0.36±0.04	0.33±0.05	0.6140
Liver (%)	2.24±0.45	2.41±0.50	2.33±0.49	0.5818
Pancreas (%)	0.25±0.05	0.28±0.05	0.27±0.05	0.5002
Caecum (%)	0.38±0.15	0.45±0.08	0.46±0.19	0.2097

[a, b]Within row means with no common superscript differ significantly ($P < 0.05$). No significant differences in the GIT visceral morphometry of Japanese quail. Diet 1: standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean ± SD. n=24 for diet 1 and diet 2; n=25 for diet 3.

3.8.5 Femora and tibiae masses, length and mass:length ratio

Tables 3.8 and 3.9 shows results for tibiae and femora mass, length and bone mass:length ratio of male and female quail, respectively. There were no significant differences in tibiae and femora mass, length and mass: length ratio in both male and female broiler Japanese quail across all dietary treatments ($P > 0.05$).

Table 3.8: Effect of supplemental *H. sabdariffa* calyces meal on the femora and tibiae mass, length and bone mass: length of male Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	P-value
Femur:				
Mass (g)	0.26±0.02	0.26±0.02	0.26±0.02	0.6159
Length (cm)	2.50±0.14	2.06±0.16	2.04±0.19	0.9888
Mass: length ratio (g/cm)	0.10±0.11	0.10±0.01	0.01±0.01	0.1139
Tibia:				
Mass (g)	0.31±0.02	0.31±0.02	0.32±0.02	0.8054
Length (cm)	3.07±0.16	3.06±0.28	3.22±0.22	0.2169
Mass: length ratio (g/cm)	0.10±0.01	0.10±0.01	0.01±0.01	0.3931

H. sabdariffa calyces meal did not significantly alter the femoral mass, length and mass: length ratio of quail. Diet 1: standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean ± SD. n=9 for diet 1 and diet 3; n=10 for diet 2.

Table 3.9: Effect of supplemental *H. sabdariffa* calyces meal on femora and tibiae mass, length and bone mass: length of female Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	P-value
Femur:				
Mass (g)	0.23±0.03	0.24±0.03	0.24±0.03	0.8586
Length (cm)	2.83±0.25	2.36±0.18	2.23±0.19	0.1874
Mass: length ratio (g/cm)	0.10±0.01	0.10±0.01	0.11±0.01	0.2758
Tibia:				
Mass (g)	0.28±0.03	0.28±0.03	0.28±0.03	0.8799
Length (cm)	2.83±0.25	2.87±0.20	2.80±0.21	0.9953
Mass:length ratio (g/cm)	0.10±0.01	0.10±0.01	0.10±0.01	0.6475

H. sabdariffa calyces meal did not significantly alter the tibiae and femora mass, length and bone mass: length ratio in quail. Diet 1: standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean ± SD. n=15 for diet 1 and diet 3; n=14 for diet 2.

3.8.6 Erythrocyte osmotic fragility

Effects of supplemental *H. sabdariffa* calyces meal on the erythrocyte osmotic fragility of male and female Japanese quail are presented as fragiligrams in Figures 3.1 and 3.2, respectively. The minimum fragility (< 4% haemolysis) took place at 0.50% PBS concentration, mean corpuscular fragility (50% haemolysis) at 0.45% PBS and maximum osmotic fragility (> 95% haemolysis) at 0.30% PBS concentration for male Japanese quail. Similarly, the minimum fragility (< 4% haemolysis) took place at 0.50% PBS concentration, mean corpuscular fragility (50% haemolysis) at 0.5% PBS and maximum osmotic fragility (> 95% haemolysis) at 0.30% PBS concentration for female Japanese quail.

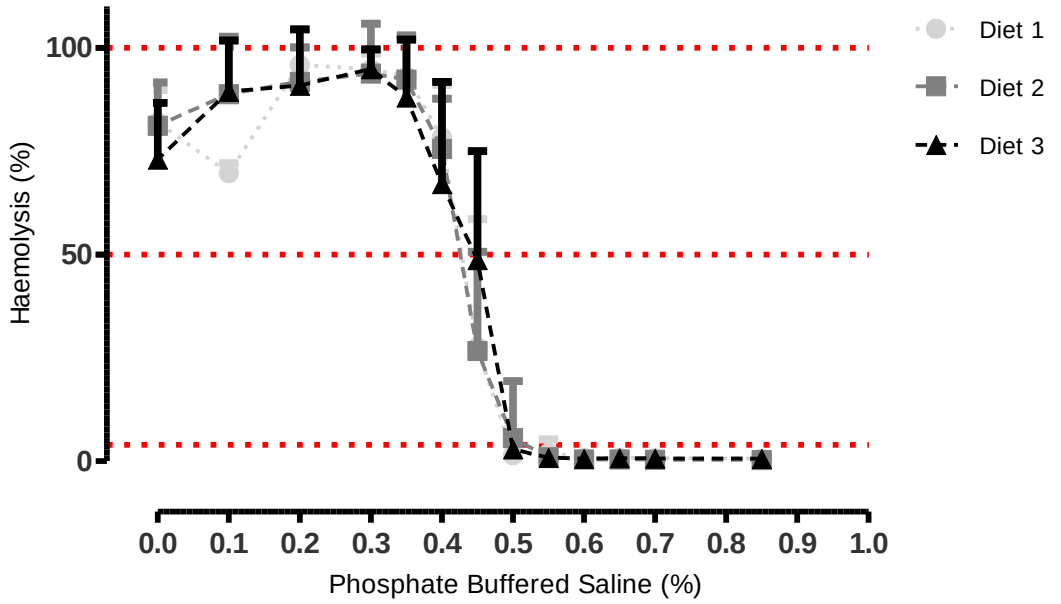

Figure 3.1:Effect of *H. sabdariffa* calyces meal on the erythrocyte osmotic fragility of male Japanese quail

Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. PBS- Phosphate buffered saline. Data is expressed as mean±SD. n= 9 for diet 1; n=10 for diet 2 and diet 3.

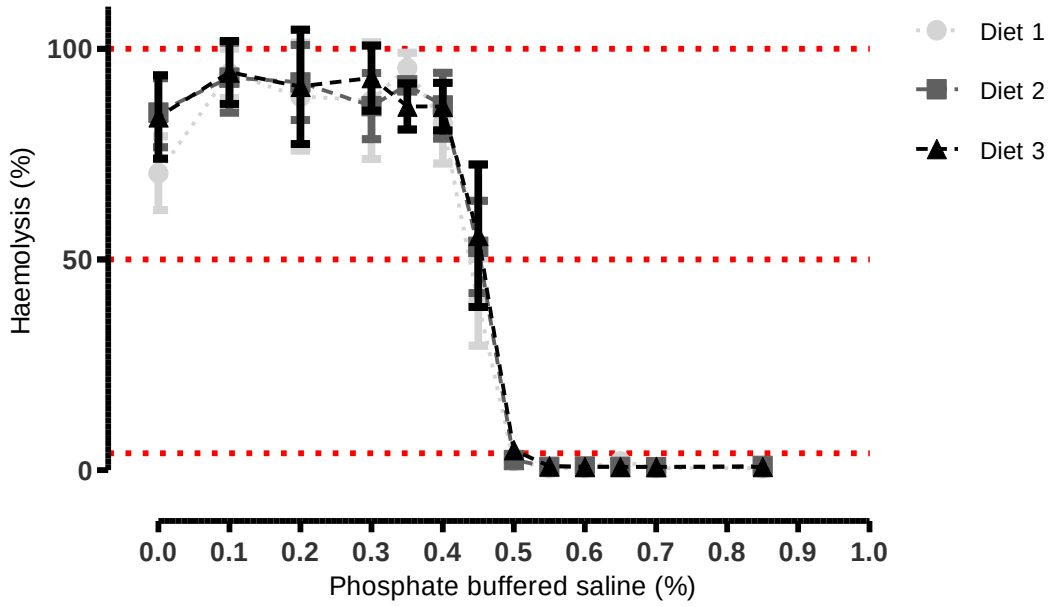

Figure 3.2: Effect of *H. sabdariffa* calyces meal on the erythrocyte osmotic fragility of female Japanese quail

Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. PBS- Phosphate buffered saline. Data is expressed as mean±SD. n= 15 for diet 1 and diet 3; n=14 for diet 2.

3.8.7 Haematocrit

Figures 3.3 and 3.4 show the effects of supplemental *H. sabdariffa* calyces meal on the haematocrit of male and female Japanese quail, respectively. The haematocrit of the male Japanese quail was similar ($P > 0.05$) across dietary treatments. However, the haematocrit of female Japanese quail fed diet 2 was significantly lower ($P = 0.0247$) compared to that of counterparts fed the control diet.

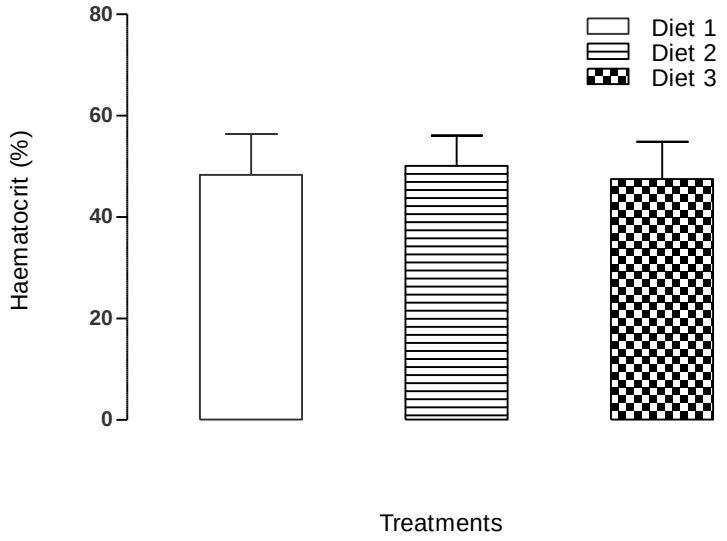

Figure 3.3: Effect of supplemental *H. sabdariffa* calyces meal on the haematocrit of male Japanese quail

No significant differences on the haematocrit of quail across all dietary treatments. Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean±SD. n= 9 for diet 1; n=10 for diet 2 and diet 3.

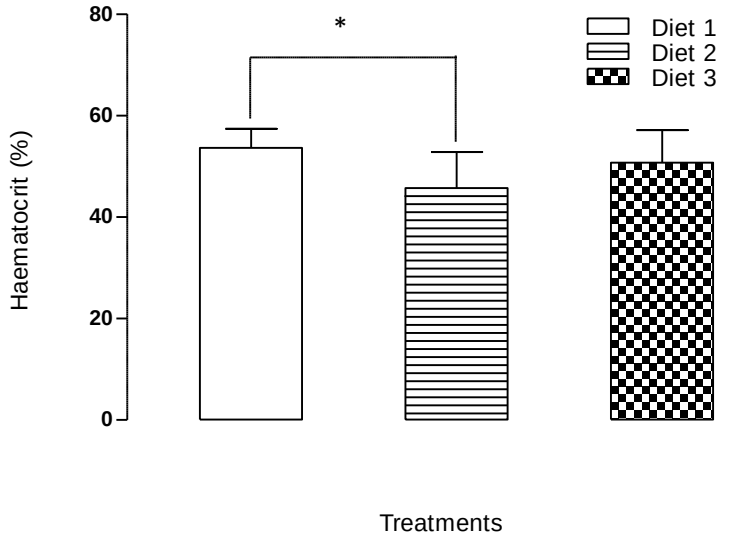

Figure 3.4: Effect of supplemental *H. sabdariffa* calyces meal on the haematocrit of female Japanese quail

Haematocrit of quail fed diet 2 was significantly lower than that of quail fed diet 1. Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean±SD. n= 15 for diet 1 and diet 3; n=14 for diet 2.

3.8.8 Surrogate markers of health

The effect of supplemental *H. sabdariffa* calyces meal on the serum concentrations of uric acid, calcium, total protein, albumin, globulin, malondialdehyde and AST activity of male and female Japanese quail are shown in tables 3.10 and 3.11, respectively. Dietary *H. sabdariffa* calyces meal had no effect ($P > 0.05$) on the serum levels of uric acid, calcium, total protein, albumin, globulin, malondialdehyde and AST activity of male and female Japanese quail.

Table 3.10: Effect of supplemental *H. sabdariffa* calyces meal on the serum concentration of uric acid, calcium, total protein, albumin, globulin, malondialdehyde and AST activity of male Japanese quail

Marker	Diet 1	Diet 2	Diet 3	P-value
Uric acid (µmol/L)	429.75±82.49	443.11±70.91	334.22±56.62	0.6177
Calcium (mmol/L)	2.31±0.83	2.71±0.62	2.77±0.58	0.5453
Total protein (g/L)	31.63±7.78	32.00±4.44	33.22±5.26	0.9645
Albumin (g/L)	12.63±2.56	12.78±1.86	14.00±2.69	0.8446
Malondialdehyde (nmol/ml)	0.86±0.10	0.90±0.13	0.97±0.14	0.1423
Globulin (g/L)	19.00±5.50	19.22±4.35	19.22±3.34	0.9978
AST (u/L)	356.87±63.80	415.56±33.59	391.67±189.91	0.6937

H. sabdariffa calyces meal did not significantly alter the general health of quail ($P > 0.05$). Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. AST – Aspartate transferase. Data is expressed as mean ± SD. n=24 for diet 1 and diet 2; n=25 for diet 3. For Malondialdehyde concentration, n=8 for diet 1 and 3; n=10 for diet 2.

Table 3.11: Effect of supplemental *H. sabdariffa* calyces meal on the concentration of uric acid, calcium, total protein, albumin, globulin, malondialdehyde and AST activity of female Japanese quail

Marker	Diet 1	Diet 2	Diet 3	P- value
Uric acid (µmol/L)	432.92±212.84	340.25±105.33	388.50±197.82	0.2165
Calcium (mmol/L)	3.12±0.81	3.12±0.81	2.79±0.94	0.4399
Total protein (g/L)	39.75±4.27	35.25±9.88	37.07±7.06	0.8378
Albumin (g/L)	14.67±1.78	13.67±2.87	13.64±3.27	0.4097
Globulin (g/L)	25.08±3.37	21.92±7.49	23.21±4.56	0.2055
Malondialdehyde (nmol/ml)	0.84±0.22	0.76±0.25	0.87±0.19	0.4345
AST (u/L)	257.58±67.93	255.00±83.36	270.50±106.55	0.9023

Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. AST – Aspartate transferase. Data is expressed as mean ± SD. n=24 for diet 1 and diet 2; n=25 for diet 3. For malondialdehyde concentration, n=14 for diet 1, n=12 for diet 2 and n=13 for diet 3.

3.9 Discussion

3.9.1 *Hibiscus sabdariffa* calyces total phenolic compounds content

The content of phenolic substances differs with species, part of plant, environment and climate (Kumar et al., 2017). Findings from our study showed the aqueous *H. sabdariffa* calyces to contain 23.75±0.15 mgGAE/g while the methanol: ethanol extract contained 25.94±0.57mgGAE/g total phenolics. Zhen et al., (2015) reported 18.98mgGaE/g as the minimum total phenolic content in *H. sabdariffa* extracts. Alhakmani et al., (2013) reported *M oleifera* flowers alcoholic extract total phenolic content to be 19.31mgGAE/g. Therefore, the total phenolic content in *H. sabdariffa* calyces used in our study is comparable to that reported by others (Zhen et al., 2015) and is also comparable to that of *M. oleifera* (Alhakmani et al., 2013). This implies that the *H. sabdariffa* calyces meal used in the current study can be exploited in poultry production to increase the growth performance and improve health of Japanese quail as has been done with other plant extracts with a similar phenolic compounds content.

3.9.2 Growth performance and feed economy

Successful poultry production is measured by achieving optimal growth of birds with minimum feed cost as a result of efficient utilisation of feed. One of the greatest challenges facing poultry producers is the expensive feed, hence the need to ensure efficient feed utilisation (Thirumalaisamy et al., 2019). Supplemental *H. sabdariffa* calyces meal did not significantly alter the terminal weight, total body weight gain and overall ADG of male broilers across dietary treatments. Unigwe et al., (2011) reported that *H. sabdariffa* calyces in drinking water enhanced the growth and feed utilisation in broiler chickens. In the current study, the diets supplemented with *H. sabdariffa* calyces did not enhance growth of Japanese quail. The reports made by Unigwe et al., (2011) were for broiler chickens, therefore the differences in the current study's findings can be attributed to anatomical differences in quail when compared to that of chicken and pH of the GIT which affects feed digestion and absorption (Mabelebele et al., 2017). Although *H. sabdariffa* calyces are known to contain

bioactive compounds that have been reported several times to enhance growth of birds, in the current study, the *H. sabdariffa* did not affect growth of quail. Although the crude protein of diet 3 was the lowest it still ranged within the recommended dietary needs of quail. This is supported by Wen et al., (2017) who reported that it was possible to formulate the low-protein diets containing about 22.0% CP for growing meat quails without adverse effects on growth and carcass yields of meat quails. Blake et al. (2013) also reported that bobwhite quail appeared to be unaffected by major dietary changes in protein level and a 26% CP diet may provide adequate nutrition for the 0 to 2 week starting period, followed by a 24% CP diet for a 2 to 4 week period, and a 20% CP diet thereafter. In this study, the 10% supplemented diet had the lowest crude protein of 22%. The quail used in our study were 5-week old, implying that even the diet with the lowest CP was still adequate enough to support and maintain growth and productivity. Additionally, animals respond differently to plant material in their feed as a result of the type of the active constituents, its concentration in the diet as well as the rate of its metabolic conversion to metabolites and consequent excretion (Dhanasekaran et al., 2020). Therefore, it can be inferred that the *H. sabdariffa* calyces meal in the current study did not stimulate growth of Japanese quail.

The total feed intake of male and female Japanese quail fed diets 2 and 3 was significantly higher than that of counterparts fed the control diet and the FCR was higher in the female Japanese quail fed the test diets. One of the challenges associated with non conventional feed resources is the presence of antinutritional factors. Antinutritional factors hinder the absorption and utilisation of nutrients by animals and thus impair the animal's growth and productive performance. Phytochemical characterisation of *H. sabdariffa* calyces meal showed the presence of gossypol (Al-Wandawi et al., 1984 and Bakheit, 1989), tannins and phytate (El-Adawy and Khalil, 1994 and Yagoub, 1998) and protease inhibitors (Abu-Tarboush and Ahmed, 1996), antinutritional factors that reduce digestibility of feed, compromise the bioavailability of nutrients and cause flatulence (Abu-Tarboush and Ahmed, 1996). Additionally, many fibrous ingredients incorporated in poultry feed to reduce production costs have low digestibility and cause poor growth and reduced egg production in

poultry (Singh et al., 2021). Therefore the crude fibre and crude protein in the experimental diets could have had confounding effects on the growth of quail. The increased feed intake of quail fed diets 2 and 3 compared to that of counterparts fed the control diet in view of similar growth performance and a high FCR implies that incorporating *H. sabdariffa* calyces meal in Japanese quail feed can result in decreased profitability.

3.9.3 Long bone indices

Impaired bone development limits poultry growth performance, increase mortality resulting in decreased profitability (Mesa et al., 2017). Breeds of broiler poultry are selected for fast growth. This high growth performance result in a quicker deposition of muscle on a poorly developed skeletal system characterised by less dense and porous bones (Williams et al., 2004) which can result in skeletal deformities. Tibiae are strongly loaded by muscles and are more prone to various mineralization disorders that can result in fractures (Whitehead, 2004). My findings showed similarities in tibiae and femora mass, length and mass: length ratio in the Japanese quail across dietary treatments. Ray et al., (2013) reported Japanese quail mean tibiae mass and length to be 0.59±0.053g and 4.74±1.10cm respectively and their femora mass and length to be 0.511±0.051g and 3.73±0.75cm, respectively (Skrobanek et al., 2005). My study findings revealed tibiae mass and length ranging from 0.28±0.03 - 0.31±0.02g and 2.80±0.21 – 3.22±0.22cm, respectively femora mass and length ranging from 0.23±0.03 – 0.26±0.22g and 2.04±0.19 – 2.50±0.14cm, respectively which are lower than those reported (Ray et al., 2013; Skrobanek et al., 2005). The lower mass and shorter tibiae and femora reported in my study compared to other studies could be speculated to be due to differences in the rearing environments as well as possible differences in the genotypes of the bird populations. The *H. sabdariffa* calyces meal used in the current study had polyphenolics. The latter enhance bone morphometry (Sharma et al., 2015). However, in this study the polyphenolics in the meal did not elicit either a positive and or negative effect on femora and tibiae masses and lengths of the Japanese quail. The shorter and lower mass of the bones of Japanese quail in the current study could imply that the growth of Japanese quail in this study was limited.

3.9.4 Viscera morphometry

The GIT is the first point of contact of feed and any other ingested material in poultry and animals and would naturally be expected to show responses to whatever material is ingested. Some vertebrates increase the size of the digestive organs as a result of the high fibre content in their diet (Jha et al., 2019). In my study, supplemental *H. sabdariffa* calyces meal at 10% significantly increased the caeca mass of male Japanese quail. Moen et al., (2016) reported that caeca lengthen in response to an increase in dietary fibre mainly because it is the site for fermentation and breakdown of cellulose. The findings on caeca mass of male Japanese quail fed diet 3 suggests that the high fibre previously reported for the *H. sabdariffa* calyces meal (Da-Costa-Rocha et al., 2014) and as seen in the crude fibre content of diet 3 (Table3.1) had an influence on the size of the caeca of the quail. The optimum functionality of the GIT directly impacts nutrient absorption and ultimately growth of birds (Lilehoj et al., 2018). Supplemental *H. sabdariffa* calyces meal in this study did not have any effect on the proventriculus, ventriculus, small and large intestines, liver and pancreas masses relative to body weight of Japanese quail. The small and large intestine lengths relative to body weight were not significantly altered by supplemental *H. sabdariffa* calyces meal. Toxic feeds can negatively impact growth of the GIT (Dhanasekaran et al., 2020; Lilehoj et al., 2018).The similarity in GIT viscera masses and intestine (small and large) lengths could be attributed to the fact that the *H. sabdariffa* calyces meal was non-toxic to the GIT.

3.9.5 Health profile

Haematocrit and erythrocyte osmotic fragility

Incorporating phytogenic feed additives in poultry production can cause nutrient deficiencies as a result of the antinutritional factors found in them such as tannins. Tannins are potentially toxic and bind to proteins, carbohydrates and minerals making them biologically unavailable (Schofield et al., 2001). Reduced availability of iron, for instance, may lead to the development of anaemia in the birds. In healthy mature Japanese quail, haematocrit ranges from 25% to 66% (Agina et al., 2017, Ayoola et al., 2015). In the current study, there were

no significant differences in the haematocrit values of quail in all dietary treatments in male Japanese quail ($P > 0.05$). The haematocrit values of male Japanese quail in the current study ranged from 44% – 56% in both male and female Japanese quail. Supplemental *H. sabdariffa* calyces meal at 5% significantly reduced ($P = 0.0247$) the haematocrit of female Japanese quail. Although the female Japanese quail fed diet 2 had a significantly lower haematocrit compared to that of quail fed diet 1, the haematocrit values remained in the normal range previously reported (Agina et al., 2017; Ayoola et al., 2015).

Red blood cell membranes have a high concentration of polyunsaturated fatty acids in their membrane making them sensitive to oxidative stress (Pandey and Rizvi, 2011). Pathological conditions resulting in increased lipid peroxidation have been shown to affect the sensitivity of erythrocytes to osmotic pressure (Singh et al., 2019). Measurement of erythrocyte osmotic fragility is thus used as a diagnostic tool in the determination of the capacity of the cell membranes to resist lysis in haemolytic diseases and pathophysiological conditions that result in the disruption of erythrocyte cell membranes (Singh et al., 2019). In the current study, the red blood cell fragility indices were similar across the groups thus implying that the dietary interventions did not negatively impact the erythrocyte membranes.

Diet is one of the several factors that affect the serum concentrations and/or activities of proxy measurements of liver and kidney function as well as general health (Behera et al., 2019). The amino transferases alanine transferase and aspartate transferase are intracellular enzymes found in high concentration in hepatocytes where they catalyse the oxidative decarboxylation and transamination reactions (Kwo et al., 2017). When the membrane integrity of hepatocytes is disrupted, these and other enzymes escape into the surrounding fluid compartment (Contreras-Zentella and Hernandez-Munoz, 2016). The liver also functions as a glucostat – helping maintain blood glucose homeostasis (Kwo et al., 2017) in addition to its detoxification role where it converts ammonia to urea and or uric acid (Behera et al., 2019). My findings show that dietary *H. sabdariffa* calyces meal did not affect the uric acid, calcium, total protein, albumin, globulin and AST of male and female Japanese quail. Furthermore, the meal did not impact serum malonaldehyde concentration of the Japanese quail across dietary treatments. The similarities in the Japanese quail's uric acid, calcium,

total protein, albumin, globulin, AST and serum malonaldehyde concentration suggest that dietary *H. sabdariffa* calyces meal did not damage and/or compromise liver and kidney function. Agina et al., (2017) reported serum AST values of 619.00±1.97U/L and 584.47±1.99U/L as well as serum uric acid concentrations of 12.70 – 105.40µmol/L and 76.40 – 292.70µmol/L, in male and female Japanese quail, respectively. In my findings, the serum AST values ranged from 356.87±63.80 – 415.56±33.59U/L and 255.00±83.36 – 270.50±106.55U/L in male and female Japanese quail, respectively while serum uric acid concentration ranged from 334.87±63.80 – 415.56±33.59µmol/L and 340.25±105.33 – 432.92±212.84µmol/L in male and female Japanese quail, respectively. Mukhtar et al., (2012) reported that *H. sabdariffa* seed did not alter serum constituents of broiler chicken. Supplemental *H. sabdariffa* calyces meal did not alter the male and female Japanese quails' serum malonaldehyde concentrations. Previous studies have reported a reduction in serum malonaldehyde concentration of broiler chicken whose diets were with products from plants rich in polyphenols (Lee et al., 2019). Although *H. sabdariffa* is polyphenol-rich with significant antioxidant activity, in the current study supplementing Japanese quail finisher diets with its calyces meal at 5% and 10% did not affect the malonaldehyde concentration of the birds. Malondialdehyde concentration increases as an indicator of stress in poultry. These findings imply that using *H. sabdariffa* calyces meal at 5% and 10% in quail diets would not guarantee reduced effects of stress in Japanese quail.

3.10 Conclusion

Although *H. sabdariffa* calyces meal could be used to supplement Japanese quail finisher feeds without the risk of compromising the birds' growth performance and health, caution has to be taken as its use potentially increases feed costs due to poor feed utilisation efficiency.

In the next chapter, I interrogated the effects of supplemental *H. sabdariffa* calyces meal on the carcass yield and meat quality of Japanese quail.

4. CHAPTER FOUR - EFFECT OF *HIBISCUS SABDARIFFA* CALYCES MEAL ON MEAT QUALITY OF JAPANESE QUAIL

4.0 Introduction

The consumption of quail meat as an alternative to chicken is increasing globally (Onyewuchi et al., 2013). The observed increase in the consumption of quail meat is premised on its comparatively lower fat and caloric content and higher protein content (Aminzade et al., 2012). The lower caloric and fat content of quail meat makes it a more healthful product when compared to broiler chicken meat (Onyewuchi et al., 2013). The intensive nature of commercial poultry production results in metabolic challenges in the birds, including oxidative stress, which affects feed intake and decreases productivity as well as product quality (Mashaly et al., 2004). In order to alleviate some of the metabolic challenges associated with intensive poultry production, synthetic feed additives are used for health and nutritional benefits to the poultry (Kuldeep et al., 2014). Synthetic feed additives with antioxidant properties, for example, ethoxyquin, butylated hydroxy toluene and butylated hydroxy anisole are routinely incorporated into poultry feeds for the purposes of preserving feed against rancidity, improving bird welfare by mitigating stress and to improve meat and egg quality (Fotina et al., 2013; Aminzade et al., 2012). However, due to health concerns regarding perceived and known negative effects synthetic feed additives and their metabolic residues have on consumer health (Shahid et al., 2013), consumers advocate for the use of natural plant-derived products with nutraceutical properties which are deemed safer (Shahid et al., 2013; Blaszozyk et al., 2013). The health beneficial properties (immunomodulating, antioxidant and antiobesity) of these plant derived products are ascribed to the presence of phytochemicals such as phenols, anthocyanins and flavonoids (Feofilova and Nesteruk, 2013). Plant-derived meals and extracts could therefore be exploited as natural sources of nutraceuticals for use in poultry production. *H. sabdariffa* calyces meals and extracts have been shown to contain flavonoids, anthocyanins and organic acids; phytochemicals with health beneficial biological activities including among many, antimicrobial, antioxidant, antianemic and antiobesity (Da Costa Rocha et al., 2014). Despite several reports on the antioxidant and other nutraceutical properties of *H. sabdariffa* calyces (Ologundudu and Abi, 2005), the potential benefits of *H. sabdariffa* calyces meal as a dietary supplement in Japanese quail feeds have not been interrogated. In chapter 3, the impact of *H. sabdariffa* calyces meal on growth performance and general health of Japanese quail was

reported. This study investigated the effect of supplementing a standard quail finisher diet with *H. sabdariffa* calyces meal on meat yield, pH, colour, water holding capacity, tensile strength (tenderness), proximate and fatty acid content of Japanese quail.

4.1 Study objectives

The specific objective of this study was to investigate the effects of dietary supplementation with *H. sabdariffa* calyces meal on the Japanese quail's meat yield as well as the meat's pH, colour, water holding capacity, tenderness, proximate (dry matter, crude protein, ether extract and ash) and fatty acid content.

4.2 Study hypotheses

H_0: Dietary supplementation with *H. sabdariffa* calyces meal does not affect meat yield as well as the meat's pH, colour, water holding capacity, tenderness, proximate (dry matter, crude protein, ether extract and ash content) and fatty acid content of Japanese quail.

H_1: Dietary supplementation with *H. sabdariffa* calyces meal promotes meat yield as well as the meat's pH, colour, water holding capacity, tenderness, proximate (dry matter, crude protein, ether extract and ash content) and fatty acid content of Japanese quail.

4.3 Materials and methods

4.3.1 *Hibiscus sabdariffa* calyces: source and preparation

H. sabdariffa was sourced and prepared as described in Chapter 3, under subheading 3.31.

4.3.2 Feed ingredients and diet formulation

The sourcing of feed ingredients and formulation of diets was carried out as described in Chapter 3, under subheading 3.34.

4.3.3 Ethical approval and study site

Ethical approval was sought and granted as described in Chapter 3, under subheading 3.3.5 and the study site details are as described in Chapter 3, under subheading 3.3.5.

4.3.4 Quail and quail management

Sourcing of Japanese quail was done as described in Chapter 3, under subheading 3.3.6 and management of quail during the experimental period was carried out as described in Chapter 3, under subheading 3.3.6.

4.3.5 Experimental design

The experiment of this part of the study was designed as described in Chapter 3, under subheading 3.3.7.

4.3.6 Terminal procedures

After 28 days of the feeding trial, the quail were weighed, fasted for 4 hours, and then humanely slaughtered using a guillotine (Harvard apparatus, Holliston, Massachusetts, United States). After bleeding out, feathers were manually plucked. Following plucking of feathers each carcass was eviscerated and then weighed on an electronic balance (Snowrex EQ-1200, Clover Scales Pty Ltd, Johannesburg, South Africa). Dressing percentage was calculated as described by Kharthika et al. (2017) using the formula:

$$Dressing\ \% = \frac{carcass\ weight\ of\ quail}{live\ weight\ of\ quail}\ x\ 100$$

4.4 Determination of the physical quality traits of the meat

4.4.1 Determination of pH and colour

Thirty minutes after slaughter, the initial pH (pH_i) of the breast and thigh meat of each carcass was determined using a pH meter (following a two-point calibration at pH 4 and pH 7) with a piercing electrode at 24^{o}C (Crison pH25, CRISON Instruments, S.A, Spain) as per the manufacturer's instructions. The colour of the meat [lightness (L*), redness (a*) and yellowness (b*)] were also measured 30 mins post-slaughter on the breast and thigh of each carcass using a Lovibond Colour meter (LC100 Spectrophotometer, Lasec, SA, China) as recommended by the Commission Internationale deI'Eclairage (CIE) (1976). The carcasses were then chilled at 4^{o}C for 24 hours following which ultimate pH (pH_u) and colour were measured on the breast and thigh meat of each carcass as described above. The decrease in pH over 24 hours was calculated by subtracting pHu from pHi. The measurements were done on 3 different points, and a mean computed from the 3 values for each bird. The mean value for pH and colout per bird (replicate) was then recorded.

4.4.2 Determination of water holding capacity

The water holding capacity of the meat was determined by centrifugation as described by Bertram et al., (2001) with some modifications. Briefly, 1±0.5g of either the breast or thigh meat were cut and placed in centrifuge tubes with 20 glass beads at the bottom to separate the meat from the liquid released by the meat. The tubes containing the meat pieces and beads were then centrifuged at 964.28g for 15 minutes (Thermo Sorvall RT 6000B centrifuge, Du Pont Instruments, Wilmington, CT, USA) following which each sample was reweighed. Each sample's water holding capacity was then computed using the equation: [100-(initial sample weight- final sample weight)/initial sample weight] × 100% (Perenlei et al., 2014).

4.4.3 Determination of tensile strength

The tensile strength of the breast meat was determined using a Texture Analyser (TA.XT2i, Stable Microsystems Ltd, Godalming, UK). Briefly, for each carcass a muscle strip (2.0 × 1.0 ×1.0 cm) was placed between 2 fixtures of the Texture Analyser and the force with which it was broken at a test speed and tension force of 0.2mm/s and 4kg, respectively was recorded. The tensile strength data was captured via the Texture Expert software for Windows, Version 1.20 as shear force.

4.5 Determination of the meat's chemical nutrient content

4.5.1 Determination of proximate content

The breast and thigh meat samples were pooled according to diet to generate a composite sample. The breast and thigh meat samples were lyophilised in a freeze drier (Custon, SSE Engineering, North America) and then milled through a 2 mm screen. The quail breast and thigh meat's dry matter, ash, ether extract and crude protein were determined as described by the Association of Analytical Chemists (AOAC, 2000: Methods 934.01 and 930.15, 942.05, 920.39 and 954.01, respectively). Each assay was carried out in duplicate.

4.5.2 Determination of fatty acid content

The fatty acid content of the meat was determined as described by Christopherson and Glass (1969). Briefly, the milled breast and thigh meat samples were extracted with diethyl ether to obtain fat. Methanolic potassium hydroxide (2N) was used to methylate the fat and generate fatty acid methyl esters (FAMEs). The FAMEs were then injected into a gas chromatograph (GC) where they were separated on a DB-23 capillary column (90 cm × 250 µm × 0.25 µm) (Supelco; Sigma Aldrich) by a temperature gradient over 45mins. Nitrogen was used as the carrier gas. The gas chromatograph consisted of a HP6890 GC (Hewlett Packard, Bristol, UK) with a flame ionisation detector. The temperature of the detector and injector was set at 300°C. A PC equipped with CHEMSTATION software was used for quantification

(Chemistations Deutschland GmbH, Augustastrasse, Wesel, Germany). The analysis was done in duplicate.

4.6 Statistical analysis

Data for the male and female studies were analysed separately. Data are expressed as mean ±SD. Graph Pad Prism Version 5 (Graph-pad Software Inc., San Diego, USA) statistical package was used to analyse the data. A one-way ANOVA was used to analyse data. The Bonferroni post hoc test was used to compare treatment means. The level of significance was set at $P < 0.05$. The statistical model used was:

$Yij = \mu + Ti + eij;$

where Yij = dependent variable of interest (carcass weight, dressing percent, pH, colour, water holding capacity, tensile strength, proximate and fatty acid content)

μ = is the overall mean common to all observations

Ti = is the fixed effect of the ith dietary treatment (i = 1, 2, 3)

eij = is the random residual error.

4.7 Results

4.7.1 Carcass yield and physical attributes of the meat

The carcass mass and dressing percentage as well as the meat's pH, water holding capacity and tensile strength from male and female Japanese quail carcasses is shown in Tables 4.1 and 4.2 respectively. There were no significant differences in the carcass mass and dressing percentage across dietary treatments ($P > 0.05$) for both male and female Japanese quail. The pH_i of breast meat of quail fed diet 1 was significantly lower ($P = 0.0008$) than that of quail fed diets 2 and 3. However the pH_u of breast meat of both male and female Japanese quail across the dietary treatments was similar. The pH decline in breast meat of the Japanese quail was significantly greater ($P = 0.0201$) for quail fed diet 2 and 3 than that of meat from

counterparts fed diet 1. There were no significant differences ($P > 0.05$) in the pH_i, pH_u and in the pH decline of thigh meat from quail carcasses across dietary treatments. Dietary supplementation with *H. sabdariffa* calyces meal significantly increased ($P = 0.0131$) the water holding capacity of the breast meat of the quail but did not affect ($P = 0.2226$) that of their thigh meat. There were no significant differences ($P = 0.6638$) in the tensile strength of breast meat of the quail across dietary treatments.

Table 4.1: Effect of supplemental *H. sabdariffa* calyces meal on empty carcass mass, dressing percentage, pHi, pHu, water holding capacity and tensile strength of male Japanese quail breast and thigh meat

Parameter	Diet 1	Diet 2	Diet 3	P- value
Carcass mass, g	112.84±7.39	111.47±10.34	110.59±9.45	0.8679
Dressing %	69.56±2.32	66.07±7.86	69.95±6.81	0.3303
Breast:				
pH_i	6.02±0.07[b]	6.17±0.02[a]	6.21±0.15[a]	0.0008
pH_u	5.87±0.18	5.75±0.08	5.79±0.11	0.1778
pH Decrease	0.16±0.24[b]	0.42±0.16[a]	0.42±0.23[a]	0.0201
Thigh:				
pH_i	6.67±0.14	6.73±0.24	6.68±0.21	0.8225
pH_u	6.72±0.14	6.75±0.13	6.66±0.21	0.4772
pH Decrease	-0.04±0.11	-0.03±0.16	0.01±0.18	0.6739
Water holding capacity, % (Breast)	81.52±1.62[b]	83.43±1.40[a]	83.11±1.07[a]	0.0131
Water holding capacity, % (Thigh)	89.78±0.69	88.98±1.39	88.60±1.94	0.2226
Tensile strength, kg force (Breast)	0.05± 0.03	0.06±0.02	0.05±0.03	0.6638

[a, b] Within row means with different superscripts differ significantly at $P < 0.05$. pH_i and pH decrease were significantly higher in the breast of quail fed diet 3, breast pH_u was similar across all dietary treatments. water holding capacity was significantly higher in breast muscle of quail fed diets 1 and 2. No significant differences on the tensile strength of breast of quail across all dietary treatments. The pH_i, pH_u and pH decrease and water holding capacity was similar for thigh of quail across dietary treatments.; $pH_{i:}$ pH value 30mins post slaughter; $pH_{u:}$ pH value 24hrs post slaughter. Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean ± SD. n=10: Diets 2 and 3, n = 9 for Diet 1.

Table 4.2: Effect of supplemental *H. sabdariffa* meal on empty carcass mass, dressing percentage, pHi, pHu and water holding capacity of the breast and thigh meat of female Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	P- value
Carcass mass, g	119.36±12.23	123.71±5.51	131.79±7.77	0.1251
Dressing %	68.91±6.22	71.24±4.20	69.05±4.68	0.7267
Breast:				
pH_i	6.26±0.12	6.30±0.11	6.30±0.13	0.5338
pH_u	5.98±0.13	6.00±0.22	5.95±0.11	0.6053
pH Decrease	0.27±0.13	0.29±0.24	0.35±0.14	0.4343
Thigh:				
pH_i	6.63±0.23	6.62±0.12	6.61±0.16	0.9586
pH_u	6.63±0.17	6.62±0.20	6.62±0.21	0.9855
pH Decrease	0	0	0	
Water holding capacity, % (Breast)	84.28±8.43	85.84±3.18	84.97±6.57	0.8102
Water holding capacity, % (Thigh)	90.93±4.94	90.89±5.48	90.86±3.68	0.9992

[a, b] Within row means with different superscripts differ significantly at $P < 0.05$. No significant differences on the pHi, pHu, pH decrease and water holding capacity of both breast and thigh of quail across all dietary treatments. pH_i: pH value 30mins post slaughter; pH_u: pH value 24hrs post slaughter. Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean ± SD. n=15 for Diet 1 and 3, n=14 for diet 2.

4.7.2 Colour

Tables 4.3 and 4.4 show the colour of breast meat from male and female Japanese quail carcasses, respectively. There were no significant differences ($P > 0.05$) in the L*, a* and b* of the breast meat from male and female quail carcasses across dietary treatments both 30 minutes and 24 hours post slaughter.

Table 4.3:Effect of supplemental *H. sabdariffa* calyces meal on colour of breast meat of male Japanese quail, 30 minutes and 24 hour post-slaughter

Parameter	Diet 1	Diet 2	Diet 3	P-value
L^*_{30min}	41.52±2.24	41.13±1.55	43.28±3.14	0.1260
L^*_{24hr}	44.72±2.80	45.08±4.01	47.35±2.27	0.1524
a^*_{30min}	4.61±1.87	4.49±1.30	5.54±4.37	0.6786
a^*_{24hr}	4.34±2.05	4.19±2.47	2.34±1.43	0.0695
b^*_{30min}	8.41±1.71	8.09±1.15	7.88±2.06	0.7927
b^*_{24hr}	9.50±1.80	9.39±3.01	8.83±2.44	0.8178

No significant differences in the meat's L*, a*, and b* 30-min and 24-hours post-slaughter ($P > 0.05$). L^*_{30mins} is the lightness value 30mins post-slaughter; L^*_{24hrs} is the lightness value 24 hours post-slaughter; a^*_{30mins} is the redness value 30 mins post slaughter; a^*_{24hrs} is the redness value 24 hours post-slaughter; $b^*_{30\ mins}$ is the yellowness value 30 mins post-slaughter; $b^*_{24\ hrs}$ is the yellowness value 24 hrs post-slaughter. Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5% *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces w/w. Data is expressed as mean±SD. n=9 for diet 1 and n=10 for diet 2 and 3.

Table 4.4: Effect of supplemental *H. sabdariffa* calyces meal on colour of breast meat of female Japanese quail, 30 minutes and 24 hour post-slaughter

Parameter	Diet 1	Diet 2	Diet 3	P- value
L^*_{30min}	42.24±3.45[a,b]	41.92±2.95[b]	44.89±2.81[a]	0.0224
L^*_{24hr}	46.36±4.11	46.52±3.81	48.03±2.77	0.3870
a^*_{30min}	4.52±1.82[a,b]	4.77±1.93[a]	2.97±2.06[b]	0.0320
a^*_{24hr}	4.06±2.43	3.38±1.75	2.88±1.86	0.3750
b^*_{30min}	9.39±2.06	8.99±1.20	10.18±2.40	0.2582
b^*_{24hr}	11.98±2.42	8.55±11.60	12.25±2.88	0.2919

[a,b]Within row means with different superscripts are significantly different at $P < .0.05$. The L^*_{30min} of breast was significantly higher in quail fed diet 3, but L^*_{24hr} remained the same across dietary treatments. The a^*_{30min} was significantly higher in breast of quail fed diet 2 but a^*_{24hr} remained similar across dietary treatments. The b* was not significantly affected 30 mins and 24 hrs post-slaughter across dietary treatments. L^*_{30mins} is the lightness value 30mins post-slaughter; L^*_{24hrs} is the lightness value 24 hours post-slaughter; a^*_{30mins} is the redness value 30 mins post-slaughter; a^*_{24hrs} is the redness value 24 hours post-slaughter; $b^*_{30\ mins}$ is the yellowness value 30 mins post-slaughter; $b^*_{24\ hrs}$ is the yellowness value 24 hrs post-slaughter. Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5% *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean±SD. n=15 for diet 1 and 3, n=14 for diet 2.

4.7.3 Chemical nutrient composition of the meat

The proximate content of breast and thigh meat of both male and female Japanese quail carcasses is shown in Tables 4.5 and 4.6, respectively. At 10% supplementation level, *H. sabdariffa* calyces meal significantly decreased ($P < 0.0001$) the dry matter content of the breast and thigh meat of male Japanese quail. The crude protein of the breast meat from carcasses of male Japanese quail was similar across dietary treatments ($P > 0.05$). At 10% supplementation level, *H. sabdariffa* calyces meal significantly increased ($P = 0.0003$) the crude protein of the thigh meat in male Japanese quail. Dietary *H. sabdariffa* calyces meal significantly decreased the ether extract content of the breast ($P = 0.0092$) and thigh ($P = 0.0026$) but increased the ash content of the breast ($P= 0.0228$) and thigh ($P = 0.0007$) meat of male Japanese quail (Table 4.5). Supplemental *H. sabdariffa* calyces meal had not effect ($P > 0.05$) on the dry matter, crude protein, ether extract and ash content of breast and thigh meat of female Japanese quail (Table 4.6).

Table 4.5: Effect of supplemental *H. sabdariffa* calyces meal on the proximate content of breast and thigh meat of male Japanese quail

Proximate component (%)	Diet 1	Diet 2	Diet 3	P-value
Breast:				
Dry matter	27.77±0.01[b]	28.14±0.07[a]	27.32±0.03[c]	<0.0001
Crude protein	79.87±0.39	80.23±0.65	80.31±0.29	0.6496
Ether extract	9.36±0.30[a]	8.87±0.37[a]	7.19±0.09[b]	0.0092
Ash	5.02±0.02[b]	5.12±0.00[a]	5.03±0.02[a,b]	0.0228
Thigh:				
Dry matter	29.30±0.01[a]	29.13±0.01[b]	26.00±0.01[c]	<0.0001
Crude protein	63.14±0.03[b]	64.34±0.25[b]	75.78±0.88[a]	0.0003
Ether extract	23.46±0.52[a]	21.71±1.29[a]	12.94±0.73[b]	0.0026
Ash	3.85±0.00[c]	4.06±0.02[b]	4.28±0.03[a]	0.0007

[a, b, c]Within row means with different superscripts differ significantly ($P < 0.05$). The crude protein of breast meat remained similar across dietary treatments. *H. sabdariffa* calyces meal reduced the dry matter and ether extract of breast and thigh meat of quail. *H. sabdariffa* calyces meal increased the ash content of breast and thigh meat of quail; Diet 1: standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5% *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean±SD. n=2 for all diets.

Table 4.6: Effect of supplemental *H. sabdariffa* calyces meal on the proximate composition of breast and thigh meat of female Japanese quail

Proximate component (%)	Diet 1	Diet 2	Diet 3	P- value
Breast:				
Dry matter	26.37±1.53	26.11±1.23	26.45±1.18	0.9315
Crude protein	83.11±3.04	84.96±3.53	83.77±1.22	0.6487
Ether extract	7.79±2.51	6.19±0.77	8.53±0.17	0.1388
Ash	6.02±1.30	5.79±0.59	5.48±0.49	0.7001
Thigh:				
Dry matter	27.65±4.24	26.24±1.92	25.53±1.80	0.5918
Crude protein	70.86±8.78	72.13±6.39	76.69±2.51	0.6825
Ether extract	23.20±7.75	18.31±1.59	17.69±0.58	0.2297
Ash	4.04±0.65	4.32±0.03	4.41±0.15	0.4076

[a, b, c]Within row means with different superscripts differ significantly ($P < 0.05$). There were no significant differences in the dry matter, crude protein, ether extract and ash content of breast and thigh meat of quail. Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5% *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean±SD. n= 2 for all diets.

4.7.4 Fatty acid content of breast and thigh meat of Japanese quail

Table 4.7 and 4.8 show the fatty acid content of breast and thigh meat of quail, respectively while Table 4.9 and 4.10 show the fatty acid profiles of breast and thigh meat of female quail, respectively. Dietary *H. sabdariffa* calyces meal did not affect ($P > 0.05$) the saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) content but at 10% supplementation it (*H. sabdariffa* calyces meal) decreased ($P = 0.0032$) the monounsaturated fatty acids (MUFAs) content of breast meat of male Japanese quail (Table 4.7). Dietary *H. sabdariffa* calyces meal significantly increased ($P = 0.0066$) the SFAs and decreased ($P = 0.0014$) the PUFAs of the thigh meat in male Japanese quail. At 5%, dietary *H. sabdariffa* calyces meal significantly decreased ($P = 0.0344$) the MUFAs in the thigh meat of male Japanese quail (Table 4.8). Dietary *H. sabdariffa* calyces meal did not affect ($P > 0.05$) the SFAs, MUFAs and PUFAs content of the breast meat in female Japanese quail (Table 4.9). Dietary supplementation with *H. sabdariffa* calyces meal in the standard Japanese quail finisher diet did not affect the SFAs ($P = 0.9286$) of the thigh meat from female Japanese quail Supplemental *H. sabdariffa* calyces meal significantly increased ($P = 0.0044$) the MUFAs content and reduced ($P = 0.0078$) the PUFAs content of thigh meat of female Japanese quail (Table 4.10).

Table 4.7: Effect of supplemental *H. sabdariffa* calyces meal on the fatty acid content of male Japanese quail breast meat

Fatty acid (%)	Diet 1	Diet 2	Diet 3	P- value
Saturated				
Lauric acid (C12:0)	0.04±0.00	0.04±0.00	0.05±0.00	-
Myristic acid (C14:0)	0.69±005	0.76±0.00	0.71±0.00	0.2237
Pentadecanoic acid (C15:0)	0.08±0.00	0.08±0.00	0.05±0.00	-
Palmitic acid (C16:0)	21.92±0.82	22.28±0.45	21.83±0.14	0.7095
Margaric acid (C17:0)	0.15±0.00	0.16±0.00	0.15±0.00	-
Stearic acid (C18:0)	8.19±0.16	8.15±0.08	8.57±0.04	0.0526
Arachidic acid (C20:0)	0.11±0.00	0.14±0.03	0.13±0.04	0.6781
Heneicosylic acid (C21:0)	0.04±0.00	0.20±0.00	0.05±0.00	-
Tricosylic acid (C23:0)	nd	nd	0.31±0.00	-
Lignoceric acid (C24:0)	0.04±0.00	0.04±0.00	nd	-
TSFA	**31.42±0.72**	**32.19±0.85**	**31.93±0.32**	**0.5689**
Monounsaturated				
Myristoleic acid (C14:1)	0.19±0.00	0.20±0.00	0.20±0.00	-
Palmitoleic acid (C16:1)	8.06±0.35	8.12±0.14	8.12±0.04	0.9544
Heptadecenoic acid (C17:1)	0.04±0.00	0.04±0.00	0.05±0.00	-
Elaidic acid (C18:1n9t)	0.23±0.00	0.20±0.00	0.15±0.00	-
Oleic acid (C18: 1n9c)	40.31±0.82	40.32±0.57	38.73±0.22	0.1142
Eicosenoic acid (C20:1)	nd	0.08±0.00	0.05±0.00	-
Docosaenoic acid (C22:1n9)	0.09±0.08	0.12±0.11	0.18±0.11	0.7351
Nervonic acid (C24:1)	0.38±0.11	0.20±0.00	0.25±0.00	0.1236
TMUFAs	**49.06±0.52^{a}**	**49.08±0.26^{a}**	**47.48±0.22^{b}**	**0.0322**
Polyunsaturated				
Linoleic acid (C18:2n6c)	14.88±0.3^{a}	13.69±0.31^{a}	9.08±7.39^{b}	0.4565
Linolelaidic acid (C18: 2n6t)	0.03±0.00	0.10±0.03	0.05±0.00	0.0631
Alpha- Linolenic acid C18:3n3)	1.15±0.05	1.00±0.06	1.02±0.00	0.0772
Gamma-Linolenic acid (18:3n6)	0.04±0.00	0.10±0.03	0.10±0.00	0.0488
Eicosadienoic acid (C20:2)	0.15±0.00	016±0.00	0.20±0.00	-
Dihomo-gamma linolenic acid (C20:3n6)	0.15±0.00	0.24±0.00	0.20±0.00	-
Eicosatrienoic acid (C20:3n3)	nd	0.20±0.00	0.05±0.00	-
Arachidonic acid (C20:4n6)	2.96±0.33^{b}	2.75±0.09^{b}	3.69±0.04^{a}	0.0341

Timnodonic acid (C20:5n3)	0.07±0.00	0.22±0.09	0.13±0.04	0.1535
Docosahexenoic (C22: 6n3)	0.12±0.11	0.50±0.31	0.53±0.04	0.1958
TPUFA	**19.37±0.35**	**18.64±0.57**	**20.15±0.14**	**0.0695**
Trans fats	0.14±0.14	0.30±0.03	0.20±0.00	0.2621
Cis fats	55.19±1.14	54.03±0.85	52.93±0.36	0.1607
Omega-3	1.33±0.08[b]	1.82±0.09[a]	1.78±0.07[a]	0.0144
Omega 6	17.96±0.54	18.10±1.45	18.19±018	0.9673
Omega-9	40.52±0.57[a]	40.66±0.42[a]	38.93±0.22[b]	0.0471
EPA	8.82±12.37	8.67±11.87	9.21±12.88	0.9990
DHA	0.12±0.11	0.50±0.31	0.31±0.36	0.4834

[a, b, c]Within row means with different superscripts differ significantly ($P < 0.05$). *H. sabdariffa* calyces meal did not alter the total SFA and PUFA of breast meat but at 10% supplemental level significantly reduced the total MUFAs of breast meat. TSFAs- Total saturated fatty acids; TMUFAs- Total mono unsaturated fatty acids; TPUFAs- total poly unsaturated fatty acids. - means that the statistical analysis was not done due to SD of 0 or the fatty acid could not be detected at all; Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5% *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean±SD. n=2 for all diets.

Table 4.8: Effect of supplemental *H. sabdariffa* calyces meal on fatty acid content of thigh meat of male Japanese quail

Fatty acid (%)	Diet 1	Diet 2	Diet 3	P-value
Saturated				
Lauric acid (C12:0)	0.03±0.01	0.05±0.00	0.06±0.00	0.0674
Myristic acid (C14:0)	0.73±0.04^{b}	0.90±0.02^{a}	0.94±0.06^{a}	0.0363
Pentadecanoic acid (C15:0)	0.07±0.00	0.09±0.03	0.09±0.00	0.4892
Palmitic acid (C16:0)	20.26±0.23^{b}	23.24±0.25^{a}	23.21±0.55^{a}	0.0059
Margaric acid (C17:0)	0.11±0.01	0.10±0.03	0.19±0.06	0.1966
Stearic acid (C18:0)	4.56±0.05^{b}	5.30±0.08^{a}	4.61±0.13^{b}	0.0063
Arachidic acid (C20:0)	0.12±0.00^{b}	0.18±0.01^{a}	0.13±0.02^{b}	0.0363
Heneicosylic acid (C21:0)	0.07±0.01^{a}	0.02±0.00^{b}	0.03±0.00^{b}	0.0076
Tricosylic acid (C23:0)	0.03±0.00	0.14±0.00	nd	-
Lignoceric acid (C24:0)	0.04±0.00^{b}	0.06±0.01^{b}	0.18±0.00^{a}	0.0004
TSFA	**26.22±0.42^{b}**	**30.37±0.31^{a}**	**29.50±0.65^{a}**	**0.0066**
Monounsaturated				
Myristoleic acid (C14:1)	0.18±0.01^{b}	0.22±0.00^{a,b}	0.28±0.02^{a}	0.0114
Palmitoleic acid (C16:1)	8.28±0.12^{b}	8.91±0.10^{b}	10.77±0.32^{a}	0.0024
Heptadecenoic acid (C17:1)	0.01±0.00	0.02±0.01	0.02±0.02	0.7332
Elaidic acid (C18:1n9t)	0.17±0.00	nd	nd	-
Oleic acid (C18: 1n9c)	45.90±0.83^{a}	43.22±0.51^{b}	43.64±0.69^{b}	0.0570
Eicosenoic acid (C20:1)	0.04±0.04	0.17±0.22	0.06±0.04	0.6180
Docosaenoic acid (C22:1n9)	0.02±0.01	0.02±0.01	0.03±0.00	0.6787
Nervonic acid (C24:1)	0.11±0.09	0.15±0.11	0.11±0.11	0.8859
TMUFAs	**54.54±0.77^{a}**	**52.58±0.28^{b}**	**54.95±0.29^{a}**	**0.0344**
Polyunsaturated				
Linoleic acid (C18:2n6c)	16.76±0.34^{a}	14.66±0.19^{b}	13.92±0.23^{b}	0.0035
Linolelaidic acid (C18: 2n6t)	0.03±0.00	0.02±0.00	0.03±0.00	0.0631
Alpha- Linoleic acid (C18:3n3)	nd	0.39±0.10	0.06±0.00	0.0129
Gamma- Linolenic acid (C18:3n6)	0.32±0.41	0.03±0.00	0.06±0.00	-
Eicosadienoic acid (C20:2)	0.03±0.02	0.02±0.01	0.05±0.02	0.4357
Dihomo-gamma linolenic acid (C20:3n6)	0.28±0.01	0.22±0.16	0.06±0.00	0.1765
Eicosatrienoic acid (C20:3n3)	nd	0.39±0.10	0.06±0.00	0.0129
Arachidonic acid (C20:4n6)	nd	0.24±0.16	0.28±0.00	0.1047
Brassic acid (C22:2)	0.12±0.12	0.10±0.08	0.07±0.02	0.8854
Timnodonic acid (C20:5n3)	nd	0.13±0.03	nd	-

Docosahexenoic acid (C22: 6n3)	0.01±0.00	0.03±0.31	0.07±0.02	0.9445
TPUFAs	**19.19±0.24[a]**	**17.11±0.01[b]**	**15.68±0.32[c]**	**0.0014**
Trans fats	0.12±0.12	0.02±0.00	0.03±0.00	0.4213
Cis fats	62.66±1.15[a]	57.88±0.69[b]	57.56±0.92[b]	0.0207
Omega-3	1.79±0.14[a]	1.81±0.15[a]	1.31±0.08[b]	0.0476
Omega-6	17.40±0.07[a]	15.17±0.19[b]	14.34±0.27[b]	0.0012
Omega -9	46.01±0.68[a]	43.25±0.50[b]	43.66±0.71[b]	0.0426
EPA	0.12±0.12	0.10±0.07	0.07±0.02	0.8854
DHA	0.02±0.00[b]	0.03±0.00[b]	0.07±0.02[a]	0.0337

[a, b, c]Within row means with different superscripts differ significantly ($P < 0.05$). *H. sabdariffa* calyces meal significantly increased the total SFA and decreased the total PUFAs in the thigh meat of quail. At 5% supplementation, total MUFAs of thigh muscle were significantly increased. TSFAs- Total saturated fatty acids; TMUFAs- Total mono unsaturated fatty acids; TPUFAs- total poly unsaturated fatty acids. -- means that the statistical analysis was not done due to SD of 0 or the fatty acid could not be detected at all. Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5% *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean±SD. n=2 for all diets.

Table 4.9: Effect of supplemental *H. sabdariffa* calyces meal on the fatty acid content of breast meat of female Japanese quail

Fatty acid (%)	Diet 1	Diet 2	Diet 3	P-value
Saturated				
Lauric acid (C12:0)	0.097±0.00	0.062±0.00	0.089±0.00	-
Myristic acid (C14:0)	3.51±0.07^{a}	0.40±0.04^{b}	0.47±0.03^{b}	< 0.0001
Pentadecanoic acid (C15:0)	0.05±0.00	0.06±0.00	0.07±0.03	0.6395
Palmitic acid (C16:0)	11.57±0.03^{c}	16.71±0.70^{b}	20.65±0.44^{a}	0.0007
Margaric acid (C17:0)	0.10±0.00	0.12±0.00	0.13±0.00	-
Stearic acid (C18:0)	6.48±0.14^{c}	9.56±0.04^{a}	8.20±0.13^{b}	0.0003
Arachidic acid (C20:0)	0.10±0.00	0.12±0.00	0.13±0.00	-
Heneicosylic acid (C21:0)	0.12±0.03	0.19±0.00	0.07±0.03	0.0504
Tricosylic acid (C23:0)	nd	nd	nd	-
Lignoceric acid (C24:0)	0.05±0.00	0.15±0.04	0.04±0.00	-
TSFA	**38.95±11.39**	**39.03±12.29**	**36.47±7.25**	**0.9708**
Monounsaturated				
Myristoleic acid (C14:1)	0.05±0.00	0.09±0.04	0.25±0.34	0.2054
Palmitoleic acid (C16:1)	2.58±0.00^{c}	5.51±0.18^{b}	7.47±0.53^{a}	0.0014
Heptadecenoic acid (C17:1)	nd	nd	0.16±0.09	0.1061
Elaidic acid (C18:1n9t)	0.07±0.03	0.15±0.04	0.13±0.00	0.1662
Oleic acid (C18: 1n9c)	21.65±0.38^{c}	32.90±1.53^{b}	40.88±0.47^{a}	0.0006
Eicosenoic acid (C20:1)	0.05±0.00	0.12±0.00	0.04±0.00	-
Docosaenoic acid (C22:1n9)	nd	0.09±0.04	0	0.0540
Nervonic acid (C24:1)	0	0.16±0.04^{a}	0	0.0135
TMUFAs	**48.59±27.52**	**45.54±7.75**	**49.34±0.27**	**0.9713**
Polyunsaturated				
Linoleic acid (C18:2n6c)	8.98±0.17^{c}	10.43±0.03^{b}	11.99±0.09^{a}	0.0003
Linolelaidic acid (C18: 2n6t)	0.07±0.03	0.06±0.00	0.04±0.00	0.4481
Alpha- Linolenic acid (C18:3n3)	1.46±0.07^{a}	1.55±0.09^{a}	0.89±0.00^{b}	0.0036
Gamma- Linolenic acid (C18:3n6)	0.05±0.00	0.06±0.00	0.09±0.00	-
Eicosadienoic acid (C20:2)	nd	nd	nd	-
Dihomo-gamma linolenic acid (C20:3n6)	0.15±0.05	0.25±0.09	0.22±0.00	0.3829
Eicosatrienoic acid (C20:3n3)	0.10±0.07	nd	0.04±0.00	0.1919
Arachidonic acid (C20:4n6)	3.46±0.00^{b}	5.54±0.13^{a}	3.61±0.09^{b}	0.0003
Timnodonic acid (C20:5n3)	0.19±0.07	0.21±0.04	0.24±0.03	0.6632

Docosahexenoic (C22: 6n3)	0.76±0.10	0.74±0.09	0.82±0.03	0.6331
TPUFA	**13.21±1.31**	**15.24±3.11**	**13.94±4.81**	**0.8405**
Trans fats	0.15±0.00	0.25±0.00	0.18±0.00	-
Cis fats	30.63±0.55^{c}	43.33±1.49^{b}	52.85±0.53^{a}	0.0004
Omega-3	1.73±0.03	1.64±0.13	2.17±0.19	0.0537
Omega 6	12.76±0.00	16.34±0.00	16.09±0.00	-
Omega-9	21.77±0.34^{c}	33.15±1.62^{b}	41.03±0.50^{a}	0.0007
EPA	0.91±0.07	0.31±0.08	0.18±0.06	0.2937
DHA	0.76±0.10	0.74±0.09	0.82±0.03	0.6331

$^{a, b, c}$Within row means with different superscripts differ significantly ($P < 0.05$). *H. sabdariffa* calyces meal did not alter the total SFA, MUFAs and PUFAs of breast meat of quail ($P > 0.05$). TSFAs- Total saturated fatty acids; TMUFAs- Total mono unsaturated fatty acids; TPUFAs- total poly unsaturated fatty acids. - means that the statistical analysis was not done due to SD of 0 or the fatty acid could not be detected at all; Diet 1: Standard Japanese quail finisher diet; Diet 2: Standard Japanese quail finisher diet + 5% *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces meal w/w. Data is expressed as mean±SD. n=2 for all the diets.

Table 4.10: Effect of supplemental *H. sabdariffa* calyces meal on the fatty acid content of thigh meat of female Japanese quail

Fatty acid (%)	Diet 1	Diet 2	Diet 3	P-value
Saturated				
Lauric acid (C12:0)	0.03±0.00^{b}	0.05±0.00^{a}	0.05±0.00^{a}	< 0.0001
Myristic acid (C14:0)	0.66±0.00^{b}	0.74±0.00^{a}	0.76±0.01^{a}	0.0035
Pentadecanoic acid (C15:0)	0.08±0.00	0.07±0.00	0.07±0.00	-
Palmitic acid (C16:0)	21.71±0.13^{c}	23.63±0.00^{a}	22.39±0.09^{b}	0.0005
Margaric acid (C17:0)	0.13±0.00	0.12±0.00	0.15±0.00	-
Stearic acid (C18:0)	6.54±0.02^{a}	5.43±0.02^{b}	5.47±0.02^{b}	< 0.0001
Arachidic acid (C20:0)	0.15±0.00	0.12±0.00	0.13±0.02	0.1017
Heneicosylic acid (C21:0)	nd	0.07±0.03	0.11±0.02	-
Lignoceric acid (C24:0)	0.06±0.02	0.04±0.02	0.02±0.00	0.1433
TSFA	**29.58±0.00**	**29.39±1.57**	**29.76±0.43**	**0.9286**
Monounsaturated				
Myristoleic acid (C14:1)	0.10±0.00	0.17±0.00	0.22±0.00	-
Palmitoleic acid (C16:1)	6.21±0.05^{b}	8.96±0.02^{a}	9.09±0.00^{a}	< 0.0001
Heptadecenoic acid (C17:1)	0.08±0.00	0.08±0.02	0.10±0.00	0.2738
Oleic acid (C18: 1n9c)	4.55±0.00^{a}	4.41±0.01^{b}	4.41±0.00^{b}	0.0006
Eicosenoic acid (C20:1)	0.10±0.00	0.08±0.02	0.10±0.00	0.3207
Docosaenoic acid (C22:1n9)	0.03±0.00	0.06±0.02	0.07±0.03	0.2352
Nervonic acid (C24:1)	0.08±0.00	0.05±0.00	0.08±0.02	0.0682
TMUFAs	**52.32±0.11^{c}**	**53.50±0.15^{b}**	**54.84±0.38^{a}**	**0.0044**
Polyunsaturated				
Linoleic acid (C18:2n6c)	15.21±0.05^{a}	13.15±0.07^{b}	12.36±0.07^{c}	< 0.0001
Linolelaidic acid (C18: 2n6t)	0.03±0.00	0.02±0.00	0.02±0.00	-
Alpha- Linoleic acid (C18:3n3)	1.28±0.02	1.23±0.02	1.23±0.06	0.3645
Gamma- Linolenic acid (C18:3n6)	0.08±0.00	0.06±0.02	0.16±0.12	0.4383
Eicosadienoic acid (C20:2)	0.09±0.02	0.10±0.07	nd	-
Dihomo-gamma linolenic acid (C20:3n6)	0.09±0.02	0.19±0.10	0.14±0.00	0.3551
Arachidonic acid (C20:4n6)	0.80±0.05	0.80±0.02	0.94±0.05	0.0701
Timnodonic acid (C20:5n3)	0.11±0.02	0.11±0.02	0.13±0.03	0.4565

TPUFAs	**17.59±0.44[a]**	**15.91±0.02[b]**	**15.49±0.07[b]**	**0.0078**
Trans fats	0.10±0.10	0.02±0.00	0.02±0.00	0.4616
Cis fats	58.90±0.21[a]	57.24±0.19[b]	57.61±0.44[a,b]	0.0263
Omega-3	1.55±0.02	1.60±0.03	1.69±0.05	0.0810
Omega-6	15.68±0.03[a]	14.24±0.02[b]	13.83±0.02[c]	< 0.0001
Omega -9	44.26±0.07	44.12±0.14	45.11±0.34	0.0362
EPA	0.11±0.02	0.11±0.02	0.13±0.02	0.3742
DHA	0.22±0.00	0.91±0.22	0.23±0.02	0.1303

[a, b, c]Within row means with different superscripts differ significantly ($P < 0.05$). TSFAs- Total saturated fatty acids; TMUFAs- Total mono unsaturated fatty acids; TPUFAs- total poly unsaturated fatty acids. - means that the statistical analysis was not done due to SD of 0 or the fatty acid could not be detected at all; Diet 1: Standard Japanese quail finisher diet; Diet 2: standard Japanese quail finisher diet + 5% *H. sabdariffa* calyces meal w/w; Diet 3: standard Japanese quail finisher diet + 10% *H. sabdariffa* calyces w/w. Data is expressed as mean±SD. n=2 per diet.

4.8 Discussion

The objectives of this study were to ascertain the effects of dietary *H. sabdariffa* calyces meal on meat yield as well as the meat's pH, colour, water holding capacity, tenderness, proximate (dry matter, crude protein, ether extract and ash content) and fatty acid content of broiler Japanese quail.

4.8.1 Meat yield

Meat yield is a function of carcass weight and dressing percentage and consumers prefer heavier carcasses (Krishnan, 2019). Meat yield as determined by carcass weight and dressing percent is dependent on factors like dietary ingredients and diet composition (Alamuoye et al., 2015). My findings showed the carcass weight of male Japanese quail ranged from 110.59±9.45 to 112.84±7.39g and that of female Japanese quail ranged from 119±12.23 to 131.79±7.77g. It has been reported that Japanese quail carcass weights range from 126.7±0.86g and 133.96±9.45g (Krishnar, 2019; Alamuoye et al. 2015) thus the carcass weight of male Japanese quail reported in my study is lower but that of female Japanese quail is within the reported range. In the current study, the dressing percentage of male Japanese quail carcass ranged from 66.07±7.86 to 69.95±6.81% and that of female Japanese quail carcass ranged from 68.91±6.22 to 71.24±4.20%. Adult Japanese quail (above 45 days in age) have been reported to have a dressing percentage that ranges between 68.42% and 75.73% (Alamuoye et al., 2015; Awan et al., 2017). The dressing percentage of quail carcasses in the current study were within the range reported in previous studies. The similarities in the Japanese quail carcass weight and dressing percent across diets suggest that dietary *H. sabdariffa* calyces meal did not compromise meat yield. The findings of the current study imply that *H. sabdariffa* calyces meal can potentially be used as a feed supplement in Japanese quail production without compromising meat yield of quail.

4.8.2Physical quality parameters of the meat

The decline of pH is an important post mortem biochemical change that is important for the conversion of muscle to meat (Warner, 2016). On slaughter, the carcass of an animal's

metabolism shifts from aerobic to anaerobic due to the depletion of oxygen (Warner, 2016). Under anaerobic metabolic conditions muscle glycogen is converted to lactic acid resulting in the build-up of lactic acid reducing the meat pH (Alvarez et al., 2019; Warner, 2016). In meat from the carcasses of well-nourished quail, according to Terlouw et al., (2008), normal pH becomes stable 24 h following death. However, there are intrinsic differences in the pH characteristics that are dependent on muscle type. Muscles vary in the composition of fibre types (slow, red, oxidative versus fast, white, and glycolytic) as well as in length, width, biochemistry and chemical composition (Listrat et al., 2016). Fibres within the same muscle go into rigor mortis at differing rates (Warner, 2016). The breast muscle is characterised by having a higher relative proportion of light fibres as compared to thigh muscle (Barbut et al., 2015). An increase in glycolytic muscle fibres in muscle bundles enhances meat pH decrease (Genchev et al., 2010).

In this study, the pH of quail breast meat decreased from a range of 6.02- 6.21 to a range of 5.75- 5.87, 24 hours after slaughter. The initial pH of thigh meat of quail in the current study ranged between 6.67- 6.73 and the ultimate pH ranged between 6.66- 6.75. The pH of quail breast meat decreased from a range of 6.26-6.30 to 5.95-6.00. The ultimate pH of Japanese quail meat has been reported to be 5.91±0.03 while that of thigh muscle is 6.58± 0.04 (Genchev et al., 2010; Terlouw et al., 2008; Boni et al. 2010; Narinc et al., 2013). Based on the previously reported pH ranges it can be inferred that the ultimate pH of breast muscle of both male and female quail in the current study was in the normal range suggesting that supplemental *H. sabdariffa* calyces did not affect the biochemical processes that lead to the conversion of breast muscle to meat. The final pH of thigh meat of male and female quail was above the normal range reported in previous studies. The higher final pH values of the thigh muscle of quail in the current study could possibly be as a result of the presence and/or dominance of type IIb fibres (Pethick et al., 2005). Type IIb fibres are known to have low glycogen content and are also susceptible to stress induced glycogen depletion which resulted in a slower decline of pH in the thigh muscles of quail in this study.

In order to minimise stress during the feeding trial the birds were provided with adequate (recommended) space and an enriched environment in the pens. Additionally, while awaiting

slaughter the birds were kept in their pens away from where slaughter took place, with very low noise and correct handling to avoid inducing stress on the quail. However, this does not take away the stress from other factors such as restricted space for flying, hence the the thigh meat of quail in this study had a higher than previously reported pHu since the quail depended mostly on their legs more for movement around the pens. Supplemental *H. sabdariffa* calyces meal into the standard quail finisher diet did not interfere with the conversion of muscle to meat in the breast and thigh.

The lightness (L*) of meat distinguishes between lighter and darker colours (American Meat Science association, 2011). Consumers discriminate against either too light or too dark meat (Ngapo et al., 2004) thus the L* value is a relatively good indicator of whether the meat will be acceptable or not. Dogan et al., (2013) characterised quail meat lightness as follows: L*>53 pale, 35.89<L*<52.34 normal and L*<35.89 as dark. Based on the classification by Dogan et al., (2013), the breast meat of Japanese quail from across dietary treatments were in the dark colour range 30 minutes post-slaughter. However, 24 hours post-slaughter they were in the normal range. The increase in the lightness (L*) values of breast meat with time is attributed to the transformation of muscle into meat (Garcia et al., 2010). Meat pH and colour are highly correlated. Higher meat pH is associated with darker meat while lower meat pH values are associated with lighter meat. The decline in pH post-slaughter is one of the major biochemical changes whose effect is to lighten meat (Muchenje et al., 2009, Swatland, 2004). With post mortem decline in meat pH, proteins denature causing the myofilament lattice to shrink, increasing the myofibrillar index that goes beyond that of the sarcoplasm and increases light scattering. The increased light scattering, leads to meat becoming lighter (Swatland, 2004). The ultimate colour of breast meat of quail from across dietary treatments in the current study was in the normal range (Tables 4.3 and 4.4) thus it can be inferred that *H. sabdariffa* calyces meal can be used as a dietary supplement without affecting quail meat colour. Redness (a*) of meat determines its acceptability by consumers (Mir et al., 2017). The redness of meat is largely dependent on the content of myoglobin in muscle (Çelen et al., 2016). High myoglobin content in the muscle is associated with an increase in the redness of meat leading to high acceptability (Neethling et al., 2017). The redness of breast meat of quail 24 hours post-slaughter in the current study did not differ between dietary treatments but

ranged from 2.34±1.43 to 4.34±2.05 for meat from males and 2.88±1.86 to 4.06±2.43 for meat female Japanese quail. Previous studies have reported the redness of Japanese quail meat in the range of 3.09-6.79 (Gevrekçi et al., 2009; Mnisi and Mlambo, 2018). The redness of quail meat in the current study is in the range reported in previous studies (Gevrekçi et al., 2009; Mnisi and Mlambo 2018). The yellowness of breast meat in the current study ranged from 8.83±2.44 to 9.50±1.80 for males and 8.55±1.60 to 12.25±2.88 for females. The yellowness values of breast meat of quail in the current study was within the range (7.74 to 12.72) reported by Ribarski and Genchev, (2013), Genchev et al., (2008) and Narinc et al., (2013). Supplemental *H. sabdariffa* calyces meal did not alter or negatively compromise the meat colour (L*, a*, b*) of quail. Therefore, breast meat of both male and female Japanese quail across dietary treatments had normal colour (Tables 4.3 and 4.4).

The meat's ability to retain moisture as determined by water holding capacity (Huff-Lonergan and Lonergan, 2005) depends on ultimate pH, protein oxidation and post mortem proteolysis (Wolmarans, 2011). A high ultimate pH results in meat proteins with higher than normal ability to retain moisture which results in dark, dry and firm meat (Barbut, 2015). A fast drop of pH post mortem (low final pH), results in meat proteins with low water holding capacity and, pale, soft exudative meat (Barbut, 2015). Awan et al., (2017) reported the water holding capacity of quail meat to range from 74.71±0.72 and 76.23±0.86%. In this study, the water holding capacity of both the breast and thigh meat of quail (both male and female) were relatively higher than those reported in the previous studies. This can be as a result of the higher pH values obtained from both the breast and thigh meat of quail in this study when compared to previous studies.

High ultimate pH in meat manifests as reduced water loss (Lawrie, 2006), this could explain the higher water holding capacity observed in the thigh than in the breast meat. While post-mortem pH and protein denaturation are the main determinants of water holding capacity, other factors also contribute to the water retaining properties of meat. Meat with higher intramuscular fat have a higher water holding capacity (Wolmarans, 2011). The thigh muscle of poultry has more intramuscular fat compared to the breast meat (Barbut, 2015). In the current study, the thigh meat of quail had a relatively higher content of fat and a higher water

holding capacity compared to the breast meat. Therefore, the findings in this study show the previously reported association between intramuscular fat content and water holding capacity.

Consumers strongly associate the freshness of meat with its tenderness (Mennecke et al., 2007). Consumers prefer tender meat and can pay a higher price in the market place for meat which has guaranteed tenderness (Miller et al., 2001). The tenderness of meat is influenced by three major factors: the degree of contraction of muscle sarcomeres, the integrity/degradation of the myofibrillar structure and (3) the connective tissue content, a determinant of "background toughness" (Koohmaraie et al., 2002; Sentandreu et al., 2002). The background toughness of meat is the resistance to shearing of the unshortened muscle (Marsh and Leet, 1966). Muscle elasticity decreases during onset of rigor, and by the end of the process (rigor mortis), the tissue reaches its maximum toughness. The tensile strength of meat is a simple measure of meat tenderness (Kharthika et al., 2016; Kaye, 2014). In this study supplemental *H. sabdariffa* calyces meal neither increased nor decreased the tensile strength of the Japanese quail breast meat thus it can potentially be utilised without the risk of compromising the tenderness of Japanese quail breast meat

4.8.3 Proximate and fatty acid content of the meat

The consumption of poultry meat is gaining popularity compared to other meat types as a result of its desirable nutritional characteristics such as low lipid content and relatively high concentration of polyunsaturated fatty acids (Bourre, 2005). The nutritional value of meat is one of the key determinants in consumer choices. Findings on the determined proximate components of the male Japanese quail breast meat from the current study show that across dietary treatments, on a dry matter basis, the breast meat from the Japanese quail carcasses, had a crude protein, ether extract and ash content which ranged from 79.87±0.39 to 80.31±0.29; 7.19±0.09 to 9.36±0.30 and 5.02±0.02 to 5.12, respectively. The thigh meat of male Japanese quail in the current study, on a dry matter basis, had a crude protein, ether extract and ash content which ranged from 63.14±0.03 to 75.78±0.88; 12.94±0.73 to 23.46±0.52 and 3.85 to 4.28±0.03 respectively. In female Japanese quail, the breast meat on a dry matter basis had a crude protein, ether extract and ash content ranging from 83.11±3.04 to 84.96±3.53; 6.19±0.77 to 8.53±0.17 and 5.48±0.49 to 6.02±1.30, respectively. The thigh meat

of female quail on dry matter basis had a crude protein, ether extract and ash content ranging from 70.86±8.78 to 76.69±2.51; 17.69±0.58 to 23.20±7.75 and 4.04 to 4.41±0.15, respectively. Japanese quail meat is reported to contain 80.81 to 96.14% crude protein, 2.85 to 13.91% ether extract and 2.60 to 6.21% ash (Raji et al., 2015; Genchev et al., 2008; Khalifa et al., 2015). The crude protein, ether extract and ash of breast meat of Japanese quail (both male and female) from the current study falls within the ranges reported by other researchers but the ether extract of thigh muscle of quail in this study is above that which has been reported by other researchers. It can therefore be inferred that dietary *H. sabdariffa* calyces meal could have favoured the deposition of more fat on the thigh muscle of the Japanese quail. It is important to note that both the breast and thigh muscle of male Japanese quail fed diet 3 (90% standard quail finisher diet + 10% *H. sabdariffa* calyces meal) had the lowest fat content (7.19±0.09 and 12.94±0.73, respectively). Thus, *H. sabdariffa* calyces meal in quail finisher diets can potentially be used to reduce the fat content in both breast and thigh meat cuts without compromising the meat's protein and ash content. This implies that *H. sabdariffa* calyces meal can be exploited to produce low fat Japanese quail meat potentially satisfying the consumers' demand for lean and healthier meat cuts (Mothershaw et al., 2009). Such meats would contribute towards reduced risk of diet-induced metabolic diseases (Walker et al., 2005).

Fatty acid composition plays a critical role in meat quality as it contributes to the sensory attributes, nutritional value as well as oxidative stability of the meat (Nardoia et al., 2015). In the current study, breast meat of male Japanese quail was found to have total saturated, monounsaturated and polyunsaturated fatty acid content in the range of 31.42±0.72% to 32.19±0.85%, 47.48±0.22% to 49.08±0.26% and 18.64±0.57% to 20.15±0.14%, respectively. The thigh meat of male Japanese quail in this study was found to have total saturated, monounsaturated and polyunsaturated fatty acids in the range of 26.22±0.42 to 30.37±0.31, 52.58±0.28 to 54.95±0.29 and 15.68±0.32 to 19.19±0.24, respectively. The breast meat of female Japanese quail in the current study contained SFA, MUFA and PUFAs in the range of 36.47±7.25 to 38.95±11.39; 45.54±7.75 to 49.34±0.27 and 13.21±1.31 to 15.24±3.11, respectively. Meat from Japanese quail has been reported to contain 28.40±0.56 to 35.28±0.71 saturated fatty acids, 40.01±2.13 to 53.45±1.20 monounsaturated fatty acids and 17.98±0.90

to 26.26±1.13 polyunsaturated fatty acids (Tavaniello et al., 2017; Khalifa et al., 2016). The total saturated, monounsaturated and polyunsaturated fatty acids of Japanese quail meat (both male and female) in the current study fall within the range previously reported. Although the SFAs of thigh of male Japanese quail fed diets 2 and 3 were significantly higher than that of quail fed diet 1, the values still fall within those reported by Tavaniello et al., (2017) and Khalifa et al., (2016). Health conscious consumers prefer meat with low saturated fat content (Mothershaw et al., 2009), therefore caution should be exercised at supplementing finisher Japanese quail feed with *H. sabdariffa* calyces meal as it might result in Japanese quail thigh meat with higher total saturated fatty acids content. Excessive intake of foods, meat included, with a high concentration of SFAs increases the risk of developing metabolic derangements (Monteiro and Azevedo, 2010). Notably, the dominant SFA in both cuts (breast and thigh muscle) across all dietary treatments was palmitic acid which is the least harmful to human health as it has been reported to have a much lower effect on raising blood cholesterol concentrations when compared to other SFAs (Fattore and Fanelli, 2013). Palmitic acid increases both the low density and high density lipoproteins implying that the ratio of LDL: HDL (the main predictor of CVD risk) remains unaffected (Fattore and Fanelli, 2013). *H. sabdariffa* seed has been shown to contain a high concentration (20.48%) of palmitic acid (Da- Costa- Rocha et al., 2014). I therefore speculate the *H. sabdariffa* calyces meal could also have contained a high concentration of the palmitic acid which could have contributed to the high percentage palmitic acid content in the thigh meat of quail fed diets 2 and 3.

Polyunsaturated fatty acids in foods increase susceptibility to lipid peroxidation (Repetto et al., 2012) while SFAs are less susceptible to lipid peroxidation (Cheng, 2016). Therefore, the preponderance of SFA and low content of PUFAs reported in the current study could help increase the shelf life of Japanese quail meat. It could be inferred that meat from the Japanese quail fed diet 2 and 3 might have a longer shelf life compared to that from quail fed diet 1.

4.9 Conclusion

Hibiscus sabdariffa calyces meal can be used as a dietary supplement without affecting meat yield of Japanese quail. Its use in quail feed improved pHu and water holding capacity of the meat therefore it can be used to produce good quality meat. Importantly, dietary *H. sabdariffa* calyces meal reduced the meat's total fat content thus can potentially be exploited to produce lean meat with better keeping quality.

I further investigated the effect of supplemental *H. sabdariffa* calyces meal on the egg production and egg quality in Japanese laying quail.

5. CHAPTER FIVE- EFFECT OF *HIBISCUS SABDARIFFA* CALYCES MEAL ON LAYING PERFORMANCE, EGG AND MEAT QUALITY OF LAYER JAPANESE QUAIL

5.0 Introduction

Poultry egg production is affected by the quality and amount of feed, intake of water, amount of light, photoperiod and duration and disease (Jacob et al., 1998). Laying hens require a balanced diet to sustain optimum production of quality eggs over time (Adi et al., 2014). Inadequate nutrition causes hens to stop laying and a lack of feed for several hours results in decreased egg production (Jacob et al., 1998). The birds' access to water is critical and an inadequate supply of it over long periods of time reduces egg production (Rault et al., 2016). Japanese laying quail require approximately 14-18 hours of light to maintain egg production and the light intensity should be adequate enough to sustain production (Wagan et al., 2018). Environmental temperatures above 26ºC pose severe problems as it reduces feed consumption, egg production, egg size and hatchability (Ayo et al., 2011). Several microbe-borne diseases including botulism, coccidiosis and infectious bronchitis reduce egg production (De Wit et al., 2011). Parasite infestation leads anaemia, reduced feed efficiency, impaired growth, low egg production and failure to withstand other diseases (Jacob et al., 1998). Japanese quail are highly sensitive to stress factors (Santos et al., 2019). During the production phase, a low external noise level should be maintained (Santos et al., 2011). Egg quality is affected by factors such as intestinal health, nutrition and environmental factors that lead to stress (Réhault-Godbert et al., 2019). Intensive egg production generates stress which in turn negatively impacts gastrointestinal functions leading to reduced absorption of nutrients, lower feed efficiency and reduced egg quality (Bouvarel et al., 2011).

Japanese quail eggs are smaller than chicken eggs but contain relatively more protein, iron, riboflavin, and vitamin B12 by weight (Lopez Sobaler et al., 2017) and they are an important source for essential nutrients including in human diets (Lopez Sobaler et al., 2017; Griffin et al., 2016). They are a good source of minerals such as iron and phosphorus (Fuller et al., 2015). Regular consumption of quail eggs reportedly modulates the immune system and help protects against diseases (Lopez Sobaler et al., 2017). Commercial pullet production targets to have high productive performance premised on optimal feed utilisation, healthy birds and the production of quality eggs (Réhault-Godbert et al., 2019). Optimisation of nutrient supply including micronutrients, especially vitamins, is critical for ensuring optimal egg production

by layer hens (Nys et al., 2018). Plant-derived meals, powders and extracts with nutraceuticals improve nutrient digestibility and absorption as well as help maintain intestinal integrity (Hassan et al., 2010). *H. sabdariffa* is one of the plants that possesses health beneficial biological activities, including among many, antimicrobial, antioxidant, hepatoprotective, nephroprotective, hypocholesterolemic and antianaemic (Da-Costa-Rocha et al., 2010) but its effect on the production and quality of Japanese quail eggs has not been evaluated. I thus sought to determine the effect of dietary supplementation with *H. sabdariffa* calyces meal on laying performance and egg quality of Japanese quail.

5.1 Study Objectives

The objectives of the study were to investigate the effect of dietary supplementation with *H. sabdariffa* calyces meal on Japanese quail:

a. egg production
b. egg mass, length, width, albumen mass, length, width and height, yolk mass, diameter and height, shell mass and thickness.

c. egg dry matter, crude protein, ether extract, ash and fatty acid content

d. Japanese quail layer hen meat quality as determined by it pH, colour, water holding capacity and tenderness.

5.2 Study Hypothesis

H_0: Dietary supplementation with *H. sabdariffa* calyces meal does not affect the laying performance (egg production, number of eggs produced and feed utilisation), egg and the quality of meat of Japanese quail.

H_1: Dietary supplementation with *H. sabdariffa* calyces meal promotes the laying performance (egg production, number of eggs produced and feed utilisation) egg and the quality of meat of Japanese quail.

5.3 Materials and Methods

5.3.1 Feed Ingredients and diet formulation

The ingredients and chemical composition of the diets used in this study are presented in Table 5.1. *Hibiscus sabdariffa* calyces were sourced and processed as described in Chapter 3, under subheading 3.3.1. Yellow maize meal and wheat bran were sourced from Herron Bridge Produce cc, Lanseria, South Africa. Soyabean meal was sourced from Opti-feeds Private Limited, Lichtenberg, South Africa. The vitamin-mineral premix and methionine were purchased from Trow Nutrition, Johannesburg, South Africa. The dietary treatments were formulated at the Central Animal Services Unit (CAS), University of the Witwatersrand, to meet the nutritional requirements of laying quail according to National Research Council recommendations (NRC, 2001). Three dietary treatments were formulated: diet 1: standard Japanese quail layer diet (SQLD) + 0% (w/w) *H. sabdariffa* calyces meal, diet 2: standard Japanese quail layer diet + 5% (w/w) *H. sabdariffa* calyces meal and diet 3: standard Japanese quail layer diet + 10% (w/w) *H. sabdariffa* calyces meal.

Table 5.1: Ingredients and chemical composition of the control and test diet

	Diet 1	Diet 2	Diet 3
Ingredients (g/kg)			
Maize meal	520.00	520.00	520.00
Soya bean meal	336.50	336.50	336.50
Wheat bran	48.00	48.00	48.00
DCP/Limestone	76.00	76.00	76.00
Vit/Mineral premix	3.00	3.00	3.00
Sodium chloride	3.00	3.00	3.00
Methionine	1.50	1.50	1.50
Vegetable oil	12.00	12.00	12.00
H. sabdariffa calyces meal	0	50.00	100.00
Total	1000	1050	1100
Chemical composition			
Dry matter (%)	91.41	90.98	93.41
Crude protein (% DM)	23.48	21.47	23.41
Ether extract (% DM)	4.13	4.64	4.49
Ash (% DM)	10.92	8.31	9.35
Calcium (% DM)	2.80	2.09	2.54
Phosphorus (% DM)	0.42	0.43	0.41
Crude fibre (%)	5.28	5.65	5.61
Energy (MJ/Kg)			
Gross energy	17.69	17.33	17.08

DM, dry matter. Vitamin-mineral premix: each kg contained vitamin A 4000 000 IU, vitamin D_3 600 000IU, vitamin E 8000IU, vitamin K_3 0.258g, vitamin B_1 0.6g, vitamin B_2 1.6g, niacin 11.94g, calcium pantothenate 3.92g, vitamin B_{12} 0.1g, vitamin B_6 0.98, choline 72.73g, folic acid 0.288g, biotin 0.0008g, MnSO4 9.92g, Zn 6.3g, Cu 0.252g, KI 0.2g, Co 0.0042g, Fe 2.1g, Se 0.0036g.

5.3.2 Ethical approval and study site

Ethical approval was sought and granted as described in Chapter 3, under subheading 3.3.5. The study site for this experiment was as described in Chapter 3, under subheading 3.3.5.

5.3.3 Birds and bird management

Ninety female Japanese quail (*Coturnix coturnix japonica*) at the age of 35-days were purchased from South Africa Quail Breeders (East London, South Africa) and used in the study. The birds were group housed in pens (10 birds per pen) in a well-ventilated, temperature and lighting controlled room. A deep litter system was used to house the birds. Wood shavings were laid on the floor of each pen as bedding. The pens were cleaned and bedding was changed once every week. The photoperiod was set at 12hours from 0600h to 1800h. The ambient temperature was maintained at 21±2°C. Upon receipt, the birds were dewormed with piperizine [Kyron Laboratories (PVT) Ltd, Johannesburg, South Africa] at 90 mg/litre of drinking water for two days and this period also served to familiarise the birds to the experimental and housing conditions. The birds were fed on their respective diet for 56 days and had *ad libitum* access to feed and drinking water.

5.3.4 Experimental design

Ninety, 35-day old Japanese quail, were in a completely randomised design, randomly allocated to and fed layer finisher diets supplemented with *H. sabdariffa* calyces meal at 0%, 5% and 10%, respectively and fed for 28 days. The quail were group housed in pens. Each diet was replicated 3 times, and each replicate had 10 Japanese quail.

5.3.5 Determination of egg laying performance

Eggs were collected twice daily and the number of eggs produced per pen was recorded. The number of eggs was used to calculate laying performance of the birds in their respective diets using the formulae below:

i. $percent\ \ laying\ quail = (number\ of\ laying\ quail \div number\ of\ experimental\ quail) \times 100$

ii. $egg\ production\ \% = (total\ number\ of\ eggs \div total\ number\ of\ layers) \times 100$ (Ali et al. 2003)

5.3.6 Determination of egg length, width and shape index

Measurement of external egg quality parameters began in the second week of the experiment following habituation. The length and width of each egg was measured using a digital Vernier callipers (Major Tech Pty Ltd, Elandsfontein, South Africa). The length was measured as the distance from the apex to the base, while the width was determined at the middle (widest) region of the egg. The mass of each egg was determined on an electronic scale **(**Presica 310M, Lasec, Johannesburg, South Africa). Each egg's shape index was computed using the equation:

s$hape\ index = (egg\ width \div egg\ length) \times 100$ (Abu Tabeekh, 2011).

5.3.7 Determination of shell mass

Immediately after determining the external egg quality parameters, an incision large enough to extract the albumen and yolk was made on the apex of the egg using a needle. The albumen and yolk were collected onto a flat plate. The eggshell mass was then measured on an electronic scale (Presica 310M, Laser, Johannesburg, South Africa) following emptying the egg contents.

5.3.8 Determination of shell thickness

The eggshell thickness was determined using a digital Vernier caliper (Major Tech Pty Ltd, Elandsfontein, South Africa) on the apex, middle and bottom part of the egg as described by Abu Tabeekh, (2011). An average of the three measurements was calculated and recorded.

5.3.9 Determination of yolk diameter, height and mass

The yolk was carefully separated from the albumen using a spoon and placed in a pre-weighed petri dish and the yolk diameter and height were determined using a digital Vernier callipers (Major Tech Pty Ltd, Elandsfontein, South Africa) as described by Abu Tabeekh, (2011). Thereafter yolk mass was determined on an electronic scale (Presica 310M, Laser, Johannesburg, South Africa). Following the determination of yolk quality parameters, egg yolks from quail from the same dietary treatment were compounded and freeze stored at 21ºC pending the determination of the proximate and fatty acid content.

5.3.10 Determination of albumen length, width, height and mass

The albumen length, width and height were determined using a Vernier caliper (Major Tech Pty Ltd, Elandsfontein, South Africa) as described by Abu Tabeekh, (2011). The albumen was emptied to a pre-weighed Petri dish and its mass determined on an electronic scale (Presica 310M, Laser, Johannesburg, South Africa). Following the determination of albumen quality parameters, egg albumens from quail in the same dietary treatment were compounded and freeze stored at -21ºC pending the determination of the proximate content.

5.3.11 Determination of yolk and albumen proximate composition

The egg yolk and albumen samples of quail fed similar diets were pooled together to make a composite sample. The pooled samples were freeze dried (Custon, SSE Engineering, North America) and then milled through a 2mm screen. The dry matter, ash and crude protein content of the egg yolk and albumen samples were determined as described by the Association of Analytical Chemists (AOAC, 2000: Methods 934.01 and 930.15, 942.05 and 954.01, respectively). Fat from the egg yolk and albumen was extracted in diethyl ether as described by the Association of Analytical Chemists (AOAC, 2000: method number 920.39). Each assay was carried out in duplicate.

5.3.12 Determination of yolk fatty acid content

The egg yolk samples of quail from similar diets were pooled together to make a composite sample for each treatment group. The pooled samples were freeze dried (Custon, SSE Engineering, North America) and milled through a 2mm screen. The milled samples were solvent-extracted with diethyl ether to obtain fat. Methanolic potassium hydroxide (2 N) was used to methylate the fat and generate fatty acid methyl esters (FAMEs) as outlined by Christopherson and Glass (1969). The FAMEs were then injected into a gas chromatograph (GC) where they were separated on a DB-23 capillary column (90 cm × 250 µm × 0.25 µm) (Supelco; Sigma Aldrich) by a temperature gradient over 45mins. Nitrogen was used as carrier gas. The gas chromatograph consisted of a HP6890 GC (Hewlett Packard, Bristol, UK) with a flame ionisation detector. The detector and injector temperatures were both set at 300°C. A PC equipped with CHEMSTATION software was used for quantification (Chemistations Deutschland GmbH, Augustastrasse, Wesel, Germany). The analysis was done in duplicate.

5.4 Terminal procedures and measurements

On day 57 of the feeding trial, terminal procedures were performed as described in Chapter 4, under subheading 4.3.6. Gastrointestinal viscera were carefully removed and blood was collected into plain and heparinised blood collection tubes. The blood was used to determine haematocrit and erythrocyte osmotic fragility. The GIT viscera morphometry, haematocrit and serum concentration of uric acid, total protein, calcium, bilirubin, globulin, albumin, malondialdehyde and AST activity measurements are detailed in Chapter 6.

5.5 Determination of physical quality traits of meat

5.5.1 Determination of pH and colour

The meat's pH and colour were determined as described in Chapter 4, subheading4.4.1.

5.5.2 Determination of holding capacity

The water holding capacity of the breast and thigh meat were determined as described in Chapter 4, under subheading4.4.2.

5.5.3 Determination of tensile strength

The tensile strength of the breast and thigh meat were determined as described in Chapter 4, under subheading 4.4.3.

5.6 Statistical analysis

Data are expressed as mean ±SD. Graph Pad Prism Version 5 (Graph-pad Software Inc., San Diego, USA) was used to analyse data. A two-way ANOVA was used to analyse weekly egg production data and a repeated measures ANOVA used to analyse within group measures. A one-way ANOVA was used to analyse data for overall egg quality and meat quality. The Bonferroni *post hoc* test was used to compare means. The level of significance was set at $P < 0.05$. The model used for data analysis was:

$Y_{ij} = \mu + T_i + e_{ij}$;

where Y_{ij} = dependent variable of interest (egg mass, length and width; shell mass and thickness; albumen mass, width, height and length, yolk mass, height and diameter, yolk and albumen proximate content and fatty acid content; meat pH, colour, WHC, tensile strength)

μ = is the overall mean common to all observations

T_i = is the fixed effect of the i^{th} dietary treatment (i = 1, 2, 3)

e_{ij} = is the random residual error.

5.7 Results

During the progression of the experiment, diet 2 and 3 each lost 1 bird leaving a total of 29 quail for each of those 2 diets.

5.7.1 Egg laying performance of Japanese quail

Table 5.2 shows the effect of *H. sabdariffa* calyces meal on the number of laying Japanese quail hens and the number of eggs produced. Supplemental *H. sabdariffa* calyces meal (5 and 10%) significantly reduced ($P = 0.0312$) the number of laying Japanese quail. Supplemental *H. sabdariffa* significantly reduced ($P < 0.0001$) the total number of eggs produced by Japanese quail throughout the experimental period. Supplemental *H. sabdariffa* calyces meal at 10% dietary inclusion significantly reduced ($P = 0.0083$) egg production rate of Japanese quail.

Table 5.2: Effect of supplemental *H. sabdariffa* calyces meal on laying performance of Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	P- value
Laying birds (%)	86.66±15.28[a]	50.00±10.00[b]	53.33±15.28[b]	0.0312
Egg production (%)	47.03±11.74[a]	47.18±9.32[a]	13.35±8.01[b]	0.0083
Total no. of eggs	223.00±37.44[a]	130.00±26.16[b]	36.00±19.00[c]	<0.0001
Feed conversion (kg feed/no. of eggs)	2.47±0.05	2.49±0.13	2.42±0.16	0.1887

[a, b]Within row means with different superscripts differ significantly (P<0.05). No significant differences in the percentage laying birds and feed conversion ratio (P> 0.05). Supplemental *H. sabdariffa* calyces meal significantly reduced egg production and number of eggs produced (P < 0.05). Diet 1 = standard Japanese quail layer diet + 0% (w/w) *H. sabdariffa* calyces meal, Diet 2 =standard Japanese quail layer diet + 5% *H. sabdariffa* calyces meal, Diet 3 = standard Japanese quail layer diet + 10% *H. sabdariffa* calyces meal. Data is expressed as mean ± SD. n= 30 for diet 1, n = 29 for diet 2 and 3.

5.7.2 Egg production

Figure 5.1 shows the effect of *H. sabdariffa* calyces meal on the weekly egg production. Supplementation of standard Japanese quail layer diet with 10% *H. sabdariffa* calyces meal delayed the onset of laying. The female Japanese quail fed diet 3 started laying an average of 8 days later and had not reached peak laying by the 8th week while the Japanese quail fed the control diet reached peak laying on the 7th week. There were no significant differences (P > 0.05) in the egg production rate of Japanese quail across dietary treatments from week 1 to 3 of the experimental period. Supplemental *H. sabdariffa* calyces meal at 10% significantly reduced (P < 0.0001) the egg production rate of Japanese quail between week 4 and 7 of the experimental period. Supplemental *H. sabdariffa* calyces meal did not significantly affect (P > 0.05) the egg production rate of Japanese quail across dietary treatments in week 8 of the experimental period.

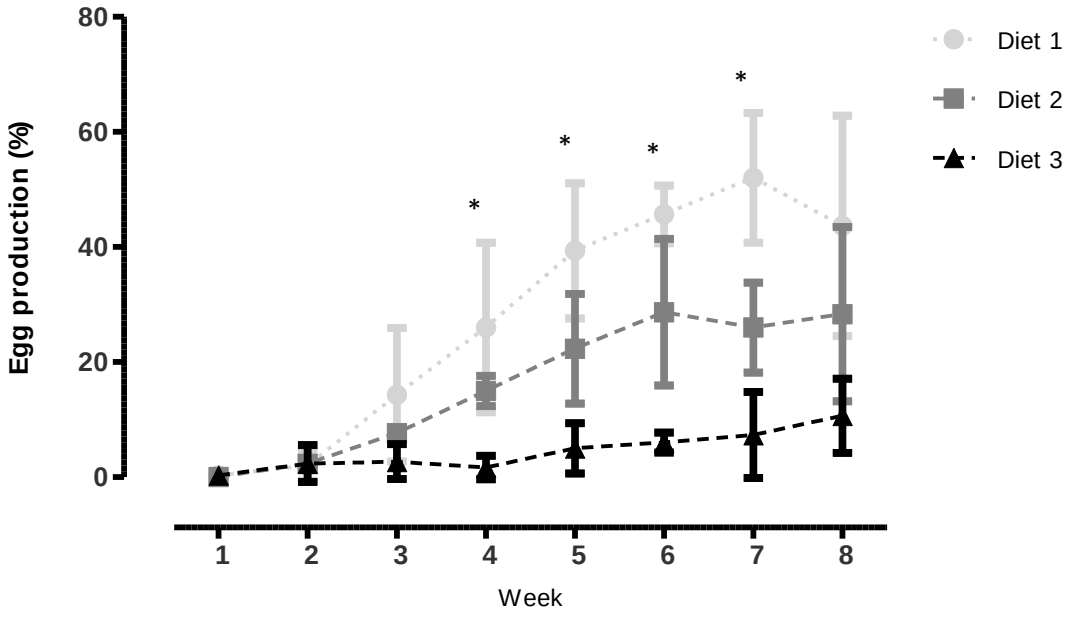

Figure 5.1: Effect of supplemental *H. sabdariffa* calyces on weekly egg production percentage

Diet 1 = Standard Japanese quail laying diet, Diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal, diet 3 = standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal. Data is expressed as means± SD. n=26 for diet 1, n=15 for diet 2 and n=16 for diet 3.

5.7.3 Egg mass

Figure 5.2 shows the effect of *H. sabdariffa* calyces meal on the weekly egg mass of Japanese quail. Supplemental *H. sabdariffa* calyces meal had no effect ($P > 0.05$) on the Japanese quail weekly egg mass. Dietary treatment and time did not contribute any significant differences throughout the experimental period.

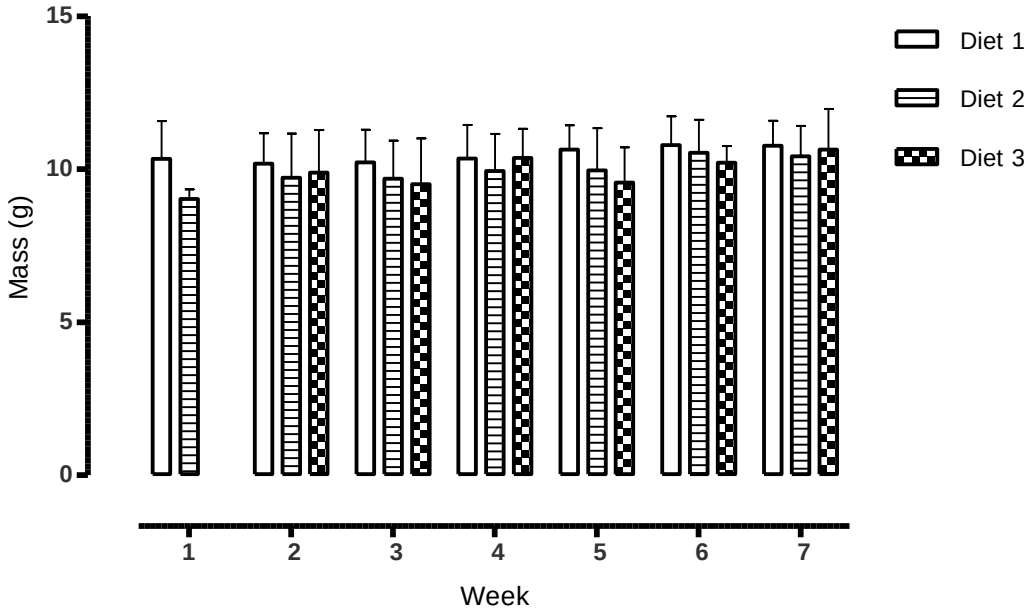

Figure 5.2: Effect of supplemental *H. sabdariffa* on the weekly mass of Japanese quail eggs

Diet 1 = standard Japanese quail laying diet, diet 2 =standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal(w/w), diet 3 = standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as means± SD. Week 1: n = 6 for diet 1 and 2, Week 2: n = 46 for diet 1, n = 21 for diet 2 and n = 7 for diet 3, Week 3: n = 71 for diet 1, n = 27 for diet 2 and n = 6 for diet 3, Week 4: n = 66 for diet 1, n = 30 for diet 2 and n = 10 for diet 3, Week 5: n = 44 for diet 1, n = 22 for diet 2 and n = 10 for diet 3, Week 6: n = 53 for diet 1, n = 28 for diet 2 and n = 17 for diet 3, Week 7: n = 48 for diet 1, n = 26 for diet 2 and n = 20 for diet 3.

5.7.4 Egg length

Figure 5.3 shows the effect of *H. sabdariffa* calyces meal on the weekly egg length of Japanese quail. At the beginning of the experiment only birds from diet 1 and 2 laid eggs and there were no significant differences noted between the egg lengths ($P > 0.05$). Dietary treatment and time did not contribute to any variation in the length of quail eggs ($P > 0.05$).

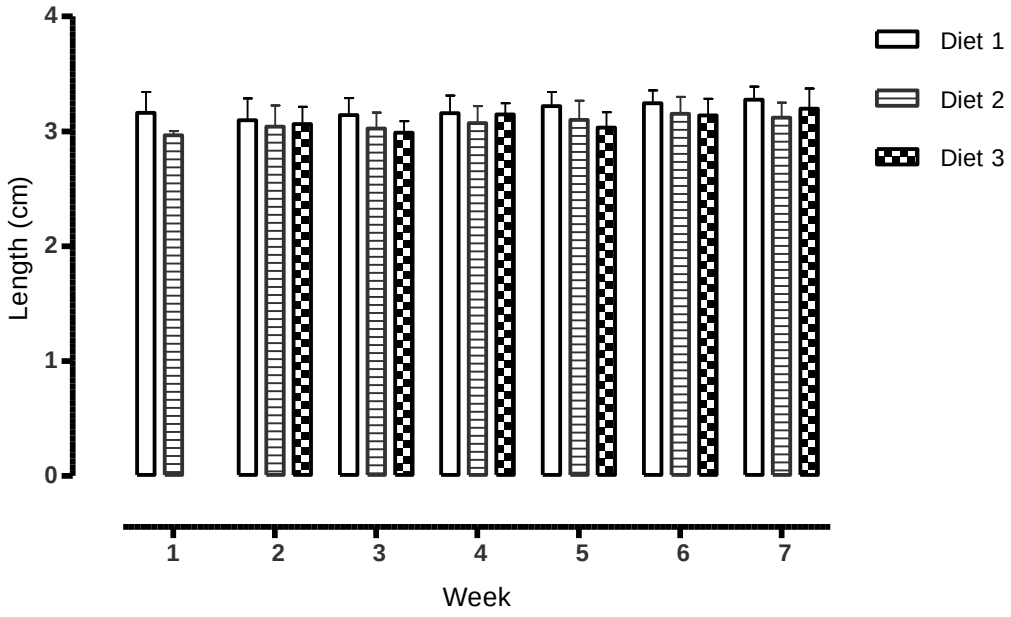

Figure 5.3: Effect of supplemental *H. sabdariffa* calyces on the weekly length of Japanese quail eggs

No significant difference in egg length measured weekly ($P > 0.05$). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal(w/w), diet 3 = standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal(w/w). Data is expressed as means± SD. Week 1: n = 6 for diet 1 and 2, Week 2: n = 46 for diet 1, n = 21 for diet 2 and n = 7 for diet 3, Week 3: n = 71 for diet 1, n = 27 for diet 2 and n = 6 for diet 3, Week 4: n = 66 for diet 1, n = 30 for diet 2 and n = 10 for diet 3, Week 5: n = 44 for diet 1, n = 22 for diet 2 and n = 10 for diet 3, Week 6: n = 53 for diet 1, n = 28 for diet 2 and n = 17 for diet 3, Week 7: n = 48 for diet 1, n = 26 for diet 2 and n = 20 for diet 3.

5.7.5 Egg width

Figure 5.4 shows the effect of dietary *H. sabdariffa* calyces meal on weekly mean egg width of eggs produced by Japanese quail. No differences were noted in the width of eggs across dietary treatments (P > 0.05). Dietary treatment and time did not cause any variation in the egg widths (P > 0.05).

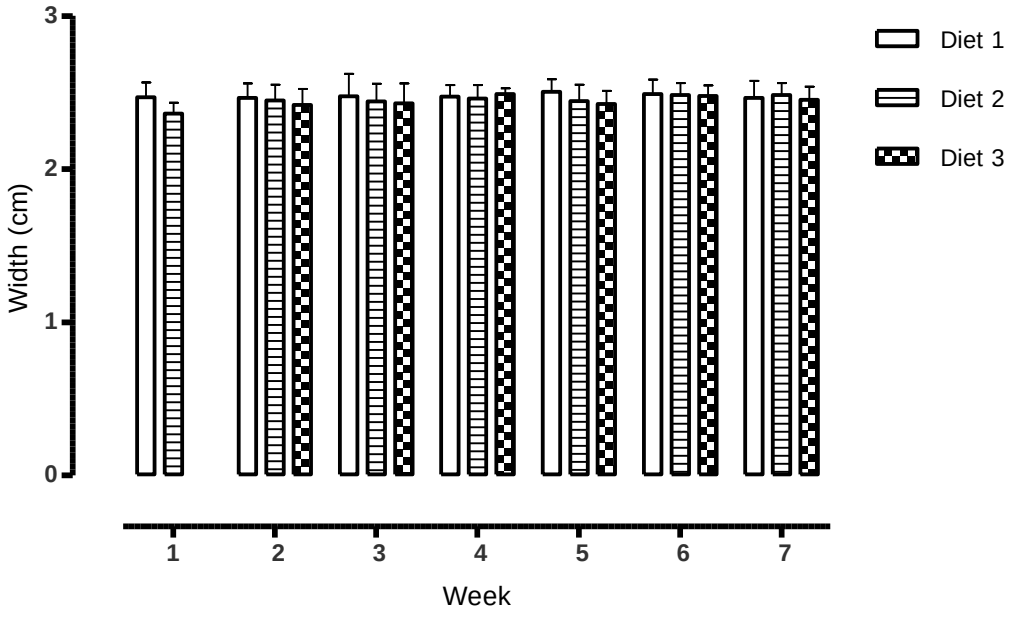

Figure 5.4: Effect of supplemental *H. sabdariffa* calyces meal on the weekly width of Japanese quail eggs

No significant differences in the width of eggs across all dietary treatments (P > 0.05). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as means± SD. Week 1: n = 6 for diet 1 and 2, Week 2: n = 46 for diet 1, n = 21 for diet 2 and n = 7 for diet 3, Week 3: n = 71 for diet 1, n = 27 for diet 2 and n = 6 for diet 3, Week 4: n = 66 for diet 1, n = 30 for diet 2 and n = 10 for diet 3, Week 5: n = 44 for diet 1, n = 22 for diet 2 and n = 10 for diet 3, Week 6: n = 53 for diet 1, n = 28 for diet 2 and n = 17 for diet 3, Week 7: n = 48 for diet 1, n = 26 for diet 2 and n = 20 for diet 3.

5.7.6 Egg shell mass

Figure 5.5 shows the effect of *H. sabdariffa* calyces meal on the mean weekly mass of egg shells produced by Japanese quail. There were no significant differences in the shell masses of eggs produced by birds across the dietary treatments (P > 0.05). Dietary treatment and time did not influence results of the shell mass (P > 0.05).

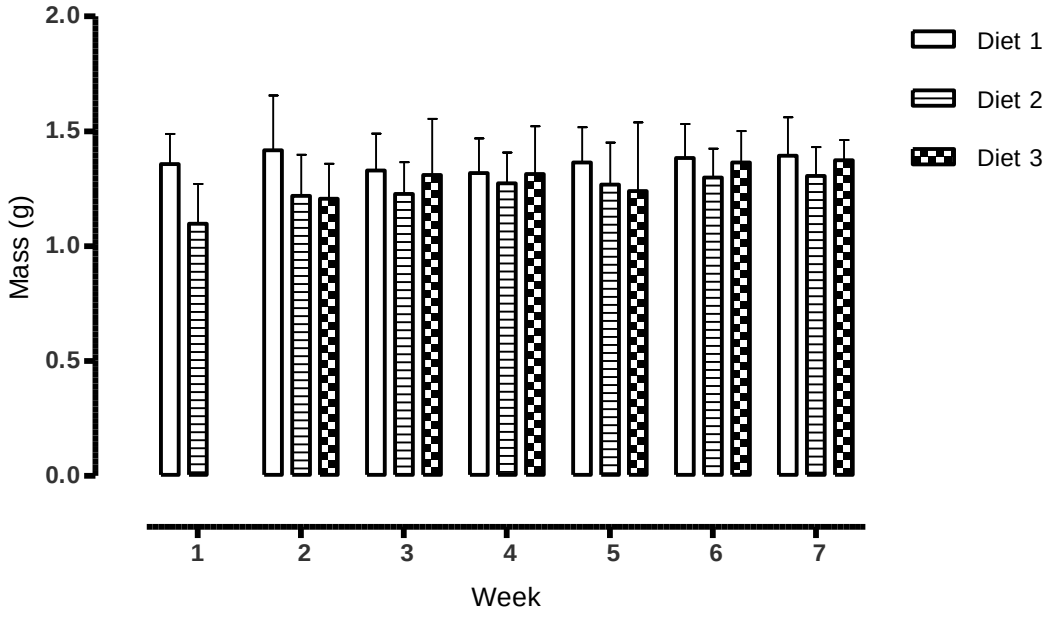

Figure 5.5: Effect of supplemental *H. sabdariffa* calyces meal on the weekly mass of the Japanese quail egg shell

There were no significant differences in the shell masses of eggs produced by birds across the dietary treatments (P > 0.05). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as means± SD. Week 1: n = 6 for diet 1 and 2, Week 2: n = 46 for diet 1, n = 21 for diet 2 and n = 7 for diet 3, Week 3: n = 71 for diet 1, n = 27 for diet 2 and n = 6 for diet 3, Week 4: n = 66 for diet 1, n = 30 for diet 2 and n = 10 for diet 3, Week 5: n = 44 for diet 1, n = 22 for diet 2 and n = 10 for diet 3, Week 6: n = 53 for diet 1, n = 28 for diet 2 and n = 17 for diet 3, Week 7: n = 48 for diet 1, n = 26 for diet 2 and n = 20 for diet 3.

5.7.7 Egg shell thickness

Figure 5.6 shows the effect of *H. sabdariffa* calyces meal on the mean weekly mass of egg shells of Japanese quail. There were no significant differences in the shell thickness of eggs from quail across dietary treatments throughout the entire experimental period ($P > 0.05$). Dietary treatment and time did not contribute any variations to the mass of egg shells ($P > 0.05$).

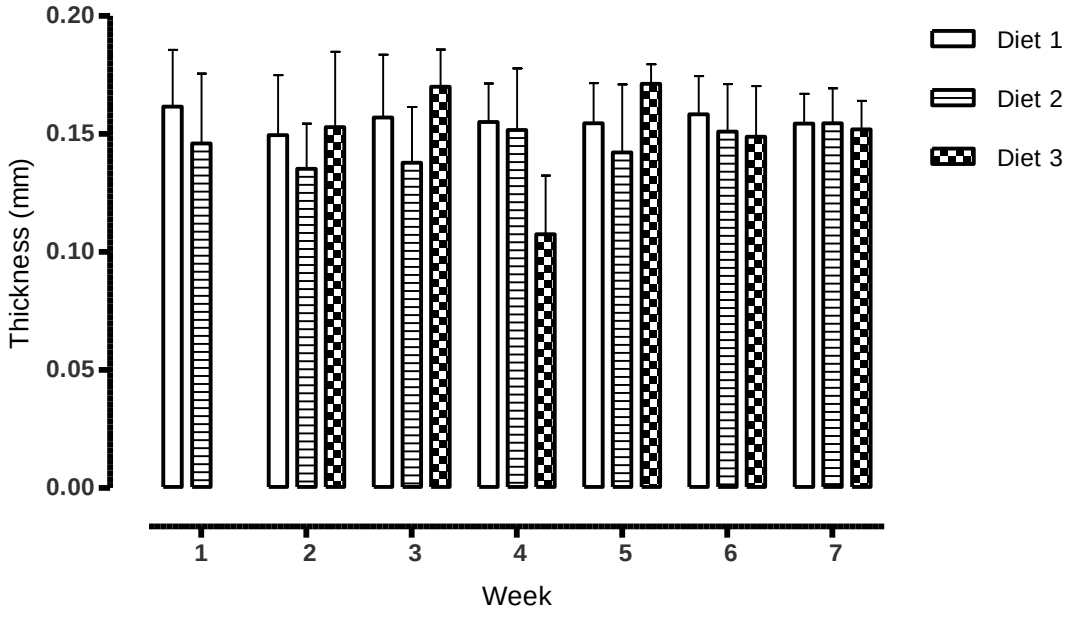

Figure 5.6: Effect of supplemental *H. sabdariffa* calyces on the weekly shell thickness of Japanese quail eggs

There were no significant differences in the shell thickness of eggs from quail across all dietary treatments throughout the entire experimental period ($P > 0.05$). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as means± SD. Week 1: n = 6 for diet 1 and 2, Week 2: n = 46 for diet 1, n = 21 for diet 2 and n = 7 for diet 3, Week 3: n = 71 for diet 1, n = 27 for diet 2 and n = 6 for diet 3, Week 4: n = 66 for diet 1, n = 30 for diet 2 and n = 10 for diet 3, Week 5: n = 44 for diet 1, n = 22 for diet 2 and n = 10 for diet 3, Week 6: n = 53 for diet 1, n = 28 for diet 2 and n = 17 for diet 3, Week 7: n = 48 for diet 1, n = 26 for diet 2 and n = 20 for diet 3.

5.7.8 Egg yolk mass

Figure 5.7 shows the effect of *H. sabdariffa* calyces meal on the weekly mass of egg yolk of Japanese quail. There were no significant differences in the weekly mass of the egg yolk of eggs from quail across all dietary treatments throughout the entire experimental period (P > 0.05).

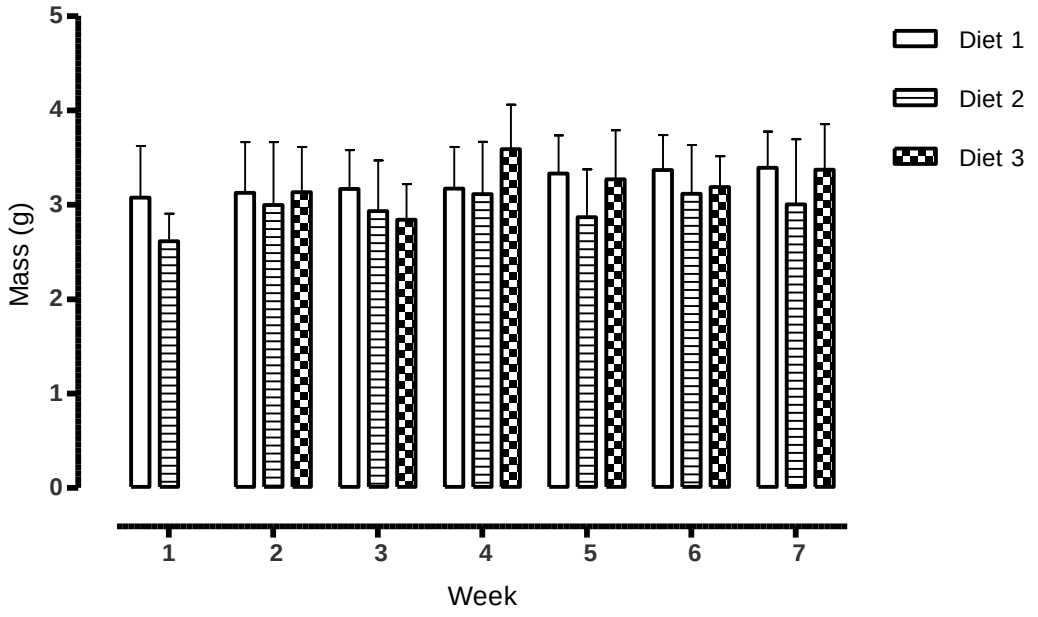

Figure 5.7: Effect of supplemental *H. sabdariffa* calyces meal on the weekly mass of the Japanese quail egg yolk

No significant differences in the weekly mass of the egg yolk of eggs from quail across dietary treatments throughout the entire experimental period (P > 0.05). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as means± SD. Week 1: n = 6 for diet 1 and 2, Week 2: n = 46 for diet 1, n = 21 for diet 2 and n = 7 for diet 3, Week 3: n = 71 for diet 1, n = 27 for diet 2 and n = 6 for diet 3, Week 4: n = 66 for diet 1, n = 30 for diet 2 and n = 10 for diet 3, Week 5: n = 44 for diet 1, n = 22 for diet 2 and n = 10 for diet 3, Week 6: n = 53 for diet 1, n = 28 for diet 2 and n = 17 for diet 3, Week 7: n = 48 for diet 1, n = 26 for diet 2 and n = 20 for diet 3.

5.7.9 Albumen mass

Figure 5.8 shows the effect of *H. sabdariffa* calyces meal on the weekly mass of egg albumen of Japanese quail. There were no significant differences in the weekly mass of the albumen of eggs from quail across dietary treatments throughout the entire experimental period (P > 0.05).

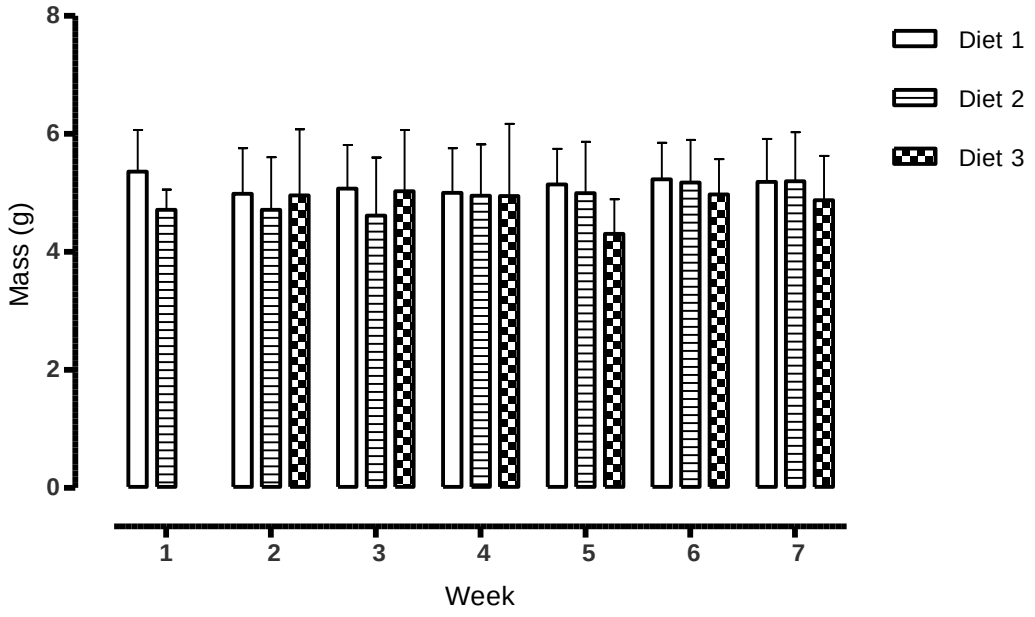

Figure 5.8: Effect of supplemental *H. sabdariffa* calyces on the weekly mass of the Japanese quail egg albumen

There were no significant differences in the weekly mass of the albumen of eggs from quail across dietary treatments throughout the entire experimental period (P > 0.05). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as means± SD. Week 1: n = 6 for diet 1 and 2, Week 2: n = 46 for diet 1, n = 21 for diet 2 and n = 7 for diet 3, Week 3: n = 71 for diet 1, n = 27 for diet 2 and n = 6 for diet 3, Week 4: n = 66 for diet 1, n = 30 for diet 2 and n = 10 for diet 3, Week 5: n = 44 for diet 1, n = 22 for diet 2 and n = 10 for diet 3, Week 6: n = 53 for diet 1, n = 28 for diet 2 and n = 17 for diet 3, Week 7: n = 48 for diet 1, n = 26 for diet 2 and n = 20 for diet 3.

5.7.10 Yolk height

Figure 5.9 shows the effect of *H. sabdariffa* calyces meal on the weekly height of yolk of Japanese quail eggs. There were no significant differences in the weekly height of yolk of eggs from quail across dietary treatments throughout the entire experimental period (P > 0.05).

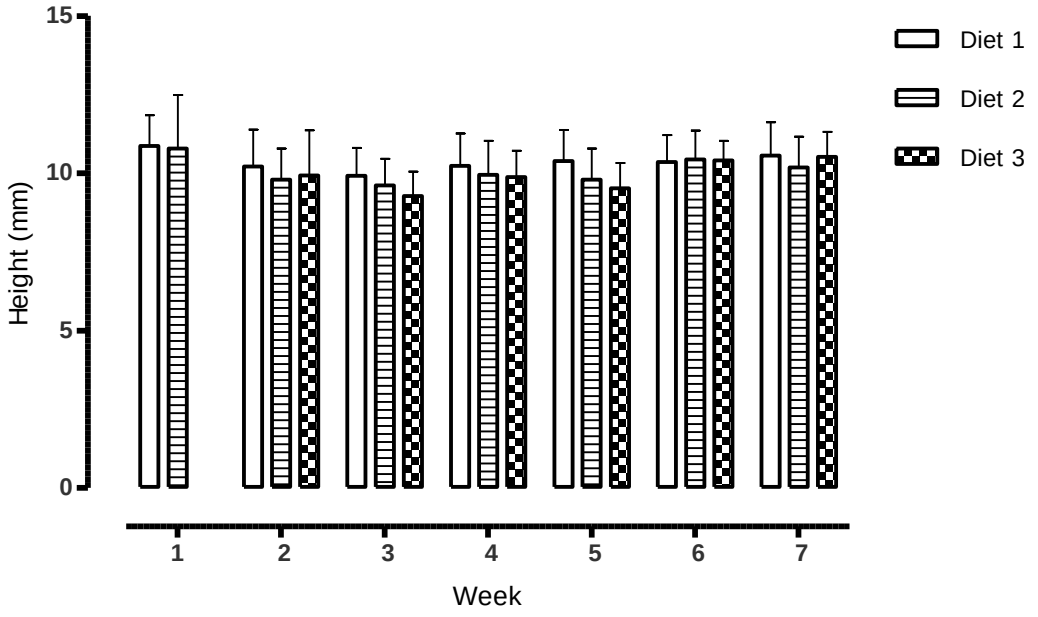

Figure 5.9: Effect of supplemental *H. sabdariffa* calyces on the weekly height of yolk in quail eggs

There were no significant differences in the weekly height of yolk of eggs from quail across dietary treatments throughout the entire experimental period (P > 0.05). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as means± SD. Week 1: n = 6 for diet 1 and 2, Week 2: n = 46 for diet 1, n = 21 for diet 2 and n = 7 for diet 3, Week 3: n = 71 for diet 1, n = 27 for diet 2 and n = 6 for diet 3, Week 4: n = 66 for diet 1, n = 30 for diet 2 and n = 10 for diet 3, Week 5: n = 44 for diet 1, n = 22 for diet 2 and n = 10 for diet 3, Week 6: n = 53 for diet 1, n = 28 for diet 2 and n = 17 for diet 3, Week 7: n = 48 for diet 1, n = 26 for diet 2 and n = 20 for diet 3.

5.7.11 Albumen height

Figure 5.10 shows the effect of *H. sabdariffa* calyces meal on the mean weekly height of albumen of Japanese quail eggs. There were no significant differences in the weekly height of albumen of eggs from quail across dietary treatments throughout the entire experimental period ($P > 0.05$).

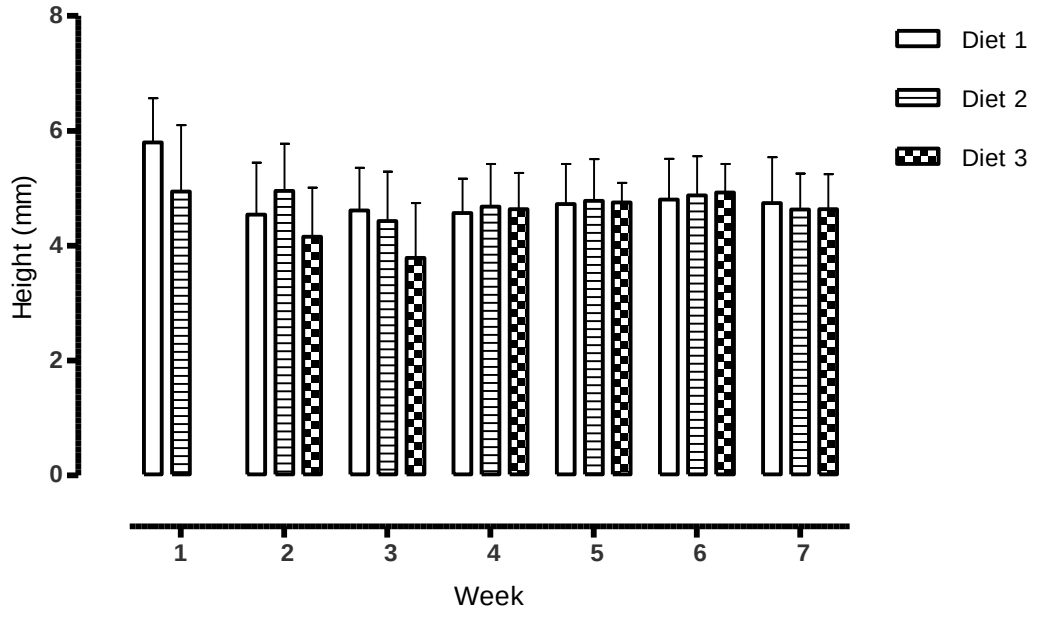

Figure 5.10: Effect of supplemental *H. sabdariffa* calyces on the weekly height of quail egg albumen

There were no significant differences in the weekly height of albumen of eggs from quail across dietary treatments throughout the entire experimental period ($P > 0.05$). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as means± SD. Week 1: n = 6 for diet 1 and 2, Week 2: n = 46 for diet 1, n = 21 for diet 2 and n = 7 for diet 3, Week 3: n = 71 for diet 1, n = 27 for diet 2 and n = 6 for diet 3, Week 4: n = 66 for diet 1, n = 30 for diet 2 and n = 10 for diet 3, Week 5: n = 44 for diet 1, n = 22 for diet 2 and n = 10 for diet 3, Week 6: n = 53 for diet 1, n = 28 for diet 2 and n = 17 for diet 3, Week 7: n = 48 for diet 1, n = 26 for diet 2 and n = 20 for diet 3.

5.7.12 Yolk diameter

Figure 5.11 shows the effect of *H. sabdariffa* calyces meal on the weekly diameter of yolk of Japanese quail eggs. There were no significant differences in the weekly diameter of yolk of eggs from quail across dietary treatments throughout the entire experimental period (P > 0.05).

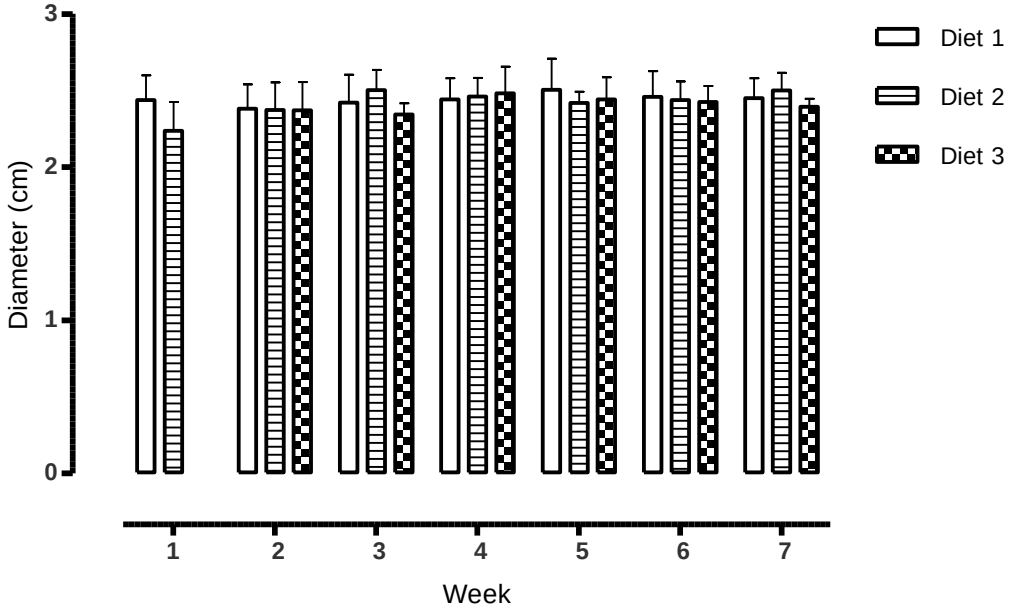

Figure 5.11: Effect of supplemental *H. sabdariffa* calyces on the weekly yolk diameter of quail eggs

No significant differences in the weekly diameter of yolk of eggs from quail across dietary treatments throughout the entire experimental period (P > 0.05). Data is expressed as means± SD. Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Week 1: n = 6 for diet 1 and 2, Week 2: n = 46 for diet 1, n = 21 for diet 2 and n = 7 for diet 3, Week 3: n = 71 for diet 1, n = 27 for diet 2 and n = 6 for diet 3, Week 4: n = 66 for diet 1, n = 30 for diet 2 and n = 10 for diet 3, Week 5: n = 44 for diet 1, n = 22 for diet 2 and n = 10 for diet 3, Week 6: n = 53 for diet 1, n = 28 for diet 2 and n = 17 for diet 3, Week 7: n = 48 for diet 1, n = 26 for diet 2 and n = 20 for diet 3.

5.7.13 Albumen length

Figure 5.12 shows the effect of *H. sabdariffa* calyces meal on weekly length of albumen of Japanese quail eggs. There were no significant differences in the weekly length of albumen of eggs from quail across dietary treatments throughout the entire experimental period (P > 0.05).

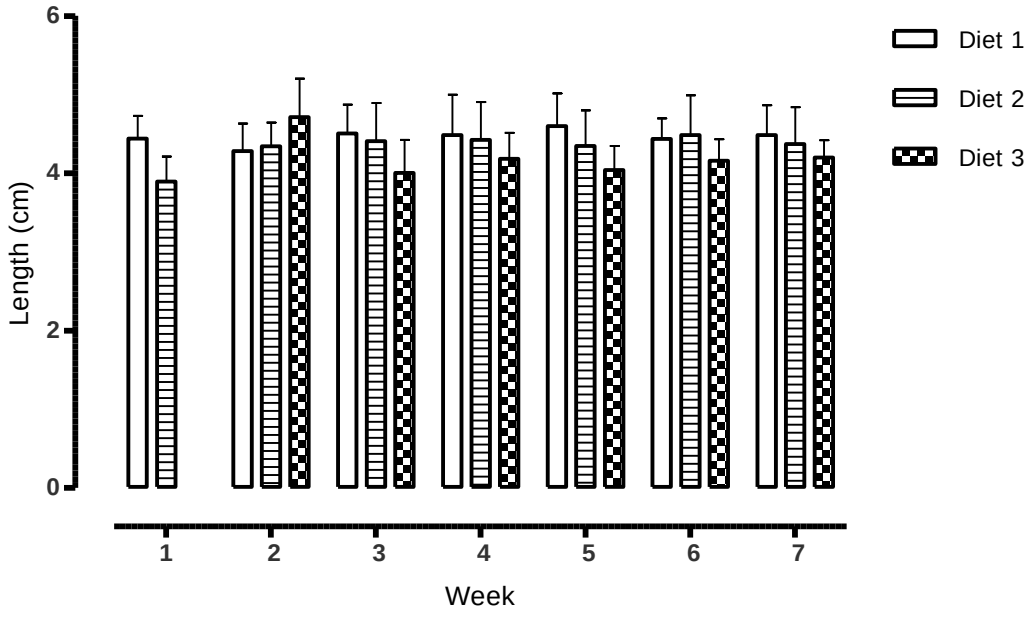

Figure 5.12: Effect of supplemental *H. sabdariffa* calyces on the weekly albumen length of quail eggs

There were no significant differences in the weekly length of albumen of eggs from quail across dietary treatments throughout the entire experimental period (P > 0.05). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as means± SD. Week 1: n = 6 for diet 1 and 2, Week 2: n = 46 for diet 1, n = 21 for diet 2 and n = 7 for diet 3, Week 3: n = 71 for diet 1, n = 27 for diet 2 and n = 6 for diet 3, Week 4: n = 66 for diet 1, n = 30 for diet 2 and n = 10 for diet 3, Week 5: n = 44 for diet 1, n = 22 for diet 2 and n = 10 for diet 3, Week 6: n = 53 for diet 1, n = 28 for diet 2 and n = 17 for diet 3, Week 7: n = 48 for diet 1, n = 26 for diet 2 and n = 20 for diet 3.

5.7.14 Albumen width

Figure 5.13 shows the effect of *H. sabdariffa* calyces meal on the weekly width of albumen of Japanese quail eggs. There were no significant differences in the weekly width of albumen of eggs from quail across dietary treatments throughout the entire experimental period (P > 0.05).

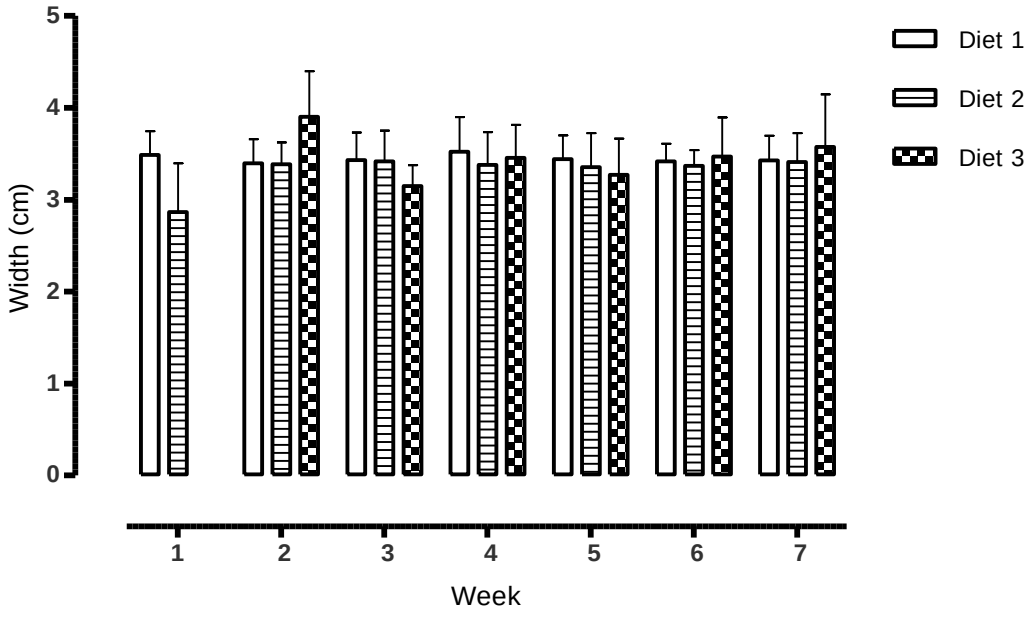

Figure 5.13: Effect of supplemental *H. sabdariffa* calyces on the width of the albumen of quail eggs

There were no significant differences in the weekly width of albumen of eggs from quail across dietary treatments throughout the entire experimental period (P > 0.05). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as means± SD. Week 1: n = 6 for diet 1 and 2, Week 2: n = 46 for diet 1, n = 21 for diet 2 and n = 7 for diet 3, Week 3: n = 71 for diet 1, n = 27 for diet 2 and n = 6 for diet 3, Week 4: n = 66 for diet 1, n = 30 for diet 2 and n = 10 for diet 3, Week 5: n = 44 for diet 1, n = 22 for diet 2 and n = 10 for diet 3, Week 6: n = 53 for diet 1, n = 28 for diet 2 and n = 17 for diet 3, Week 7: n = 48 for diet 1, n = 26 for diet 2 and n = 20 for diet 3.

5.7.15 External egg quality: overall outcomes

Table 5.3 shows the effect of *H. sabdariffa* calyces meal on the overall outcomes of external egg parameters for the Japanese quail. Egg mass and shell ratio remained similar for birds across the dietary treatments ($P > 0.05$). Egg length was significantly reduced ($P < 0.0001$) by supplemental *H. sabdariffa* calyces meal. Supplemental *H. sabdariffa* calyces meal at 10% significantly reduced ($P = 0.0139$) the width of eggs. Supplementing standard Japanese quail laying diet with *H. sabdariffa* calyces meal at 5% significantly increased ($P < 0.0001$) the shape index of quail eggs, while at 10% there were no alterations to the shape index. The shells of eggs produced by birds fed diet 2 (were significantly thinner ($P = 0.0233$) compared to those of birds fed the control diet. Supplemental *H. sabdariffa* calyces meal significantly reduced the shell mass of quail eggs ($P< 0.0001$).

Table 5.3: Effect of supplemental *H. sabdariffa* calyces meal on overall external egg parameters of Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	P-value
Egg mass (g)	10.47±1.18	10.03±1.23	10.17±1.17	0.8923
Egg length (cm)	3.19±0.17[a]	3.08±0.15[b]	3.12±0.16[b]	< 0.0001
Egg width (cm)	2.48±0.11[a]	2.46±0.09[a,b]	2.45±0.09[b]	0.0139
Shape Index (%)	77.88±4.22[b]	79.84±2.42[a]	78.64±3.52[a,b]	< 0.0001
Shell thickness (mm)	0.16±0.02[a]	0.15±0.04[b]	0.15±0.03[a,b]	0.0233
Shell mass (g)	1.36±0.18[a]	1.26±0.15[b]	1.1±0.19[b]	< 0.0001
Shell ratio (%)	13.33±5.76	12.64±1.65	12.91±1.61	0.1856

[a, b]Within row means with different superscripts differ significantly ($P < 0.05$). There were no significant differences in the egg mass and shell ratio of quail eggs across dietary treatments ($P > 0.05$). Egg length and width were reduced by supplemental *H. sabdariffa* calyces meal ($P < 0.05$). 5% *H. sabdariffa* calyces meal significantly increased shape index ($P < 0.0001$). Supplemental *H. sabdariffa* calyces meal significantly reduced shell mass of quail eggs ($P < 0.0001$). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as means± SD. n=338 for diet 1, n=165 for diet 2 and n=75 for diet 3.

5.7.16 Internal egg quality: overall outcomes

Table 5.4 shows the effect of *H. sabdariffa* calyces meal on the internal egg quality parameters of Japanese quail. There were no significant differences in the yolk diameter, albumen height, width and ratio of eggs across all dietary treatments (P > 0.05). Eggs from Japanese quail hens fed diet 2 had the lowest yolk mass and index. Eggs from Japanese quail fed diet 3 had a higher yolk ratio compared to those of Japanese quail fed diet 2. Japanese quail fed diet 3 produced eggs with the highest yolk: albumen ratio (P = 0.0022) and the lowest albumen length (P <0.0001).

Table 5.4: Effect of supplemental *H. sabdariffa* calyces meal on the overall internal egg quality parameters of Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	P-value
Yolk mass (g)	3.25±0.44[a]	3.00±0.57[b]	3.28±0.46[a]	< 0.0001
Yolk height (mm)	10.27±1.01[a]	10.01±1.03[b]	10.12±0.92[a,b]	0.0285
Yolk diameter (cm)	2.44±0.17	2.44±0.14	2.41±0.12	0.2161
Yolk index (%)	42.26±5.07	41.14±5.17	42.12±4.37	0.0599
Yolk ratio (%)	31.10±3.70[a,b]	30.11±5.63[b]	32.46±5.27[a]	0.0008
Yolk: Albumen ratio (%)	65.01±0.14[b]	62.09±0.17[b]	71.01±0.31[a]	0.0022
Albumen height (mm)	4.67±0.76	4.72±0.77	4.57±0.71	0.3637
Albumen width (cm)	3.44±0.29	3.38±0.34	3.49±0.51	0.0379
Albumen length (cm)	4.47±0.41[a]	4.40±0.50[a]	4.20±0.36[b]	< 0.0001
Albumen average (cm)	3.95±0.31	3.89±0.38	3.85±0.39	0.0258
Albumen ratio (%)	48.76±5.26	49.36±7.15	47.76±6.79	0.1622

[a, b,] Within row means with different superscripts differ significantly (P < 0.05). There were no significant differences in the yolk diameter, albumen height, width and ratio of eggs across all dietary treatments (P > 0.05). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as means± SD. n = 338 for diet 1, n =165 for diet 2 and n=75 for diet 3.

5.7.17 Egg yolk and albumen proximate content

Table 5.5 shows the effect of *H. sabdariffa* calyces meal on the proximate content of egg yolk and albumen of Japanese quail. The dry matter and protein content of the yolk and albumen of

eggs produced by birds fed on diet 3 were higher P < 0.05) than those of eggs produced by birds from the control diet. Supplemental *H. sabdariffa* calyces meal significantly reduced the ash content of yolk and albumen of eggs (P < 0.0001). The yolk and albumen of eggs from birds fed on diet 3 had a significantly lower fat content compared to that of eggs from birds fed diets 1 and 2 (P < 0.0001).

Table 5.5: Effect of supplemental *H. sabdariffa* calyces meal on the egg yolk and albumen proximate composition

Parameter	Diet 1	Diet 2	Diet 3	P-value
Dry matter (%)	27.35±0.01^{c}	27.52±0.01^{b}	26.13±0.01^{a}	< 0.0001
Ash (% DM)	4.08±0.03^{a}	3.96±0.10^{b}	3.77±0.03^{c}	< 0.0001
Protein (% DM)	47.28±0.39^{b}	46.94±0.28^{c}	47.67±0.27^{a}	< 0.0001
Ether extract (% DM)	38.10±0.23^{b}	38.27±0.06^{a}	36.39±0.09^{c}	< 0.0001

$^{a, b,c}$ Within row means with different superscripts differ significantly (P < 0.05). Diet 1 = standard Japanese quail laying diet, diet 2 = 95% standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal, diet 3= 90% standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal. Data is expressed as means± SD. For all treatment groups, composite samples were assayed in duplicate, n =2.

5.7.18 Egg yolk fat and fatty acid content

Table 5.6 shows the effect of *H. sabdariffa* calyces meal on the yolk fat content and fatty acid profile of eggs produced by Japanese quail. Although there was a statistically significant increase in the fat content of eggs yolks from birds fed the *H. sabdariffa* calyces meal diet (P < 0.0001), the difference was less than 1%. There was significant increase in the total saturated fatty acid content in the yolks of eggs produced by birds fed on diet 3 compared to the other dietary groups (P < 0.0001). The total monounsaturated fatty acids were significantly higher in the yolk of eggs produced by birds fed on the control diet (P < 0.0001) compared to those fed *H. sabdariffa* calyces meal. The yolks of eggs from birds fed diet 2 had a significantly higher total polyunsaturated fatty acid content (P < 0.0001). Supplemental *H. sabdariffa* calyces meal significantly reduced the omega 3 fats in the yolk of quail eggs (P <

0.0001) produced by birds fed the control diet. Egg yolk of birds fed diet 2 had the highest omega 6 content.

Table 5.6: Effect of supplemental *H. sabdariffa* calyces meal on the egg yolk fat and fatty acid content

Parameter	Diet 1	Diet 2	Diet 3	P-value
Fat (% DM)	51.59±0.05^{b}	51.75±0.11^{a}	51.72±0.01^{a}	< 0.0001
Saturated				
Palmitic acid (C16:0)	26.94±0.74^{b}	26.18±0.25^{c}	27.55±0.40^{a}	< 0.0001
Stearic acid (C18:0)	7.65±0.09^{b}	7.56±0.04^{c}	8.00±0.19^{a}	< 0.0001
Tricosylic acid (C23:0)	0.06±0.00^{b}	0.10±0.02^{a,b}	0.10±0.01a	< 0.0001
TSFAs	**34.87±0.63^{b}**	**34.00±0.25^{c}**	**36.44±0.53^{a}**	< 0.0001
Mono-unsaturated				
Palmitoleic acid (C16:1)	5.27±0.16^{b}	5.04±0.04^{c}	5.47±0.08^{a}	< 0.0001
Oleic acid (C18:1n9c)	46.67±0.75^{a}	46.33±0.19^{a}	45.63±0.52^{b}	< 0.0001
Nervonic acid (C24:1)	0.14±0.08^{a}	0.33±0.01^{a}	0.35±0.01^{a}	< 0.0001
TMUFAs	**52.45±0.57^{a}**	**51.75±0.18^{b}**	**51.55±0.40^{b}**	**< 0.0001**
Poly-unsaturated				
Linoleic acid (C18:2n6c)	8.63±0.05^{b}	9.00±0.04^{a}	8.33±0.03^{c}	< 0.0001
Eicosadienoic acid (C20:2)	0.11±0.00^{a}	0.14±0.01^{a}	0.14±0.00^{a}	< 0.0001
Arachidonic acid (C20:4n6)	0.89±0.01^{c}	0.99±0.03^{b}	1.16±0.01^{a}	< 0.0001
TPUFAs	**10.17±0.01^{b}**	**10.68±0.08^{a}**	**10.02±0.21^{c}**	**< 0.0001**
Trans	0.14±0.07^{b}	0.31±0.01^{a}	0.15±0.08^{b}	< 0.0001
Cis	55.30±0.80^{a}	55.34±0.23^{a}	53.96±0.56^{b}	< 0.0001
Omega 3	0.52±0.22^{a}	0.48±0.01^{b}	0.29±0.02^{c}	< 0.0001
Omega 6	9.63±0.02^{b}	10.16±0.08^{a}	9.55±0.08^{b}	< 0.0001
EPA	0.08±0.03^{b}	0.08±0.01^{b}	0.10±0.01^{a}	0.0146
Omega 9	46.75±0.80^{a}	46.53±0.19^{a}	45.75±0.15^{b}	< 0.0001

$^{a, b,c}$Within row means with different superscripts differ significantly (P < 0.05). Diet 1 = standard Japanese quail laying diet, diet 2 = 95% standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal, diet 3= 90% standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal. Data is expressed as means± SD. For all treatment groups, composite samples were assayed in duplicate, n =2.

5.7.19 Meat yield and pH

Table 5.7 shows the effect of *H. sabdariffa* calyces meal on carcass mass, dressing percent and pH of breast and thigh meat of layer Japanese quail. Supplemental *H. sabdariffa* calyces meal had no effect on the carcass mass, dressing percentage, pH_i, pH_u and pH decrease in the breast and thigh meat of Japanese quail across dietary treatments (P > 0.05).

Table 5.7: Effect of supplemental *H. sabdariffa* calyces meal on yield and pH of layer Japanese quail meat

Parameter	Diet 1	Diet 2	Diet 3	P-value
Carcass mass, g	136.19±16.28	127.43±13.71	142.13±20.66	0.0624
Dressing Percentage, %	65.52±3.79	63.29±3.79	67.01±5.28	0.1094
Breast:				
pH_i	6.14±0.22	6.20±0.19	6.03±0.23	0.1006
pH_u	5.91±0.24	5.97±0.16	5.97±0.19	0.5629
pH decrease	0.22±0.24	0.23±0.19	0.07±0.33	0.1407
Thigh:				
pH_i	6.57±0.21	6.62±0.18	6.56±0.24	0.7058
pH_u	6.64±0.34	6.68±0.19	6.65±0.25	0.9013
pH decrease	-0.07±0.22	-0.06±0.15	-0.10±0.24	0.8567

Supplemental *H. sabdariffa* calyces meal did not significantly affect the carcass mass, dressing percentage, pH_i, pH_u and pH decrease in the breast and thigh muscles of quail across dietary treatments ($P > 0.05$). Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal(w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as mean±SD. n= 30 for diet 1, n=29 for diet 2 and diet 3.

5.7.20 Meat colour

Table 5.8 shows the effect of *H. sabdariffa* calyces meal on the L*, a* and b* colour parameters (30 mins and 24 hours post-slaughter) of breast and thigh meat of layer Japanese quail. Supplemental *H. sabdariffa* calyces meal did not affect the L*, a* and b* colour parameters of the breast and thigh meat of quail across dietary treatments 30 mins and 24 hours post slaughter ($P > 0.05$ There was a significant increase in the a* of thigh meat of quail fed on diets 2 and 3, 24 hours post slaughter ($P = 0.0032$).

Table 5.8: Effect of supplemental *H. sabdariffa* calyces meal on the colour of breast and thigh meat of Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	P-Value
Breast:				
30 mins				
L*	43.49±2.47	44.43±1.96	42.78±2.45	0.1686
a*	3.92±1.92	3.17±1.38	4.24±2.63	0.3475
b*	10.21±2.01	10.94±2.60	9.79±1.90	0.3411
24 hrs				
L*	47.55±2.94	47.14±3.08	47.43±2.91	0.9460
a*	3.14±2.52	3.06±2.95	4.21±3.18	0.3834
b*	12.99±2.79	13.53±2.53	12.97±2.31	0.7928
Thigh:				
30 mins				
L*	48.63±3.58	47.67±3.29	47.82±3.84	0.6561
a*	0.50±1.92	0.24±1.63	1.06±1.75	0.4387
b*	8.74±2.11	7.94±2.89	7.84±3.03	0.4748
24 hrs				
L*	45.19±3.91	44.43±2.96	45.49±1.99	0.6325
a*	0.07±1.26[b]	1.74±1.95[a]	1.33±1.59[a]	0.0032
b*	6.88±2.91	7.93±2.37	7.48±3.34	0.5401

[a, b]Within row means with different superscripts differ significantly ($P < 0.05$. Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as mean±SD. n= 30 for diet 1, n=29 for diet 2 and diet 3.

5.7.21 Meat's water holding capacity and tensile strength

Table 5.9 shows the effect of *H. sabdariffa* calyces meal on the water holding capacity and tensile strength (24 hours post-slaughter) of breast and thigh meat of Japanese quail. There were no significant differences in the water holding capacity and tensile strength of the breast and thigh meat of Japanese quail across dietary treatments ($P > 0.05$).

Table 5.9: Effect of supplemental *H. sabdariffa* calyces meal on the water holding capacity and tensile strength of the breast and thigh meat of layer Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	P- value
Breast:				
Water holding capacity (%)	84.44±3.08	84.52±3.15	81.33±31.17	0.0825
Tensile strength (kg force)	0.04±0.03	0.06±0.07	0.11±0.11	0.1469
Thigh:				
Water holding capacity (%)	89.74±3.74	90.56±2.68	90.72±2.11	0.8105
Tensile strength (kg force)	0.09±0.12	0.10±0.12	0.09±0.10	0.9370

Supplemental *H. sabdariffa* calyces meal did not significantly affect (P >0.05) the water holding capacity of breast and thigh meat of Japanese quail. Diet 1 = standard Japanese quail laying diet, diet 2 = standard Japanese quail laying diet + 5% *H. sabdariffa* calyces meal (w/w), diet 3= standard Japanese quail laying diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as mean±SD. n= 30 for diet 1, n=29 for diet 2 and diet 3.

5.8 Discussion

5.8.1 Egg production and quality

Polyphenol-rich plants, including *H. sabdariffa* calyces meal improve gut health and nutrient absorption of poultry and have been reported to improve egg production and quality of Japanese quail (Sukkharaintet al., 2011, Lin et al., 2014, Mohammed et al., 2012). In the current study, dietary supplementation with *H. sabdariffa* calyces meal at 10% inclusion level delayed the onset of laying by 8 days and reduced egg production (Figure 5.3, Table 5.2). Many fibrous ingredients incorporated in poultry feed to reduce production costs have low digestibility and cause poor growth and reduced egg production in poultry (Singh et al., 2021) hence the reduced egg production observed in Japanese quail fed diet 3. A delay in onset of laying and a reduced egg production in commercial egg production results in losses to the farmer. The use of feed additives of plant origion is limited as a result of the presence of anti-nutritional factors (ANFs) (Makkar et al., 1997). When the dose of these plant based feed additives is increased, the anti-nutritional factors block the absorption of minerals in the gut thereby hindering normal metabolism and affecting egg production, shell thickness and overall egg quality (El-Sheikh et al., 2015). Previous studies on *H. sabdariffa* calyces meal showed the presence of tannins, phytate (El-Adawy and Khalil, 1994 and Yagoub, 1998), gossypol (Al-Wandawi et al., 1984 and Bakheit, 1989) and protease inhibitors (Abu-Tarboush and Ahmed, 1996), antinutritional factors which reduce digestibility of feed, compromise the bioavailability of nutrients and cause flatulence (Abu-Tarboush and Ahmed, 1996). Although I did not measure the ANFs in *H. sabdariffa* calyces meal I speculate that *H. sabdariffa* calyces meal possibly contained ANFs which may have interfered with the bioavailability of certain nutrients as previously reported. Tannic acid has been reported to reduce the metabolizable energy of poultry diets, depress the nitrogen retention by birds and reducing the productive performance by both broilers and laying hens (Tufarelli et al., 2018). It can be inferred that the 10% inclusion level of *H. sabdariffa* in my study decreased egg production possibly due to the metabolizable energy reducing effect of ANFs such as tannins. This implies that use of *H. sabdariffa* as a supplement in laying Japanese quail may result in losses as a result of reduced egg production performance of Japanese quail.

Poor egg quality leads to downgrading, while enhanced egg quality increases egg value (Adi et al., 2014). Quail hens require nutrition and an optimal digestive system to produce good quality eggs (Guo et al., 2004). Sukkhavanit et al., (2011) reported that *H. sabdariffa* calyces extract did not affect egg weight in laying chickens. My findings are in line with reports made by Sukkhavanit et al., (2011), egg mass and shell ratio of Japanese quail were not affected by supplemental *H. sabdariffa* calyces meal ($P > 0.05$). This implies that inclusion of *H. sabdariffa* calyces meal in Japanese quail layer diets does not influence the size of the eggs produced by Japanese quail with respect to egg mass.

Adequate dietary calcium is critically important for the formation of strong eggshells which are necessary to guard against losses from breakages during handling (Pelicia et al., 2009). The absorption of calcium in the GIT has been shown to be optimised by natural feed supplements (Guo et al., 2004). Japanese quail require at least 2.5-3.0% calcium with 0.25% phosphorus during the early stage of production for optimal egg production and egg shell weight (Amoah et al., 2012). A deficiency of calcium reduces egg production and results in eggs with thin eggshells (Nys et al., 2018). Findings from the current study show that eggs produced by Japanese quail fed diet 2 had a significantly thinner eggshells compared to those of eggs produced by quail fed diet 1 and the supplemental *H. sabdariffa* calyces meal significantly reduced the eggshell mass. The mean eggshell thickness has been reported to range between 0.17 and 0.30 mm for Japanese quail eggs (Turkyilmaz et al., 2005; Erisir et al., 2015). In the current study the Japanese quail eggshell thickness ranged from 0.15±0.03 to 0.16±0.02mm. The Japanese quail eggshell thickness reported in the current study is within previously reported ranges for egg shell thickness. (Turkyilmaz et al., 2005; Erisir et al., 2015). Importantly, the eggshell thickness from quail fed *H. sabdariffa* calyces meal-based diets was lower compared to that of eggshells from Japanese quail hens fed the control diet. Trace elements play a significant role in the formation of egg shells by being part of the membrane and egg shell membrane formation process as key enzymes. Additionally, trace elements contribute to the quality of egg shells by interacting with calcite crystals (Mabe, 2003). Yang, (2012) reported that Mn supplementation (15, 35 or 55 mg/kg) resulted in an increase in eggshell thickness. I observed that supplemental *H. sabdariffa* calyces at 5% and 10% reduced the ash content of the diets from 10.92% (control diet) to

8.31% and 9.35% respectively (Table 5.1). This could possibly account for the thinner shells produced by Japanese quail fed diets 2 and 3. Previous studies have shown that egg thickness particularly thin eggs can reduce hatchability of fertile eggs (Turkyilmaz et al., 2005; Erisir et al., 2015). This implies that supplemental *H. sabdariffa* calyces meal (both 5% and 10%) in the diets of laying Japanese quail may pose the risk of economic losses to the farmer due to high breakages as well as poor hatchability of the eggs.

Despite its known high calcium content, in the current study the *H. sabdariffa* calyces meal seems not have improved the calcium content in the diets or at least in the birds for the formation of eggs with thick shells.

Although at 10% dietary inclusion *H. sabdariffa* calyces meal significantly reduced egg length and width, the mean egg length and width of eggs produced by Japanese quail in diet 3 were within the range of Japanese quail eggs as reported by Abu Tabeekh, (2011). This suggests that dietary *H. sabdariffa* calyces meal did not compromise the length and width of Japanese quail eggs.

Egg shape index is used as a criterion for determining the stiffness of the eggshell (Xiao et al., 2014). Stiffness of the eggshell is an indicator of the eggshell strength (Yan et al., 2014). Japanese quail eggs have been shown to have an egg shape index which ranges from 78.04±0.54 to 79.05±0.59% (Kanagaraju et al., 2013, Zita et al., 2012). Findings from the current study showed an egg shape index ranging from 77.84±4.22% to 79.84±2.42% which is within the normal range which suggests that dietary *H. sabdariffa* calyces meal did not compromise the mechanical propensity for breakage of the Japanese quail eggshells

Albumen and yolk quality are key determinants of egg quality (Kibala et al., 2018). The higher the internal parameters of eggs, the higher the egg quality (Honkatukia et al., 2013). The reported egg yolk mass, yolk index, albumen index and yolk:albumen ratio of Japanese quail eggs are 3.88±0.01g to 4.01±0.03g, 44.46±0.21% to 44.92±0.42%, 9.46±0.12% to 9.68±0.21% and 48.62±0.52% to 50.13±0.31% respectively (Kanagaraju et al., 2013, Zita et al., 2012). The quality of albumen is a measure of its firmness and ability to hold the yolk in the centre position of the egg. The albumen starts to lose its quality soon after the egg is laid

and it loses its thickness during storage (Honkatukia et al., 2013). In the current study, there were no significant differences on the egg albumen width of the Japanese eggs across dietary treatments. Importantly, supplementing the Japanese quail layer diet with 10% *H. sabdariffa* calyces meal increased the yolk mass, yolk ratio and yolk:albumen ratio but decreased the albumen length of Japanese quail eggs. Findings from the current study suggest that at 10% dietary inclusion *H. sabdariffa* calyces meal resulted in the production of eggs with thicker albumen and thus were of better quality compared to those of eggs produced by Japanese quail fed diets 1 and 2. It can therefore be inferred that despite decreasing egg production by the Japanese quail hens, dietary *H. sabdariffa* calyces meal caused the production of eggs with bigger yolks and improved albumen quality. At 10%, dietary inclusion *H. sabdariffa* calyces meal significantly increased the Japanese quail eggs' crude protein content but reduced their ether extract and ash content. The increase in protein content increases the nutritional value of the eggs while the decrease in fat content could mean a reduced risk of developing dietary lipid associated metabolic derangements and diseases (Wolk et al., 2017). Poultry derived products such as eggs and meat and eggs rich in polyunsaturated fatty acids have a short shelf life due to increased lipid peroxidation (Estevez et al., 2015). Findings from the current study show that supplemental *H. sabdariffa* calyces meal significantly decreased the PUFA but increased the fat content of Japanese quail egg yolks (Table 5.6). Since a higher PUFA content results in increased lipid peroxidation and product deterioration (Estevez et al., 2015), it can be inferred that dietary *H. sabdariffa* calyces can be exploited to produce Japanese quail eggs with a longer shelf life but caution has to be exercised as its use can lead to products with higher saturated fatty acid contents which can cause metabolic derangements and diseases.

In addition to producing eggs, at the end of their egg production cycle, layer hens (termed off-layers) are slaughtered for meat. Meat from chicken off-layers is characterised by lower fat, high dry matter and higher protein (Lichovnikova et al., 2009) when compared to that from broiler chicken. Findings from the current study show that while supplemental *H. sabdariffa* calyces meal had no effect on Japanese quail off layers meat's pH, water holding capacity and tensile strength, it significantly increased the meat's (breast and thigh) redness. Dietary interventions have been reported to influence the colour of chicken thigh meat (Wideman et al., 2016). *Hibiscus sabdariffa* calyces meal has been shown to contain antioxidants (Da-

Costa Rocha et al., 2014) that can potentially reduce the generation of ROS from fresh meat in storage and thus allow for longer shelf life. It can therefore be inferred that the observed increase in the redness of the meat from the Japanese quail hen carcasses could have been from the *H. sabdariffa* calyces meal bourne antioxidants that mediated reduction in ROS formation resulting in the preservation of the meat's redness. Poultry meat with darker tinge compared to a lighter colour is associated with toughness (Celen et al., 2016). Consumers have a clear preference for lighter than dark coloured poultry meat (Wideman et al., 2016).

5.9 Conclusion

At 5% dietary inclusion *H. sabdariffa* calyces meal can be used to supplement Japanese quail layer diet without compromising feed intake and egg production. However, at 10% dietary inclusion it resulted in decreased feed intake and egg production despite causing the production of eggs with better quality albumen and larger yolks. Supplemental *H. sabdariffa* calyces meal at both 5% and 10% resulted in thinner egg shells. Caution must be exercised as *H. sabdariffa* calyces meal resulted in the production of eggs with a higher lipid and saturated fatty acid content which can compromise consumer health.

The next chapter gives a narrative of the outcomes of my interrogation of the effects of supplemental *H. sabdariffa* calyces meal on the growth performance, feed utilisation, GIT viscera morphometry and general health of layer Japanese quail hens. Experimental chapter 6 is a continuation of the experimental chapter 5 and thus the same birds were used.

6. CHAPTER SIX- EFFECT OF *HIBISCUS SABDARIFFA* CALYCES MEAL ON GROWTH PERFORMANCE, GIT VISCERA AND HEALTH OF LAYER JAPANESE QUAIL

6.0 Introduction

The egg laying process demands that the body be in a good state of health and nutrition in order to have maximum egg production and good quality eggs produced (Adi et al., 2014). Egg laying depends heavily on the bird's nutrient stores, for example, calcium is drawn from the skeletal system to support the formation of eggshells (Ketta and Tumova, 2016; Whitehead, 2004). If the bird does not have adequate calcium in its skeletal stores, egg laying will lead to weak bones that cannot support the bird's body and thus the bird will easily succumb to fractures (Ketta and Tumova, 2016; Johnson, 2014).

The European Union in 2006 banned the use of synthetic antibiotics and antioxidants in poultry feed following the carry over effects associated with these feed additives such as developing antibiotic resistance (Nisha, 2008; Alloui et al., 2014) and the development of cancer in consumers (Blasczyck et al., 2013). The poultry industry are looking for alternative growth promoters which will not negatively impact poultry product quality and consumer health. Plant products, for example, meals, crude extracts and purified phytochemicals have been reported to exhibit antibiotic, antifungal, antioxidant, immune modulating (Mahfuz et al., 2017) and growth promoting effects (Voemesse et al., 2019; Abouz-Elezz et al., 2011). Considerable research has been undertaken on the potential benefits of using plant-derived products (meals, crude extracts and purified phytochemicals) as supplements in poultry feeds, especially broiler and pullet chicken feeds, targeting to boost growth performance and feed utilisation efficiency as well as to enhance bird health and product quality (Valenzuela-Grijalva et al., 2017; Sunder et al., 2013; Guo et al., 2004). However little if any research has been done to interrogate the potential benefits of the use of plant-derived products as growth and health promoters in pullet quail feeds. I investigated the potential beneficial effects of *H. sabdariffa* calyces meal on the growth performance and health of pullet Japanese quail by specifically determining its effects on body mass and tibiae and femora based growth indices, GIT viscera macromorphometry and general health.

6.1 Specific objectives

The specific objectives of the study were to determine, in pullet Japanese quail, the effects of dietary supplementation with *H. sabdariffa* calyces meal on:

a. growth performance (body weight and long bone indices), feed intake and feed utilisation efficiency, GIT viscera macromorphometry.
b. general health by determining *H. sabdariffa* calyces meal's effects on the
    i. packed cell volume
    ii. serum surrogate markers of kidney (serum uric acid concentration) and surrogate markers of liver (serum ALT and ALP activity, total bilirubin, total protein and albumin concentration) function as well as serum, calcium, phosphorus and malonaldehyde concentration.
    iii. liver lipid content

6.2 Study Hypothesis

H_0: Dietary supplementation with *H. sabdariffa* calyces meal does not affect the growth performance as measured by body mass and long bone based indices, GIT viscera macromorphometry and the health of pullet Japanese quail

H_1: Dietary supplementation with *H. sabdariffa* calyces meal promotes the growth performance as measured by body mass and long bone based indices, GIT viscera macromorphometry and the health of pullet Japanese quail

6.3 Materials and Methods

6.3.1 Feed Ingredients and diet formulation

Hibiscus sabdariffa calyces were sourced and processed as described in chapter 3, under subheading 3.3.1. The ingredients and chemical composition of the diets used in this study are as presented in Table 5.1. Ingredients were sourced as described in Chapter 5, under subheading 5.3.1 and diets formulation was done as described in Chapter 5, under subheading 5.3.1.

6.3.2 Ethical approval and study site

Ethical approval for this study was sought and granted as described in Chapter 3, under subheading 3.3.5 and a details of the study site for this experiment are described in Chapter 3, under subheading 3.3.5.

6.3.3 Quail and quail management

Sourcing of Japanese quail was done as described in Chapter 5, under subheading 5.3.3 and management of quail during the experimental period was carried out as described in Chapter 5, under subheading 5.3.3.

6.3.4 Experimental design

The experiment for this study was designed as described in Chapter 5, under subheading 5.3.4.

6.3.5 Body weight and feed intake measurement

The body weight of each bird was measured as described in Chapter 3, under subheading 3.3.8

Feed intake of each dietary group was determined twice a week and the feed intake of each bird calculated from the group feed intake calculated using the formula:

$$Feed\ intake = \left(feed\ offered(g) - residual\ feed(g)\right) \div number\ of\ quail\ in\ the\ pens$$

The feed conversion ratio (FCR) of each bird was computed using the formula:

$FCR = [Feed\ intake \div BWG\ (g)] \div No.of\ birds\ in\ the\ pen$ (Onu et al., 2004).

6.4 Terminal procedures

Termination of Japanese quail for this study was carried out as described in Chapter 3, under subheading 3.4. At termination, I identified the actively laying and non-laying birds by carefully examining the cloaca and ovaries for evidence of egg formation.

6.4.1 Determination of haematocrit

The haematocrit was determined as described in Chapter 3, under subheading 3.4.2.

6.4.2 Determination of long bone parameters

The long bone parameters were determined as described in Chapter 3, under subheading 3.5.

6.4.3 Determination of general health profile

The general health profile of the quail was determined as described in Chapter 3, under subheading 3.6.

6.4.4 Determination of liver lipid content

The frozen liver samples were thawed at room temperature for 30 minutes. Thereafter a thirty gram (30g) sample from the compounded livers was weighed into a pre-weighed plastic container and then freeze-dried in a preconditioned freeze dryer (Specht Scientific Engineering, Cologne, Germany) which was connected to a vacuum pump (Sogevac SV28, Leybold GmbH, Cologne, Germany) over 24 hours at conditioner temperature of -50ºC and shelf temperature of 26ºC. The freeze-drying was done according to the instructions given by the manufacturer. Each freeze dried liver sample was then ground using a grinder (Retsch ZM200, Retsch-Allee 1-5 42781, Haan, Germany) to pass through a 1mm sieve. A Soxtec System HT 1043 Extraction Unit (Foss Analytical, HillerØd, Denmark)was used to determine the liver lipid content of the sample according to AOAC (2001): Method number 920.39.

6.5 Statistical analysis

Data are expressed as mean ±SD. Graph Pad Prism Version 5 (Graph-pad Software Inc., San Diego, USA) statistical package was used to analyse the data. The General Linear Model (Proc GLM) of the Statistical Analysis System (SAS, 2003) was used to analyse data on weekly body weight using a repeated measures ANOVA. The model used for data analysis was:

$Yijk = \mu + Ti + Wj + TWij + eijk;$

where

Yijk = is the kth observation (body weight) of the ith dietary treatment of the j

th week

μ = is the overall mean

Ti = is the fixed effect of the ith dietary treatment (i = 1, 2…5)

Wj = is the effect of the jth week of measurement (j = 1, 2… 3…..9)

TWij = is the interaction between dietary treatment and week

eijk = is the random residual error

Data on growth performance (body weight based)

A one-way ANOVA was used to analyse data on haematocrit and serum concentration of uric acid, total protein, albumen, triglyceride, cholesterol, bilirubin, malondialdehyde as well as ALP and ALT activity. The Bonferroni *post hoc* test was used to compare treatment means. The level of significance was set at $P < 0.05$. The statistical model used was:

$Y_{ij} = \mu + T_i + e_{ij}$;

where Y_{ij} = dependent variable of interest (growth performance indices (body weight, body weight gain, average daily gain, feed intake, and feed conversion efficiency), haematocrit, GIT viscera masses and length, serum markers of health and liver lipids

μ = is the overall mean common to all observations

T_i = is the fixed effect of the i^{th} dietary treatment (i = 1, 2, 3)

e_{ij} = is the random residual error.

6.6 Results

Growth performance, GIT viscera macromorphometry and haematocrit was determined on all birds used in the experiment, while long bone indices, serum markers of health and liver lipid content was determined only on those birds that were ascertained as laying.

6.6.1 Growth performance

Table 6.1 and 6.2 show the effects of *H. sabdariffa* calyces meal on the growth performance of pullet Japanese quail hens as determined by body mass and long bone based indices, respectively. At 10% dietary inclusion supplemental *H. sabdariffa* calyces meal significantly reduced the BWG ($P = 0.0273$) and ADG ($P = 0.0325$) of pullet Japanese quail for the period week 1to 2 but increased ($P < 0.05$) their BWG ($P < 0.05$) during the period from week 3 to 6 and similarly their and ADG ($P = 0.0402$) during the period week 3 to 4. However dietary *H. sabdariffa* calyces meal had no effect on the pullet Japanese quail BWG for the period week 7 to 8 ($P = 0.2815$) as well as the trial (total) BWG ($P = 0.1342$). It however had no effect ($P > 0.05$) of the birds' ADG during the period week 5 through to 8 as well as on the trial (total) ADG of the pullet Japanese quail hens. Dietary *H. sabdariffa* calyces meal had no effect ($P>0.05$) of the femora and tibiae mass, length and mass:length ratio of the Japanese quail.

During the period week 1 to 2 of the feeding trial, supplemental *H. sabdariffa* calyces meal had no effect ($P = 0.1993$) on the bird's feed intake. However, at 10% dietary inclusion the *H. sabdariffa* calyces meal significantly reduced ($P < 0.05$) the Japanese quail hens' feed intake for the period week 3 to 8 of the trial. Supplemental *H. sabdariffa* calyces meal (5% and 10%) significantly reduced ($P < 0.0001$) the trial (total) feed intake of the hens.

Supplemental *H. sabdariffa* calyces meal did not significantly affect ($P > 0.05$) the FCR of Japanese quail from weeks 1 through to 4 of the experimental period. Supplemental *H. sabdariffa* calyces meal (10%) significantly increased ($P < 0.0001$) the FCR of Japanese quail in weeks 5 and 6. The FCR for Japanese quail across dietary treatments was not affected by

supplemental *H. sabdariffa* calyces meal in week 7 and 8 of the experimental period. Supplemental *H. sabdariffa* calyces meal did not affect ($P > 0.05$) the trial FCR of Japanese quail as well as the amount of feed per number of eggs produced.

Table 6.1: Effect of supplemental *H. sabdariffa* calyces meal on growth performance of Japanese quail

Parameter	Week	Dietary treatments			P- value
		Diet 1	Diet 2	Diet 3	
Induction body mass (g)		153.92±21.75	148.25±21.33	161.36±16.71	0.2062
Terminal body mass (g)		207.01±18.84	201.20±15.76	211.80±23.24	0.3055
Weekly BWG (g)	1 - 2	22.50±9.37[a]	20.31±7.41[a,b]	15.52±5.41[b]	0.0273
	3 - 4	20.54±3.49[b]	23.77±1.05[a,b]	27.35±14.99[a]	0.0402
	5 - 6	15.84±5.76[b]	20.34±3.79[a,b]	22.73±11.45[a]	0.0125
	7 – 8	6.60±0.00	5.99±0.76	7.57±5.20	0.2815
Total BWG (g)		**58.89±10.47**	**68.31±4.90**	**63.07±6.71**	**0.1342**
Weekly ADG (g/day)	1 - 2	1.61±0.67[a]	1.45±0.53[a,b]	1.11±0.39[b]	0.0325
	3 - 4	1.47±0.25[b]	1.70±0.07[a,b]	1.94±1.07[a]	0.0402
	5 - 6	1.13±0.41	1.45±0.27	1.08±1.09	0.2423
	7 – 8	0.47±0.00	0.43±0.05	0.40±0.57	0.7544
ADG (Trial) (g/day)		**1.24±0.1**	**1.07±0.68**	**0.97±0.25**	**0.0841**
Weekly FI (g)	1 - 2	91.51±11.64	89.90±12.61	86.46±8.34	0.1993
	3 - 4	118.07±7.49[a]	111.90±10.52[b]	111.38±8.56[b]	0.0074
	5 - 6	134.89±8.28[a]	128.01±13.89[a,b]	124.54±14.13[b]	0.0061
	7 – 8	143.51±0.45[a]	139.22±0.53[b]	136.11±1.19[c]	< 0.0001
Total FI (g)		**477.96±9.37[a]**	**458.89±7.18[b]**	**449.44±0.22[c]**	< 0.0001
Weekly FCR	1 - 2	4.71±2.52	4.88±1.7	4.13±2.69	0.6579
	3 - 4	5.89±1.31	4.72±0.55	5.67±2.44	0.0622
	5 - 6	7.33±1.14[b]	6.54±1.71[b]	22.76±15.27[a]	< 0.0001
	7 - 8	10.77±0.00	12.60±0.00	5.97±0.00	
FCR (Trial)		**6.10±2.24**	**5.15±1.02**	**5.74±1.76**	**0.2758**
Feed conversion (kg feed/no. of eggs)		2.47±0.05	2.49±0.13	2.42±0.16	0.1887

[a, b] Within row means with different superscripts differ significantly at $P < 0.05$. Diet 1: Standard Japanese quail layer diet; Diet 2: Standard Japanese quail layer diet + 5 % *H. sabdariffa* calyces meal w/w; Diet 3: Standard Japanese quail layer diet + 10% *H. sabdariffa* calyces meal w/w. BWG- Body weight gain; ADG-Average daily gain; FI - Feed intake; FCR = Feed conversion Data is expressed as mean ± SD. n=30 for Diet 1 and 29 for diet 2 and diet 3.

Table 6.2: Effect of supplemental *H. sabdariffa* calyces meal on the tibiae and femoral mass, length and bone mass: length ratio of Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	P- value
Femora				
Mass (mg)	230.00±0.03	210.00±0.03	210.00±0.03	0.2213
Length (mm)	20.60±0.14	20.60±0.16	20.04±0.19	0.9888
Mass:length ratio (mg/mm)	11.16±0.11	10.19±0.01	10.48±0.02	0.7856
Tibiae:				
Mass (mg)	260.00±0.03	260.00±0.04	260.00±0.03	0.9929
Length (mm)	24.80±0.25	24.90±0.20	24.90±0.23	0.9953
Mass: length ratio (mg/mm)	10.62±0.01	10.44±0.01	10.44±0.01	0.9839

No significant differences on the tibiae and femoral bone indices across dietary treatments. Diet 1 = standard Japanese quail layer diet, diet 2 = standard Japanese quail layer diet + 5% *H. sabdariffa* calyces meal (w/w) and diet 3 contains standard Japanese quail layer diet + 10% *H. sabdariffa* calyces meal (w/w). Data is expressed as mean ± SD. n= 15 for diet 1, n= 14 or diet 3 and n=11 for diet 3.

6.6.2 GIT viscera morphometry

Table 6.3 shows the effects of *H. sabdariffa* calyces meal on the GIT viscera macromorphometry of pullet Japanese quail. Dietary *H. sabdariffa* calyces meal had no effect ($P > 0.05$) on the macromorphometry GIT viscera and that of the accessory GIT viscera

Table 6.3: Effect of supplemental *H. sabdariffa* calyces meal on the GIT viscera morphometry relative to body mass of Japanese quail

Organ	Diet 1	Diet 2	Diet 3	P-Value
Proventriculus (% BM)	0.43±0.06	0.42±0.06	0.43±0.08	0.8863
Ventriculus (% BM)	2.02±0.42	2.18±0.33	2.21±0.28	0.2203
Small intestines (% BM)	2.74±0.56	2.53±0.82	2.73±0.39	0.5397
Small intestines (mm/g)	2.83±0.64	2.77±0.83	3.12±0.25	0.2354
Large intestines (% BM)	0.36±0.54	0.30±0.08	0.26±0.09	0.5982
Large intestines (mm/g)	0.35±0.09	0.34±0.05	0.33±0.04	0.7603
Liver (% BM)	2.96±0.63	2.95±0.63	2.50±0.85	0.0932
Pancreas (% BM)	0.27±0.06	0.26±0.07	0.27±0.11	0.9332
Caecum (% BM)	0.39±0.09	0.42±0.08	0.45±0.11	0.1224

No significant differences on the GIT viscera morphometry of quail across dietary treatments. Diet 1 = standard Japanese quail layer diet, diet 2 =standard Japanese quail layer diet + 5% *H. sabdariffa* calyces meal (w/w) and diet 3 standard Japanese quail layer diet + 10% *H. sabdariffa* calyces meal (w/w), BM – percent relative to body mass. Data is expressed as mean ± SD. n=30 for diet 1, n=29 for diet 2 and diet 3.

6.6.3 Haematocrit and serum markers of health

Figure 6.1 shows the effect of *H. sabdariffa* calyces meal on the haematocrit of pullet Japanese quail while table 6.4 shows its (dietary *H. sabdariffa* calyces meal) effect on the serum surrogate markers of liver and kidney as well as the general serum clinical chemistry of the birds. Supplemental *H. sabdariffa* calyces meal had no effect ($P > 0.05$) on the haematocrit, serum surrogate markers liver (uric acid, ALT, ALP) and kidney (albumin) as well serum calcium, phosphorus, cholesterol and triglyceride concentration of the birds.

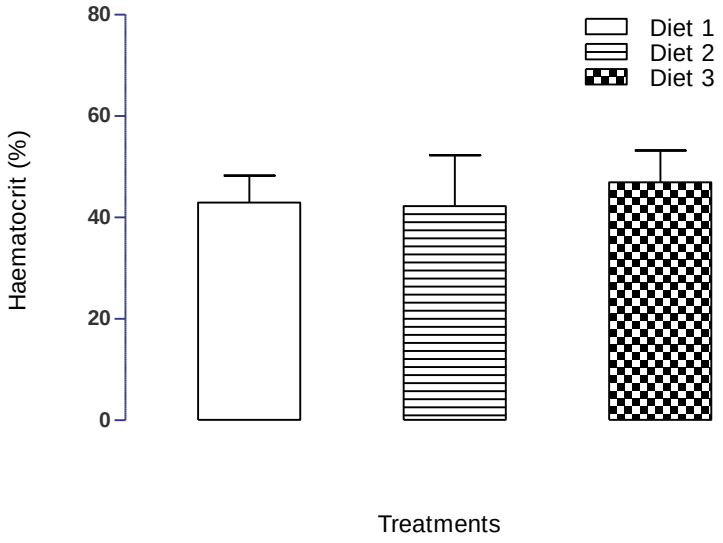

Figure 6.1: Effect of supplemental *H. sabdariffa* calyces meal on the haematocrit of Japanese quail

Diet 1= standard Japanese quail layer diet; Diet 2=standard Japanese quail layer diet + 5% *Hibiscus sabdariffa* calyces meal (w/w); Diet 3= standard Japanese quail layer diet + 10% *Hibiscus sabdariffa* calyces meal(w/w). for diet 3. Data is expressed as mean ± SD. n= 30 for diet 1; n=29 for diet 2 and diet 3.

Table 6.4: Effect of supplemental *H. sabdariffa* calyces meal on the serum surrogate markers of health of Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	P- Value
Uric acid (µmol/L)	664.00±355.49	479.00±273.30	630.73±297.30	0.3153
ALT (U/L)	24.67±46.84	nd	nd	
ALP (U/L)	441.55±533.62	322.92±387.53	721.63±546.92	0.1572
Phosphorus (mmol/L)	2.33±0.84	2.80±0.98	2.04±0.67	0.1105
Calcium (mmol/L)	3.72±0.37	3.94±0.21	3.71±0.64	0.3675
Albumin (g/L)	15.75±3.04	16.42±2.39	16.83±1.80	0.5770
Cholesterol (mmol/L)	5.14±1.13	4.17±1.09	5.00±1.40	0.1271
Triglyceride (mmol/L)	4.24±0.00	4.24±0.00	4.24±0.00	-

No significant differences on the serum surrogate markers of health across dietary treatments. Diet 1= standard Japanese quail layer diet, diet 2 = standard Japanese quail layer diet + 5% *H. sabdariffa calyces* meal (w/w) and diet 3 = standard Japanese quail layer diet + 10% *H. sabdariffa* calyces meal(w/w). ALT- Alanine transferase; ALP- Alkaline phosphatase, nd - not detected. Data expressed as mean ± SD. n=12 for diet 1 and 2 and n= 11 for diet 3.

6.6.4 Liver lipid content

Figure 6.2 shows the effect of *H. sabdariffa* calyces meal on liver lipid content of pullet Japanese quail. Supplemental *H. sabdariffa* calyces meal significantly decreased (P< 0.0001) the liver lipid content of the Japanese quail.

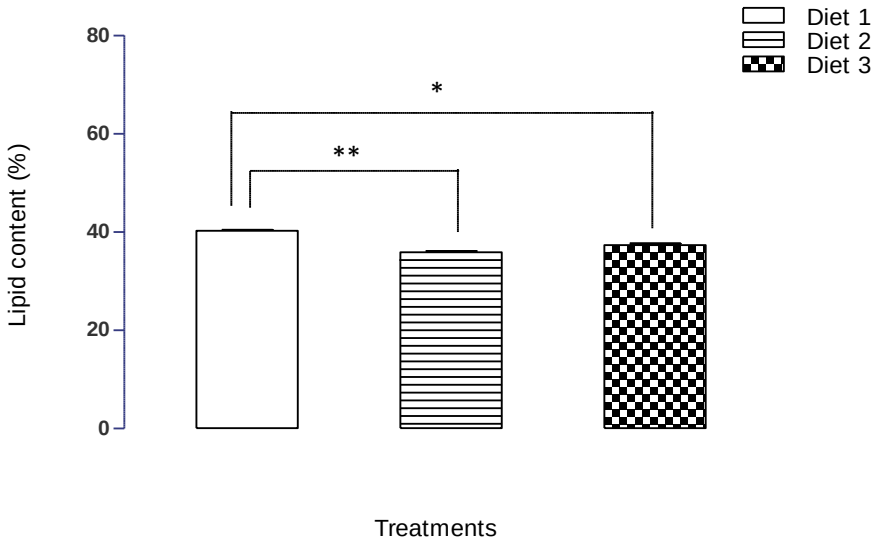

Figure 6.2: Effect of supplemental *H. sabdariffa* calyces meal on the liver lipid content of Japanese quail

Supplemental *H. sabdariffa* calyces meal significantly decreased (P < 0.05) the lipid content of Japanese quail livers. Diet 1- standard Japanese quail layer diet, diet 2- standard Japanese quail layer diet + 5% *H. sabdariffa* calyces meal(w/w) and diet 3- standard Japanese quail layer diet + 10% *H. sabdariffa* calyces meal(w/w). Data expressed as mean ± SD. n=26 for diet 1; n=15 for diet 2 and n= 16 for diet 3.

6.7 Discussion

My discourse on the discussion dwells on interpreting findings of the current study, explaining the possible mechanisms underlying the findings, making relevant comparisons and drawing inferences on the effect of supplemental *H. sabdariffa* calyces meal on growth performance, GIT visceral and GIT accessory viscera macromorphometry and health of pullet Japanese quail. Supplemental *H. sabdariffa* calyes meal did not affect ($P > 0.05$) the trial BWG, the bone indices (tibiae and femorae mass, length and mass:length ratio) of Japanese quail as well as on the trial ADG of Japanese quail. Supplemental *H. sabdariffa* calyces meal at both 5% and 10% significantly reduced ($P < 0.05$) the trial feed intake of Japanese quail but did not affect ($P = 0.2758$) the FCR of Japanese quail. Supplemental *H. sabdariffa* calyces meal did not significantly affect the haematocrit, serum surrogate markers liver and kidney as well as serum clinical chemistry of the birds but significantly reduced ($P < 0001$) the liver lipids of Japanese layer quail.

6.7.1 Growth performance

Growth promoters are incorporated in poultry feeds in order to improve productive performance (Katz and Baltz, 2016) and bird health (Dhama et al., 2014). Body mass and body mass based indices such as terminal body weight, BWG and ADG are some of the tools used to determine the growth performance of poultry and livestock. Energy and other nutrients assimilated from the GIT are critical for poultry growth performance and egg production (Li et al., 2014; Mahfuz et al., 2017) hence the need for an optimal GIT environment and function to support nutrient digestion and absorption.

The addition of *H. sabdariffa* calyces meal in the drinking water of broiler chicken improved their growth performance (Usman et al., 2016) and the use of its extracts as dietary supplements have been shown to improve digestibility of broiler chicken diets (El- Husseiny et al., 2002) and to improve the average daily weight gain of chickens (Unigwe, 2011). The positive impact of *H. sabdariffa* in chicken growth performance is ascribed to phytochemicals in the *H. sabdariffa* calyces meals and or extracts. These phytochemicals have beneficial

biological activities including among many, antibacterial, antifungal, antioxidant, as well as immune and digestive system modulation (Mahfuz et al., 2017; Li et al., 2014). While phytochemicals in plant-derived feed additives exhibit beneficial biological properties, when in high concentrations they impart flavours and odours to feed that decrease acceptability and feed intake negatively impacting dietary nutrient supply and poultry growth performance (Valenzuela – Grijalva et al., 2017). My findings show that supplemental *H. sabdariffa* calyces meal had no effect on the terminal body weight, total BWG, trial ADG and trial FCR of the Japanese quail (Table 6.1). Interestingly, the total FI of the pullet Japanese quail decreased with increasing dietary *H. sabdariffa* calyces meal (Table 6.1). Phytogenics have been shown to increase feed utilisation efficiency in broiler chicken via thinning of the gut mucosal surface resulting in increased nutrient absorption via the apical surface of the absorptive enterocytes (Yang et al., 2015). The observed decrease in total FI with an increase in supplemental dietary *H. sabdariffa* calyces meal but with similarities in the Japanese quail's terminal body weight, total BWG, trial ADG and FCR (trial) could be due to the fact that Japanese quail in diet 1 had the highest egg productivity so possibly the nutrients were most likely being channelled into egg production otherwise it suggests that *H. sabdariffa* calyces meal improved feed utilisation efficiency of the birds. *Hibiscus sabdariffa* calyces meal has a high concentration of polyphenols which can negatively impact the smell and flavour of feed (Formagio et al., 2013) and hence FI and growth performance (Wafar, 2013). My study findings show that its use as a feed supplement at 5% and 10% in pullet quail feeds seems to have enhanced feed utilisation efficiency of the bird as there was similar growth [terminal body weight, total BWG, trial ADG and FCR (trial)] but with reduced total FI. I speculate that phytochemical content of the meal favoured positive associative effects that manifested with an improvement in the efficiency with which the feed was utilised. Importantly, these findings suggest dietary *H. sabdariffa* calyces meal might have enhanced nutrient digestion, absorption and utilisation in the birds. However, Sukkhavanit et al., (2011) reported that *H. sabdariffa* calyces meal in laying hen (chicken) diets did not affect the feed utilisation efficiency of the hens. The differences in our findings could be as a result of the dose given to the pullets, Sukkhavanit et al., (2011) used a maximum dose of 4% while in my study I went up to 10% inclusion level. My findings demonstrate that *H. sabdariffa* calyces meal at 5% and 10% can

be used to supplement standard layer quail feed to mediate an improvement in feed utilisation of laying Japanese quail.

Hydration status and gut fill impact the use of body mass and indices derived from it as a measure of growth performance (Ugras, 2020). Long bones, for example, femora and tibiae respond to stimulation by growth hormone in a dose-dependent manner (Lindsey and Mohan, 2015) thus long bone indices can be used as a more accurate, relative to body mass, proxies for growth performance. Establishing and maintaining a strong skeleton is vital to ensure optimal productive laying hens. The continual loss of structural bone during the egg laying period results in weakening of the skeleton and increased risk of fracturing (Świątkiewicz et al., 2014). The provision of a diet with adequate in calcium and phosphorus in a bioavailable form (Johnson, 2014) is essential in pullet production. Polyphenol rich plants such as *Moringa oleifera* have been reported to increase tibiae bone weight and ash percent (Mahfuz and Piao, 2019). Supplemental *H. sabdariffa* calyces meal can be used as a supplement in Japanese quail kept for eggs as it does not alter the tibiae and femoral bone indices of health. My study findings show that supplemental *H. sabdariffa* calyces meal did not affect the mass, length and mass:length ratio of the femora and tibiae of the quail across dietary treatments. The similarities observed in the long bone indices imply that the *H. sabdariffa* calyces meal neither compromised nor promoted the growth performance of the pullet Japanese quail. Supplemental *H. sabdariffa* calyces meal can therefore be used in pullet Japanese quail feeds without the risk of compromising growth performance and possibly skeletal integrity.

6.7.2 GIT viscera macromorphometry

The gut is responsible for mediatiating the uptake of nutrients and their use by poultry including Japanese quail (Adi et al., 2014; Rehault-Godbert et al., 2019). Therefore, a functionally efficient and healthy gut is key in order to achieve optimum bird productive performance (Rehault-Godbert et al., 2019). In chicken gut health, a major determinant of chicken growth performance, GIT resident commensal microbiota, particularly that buried in the mucus layer and or adhering to the digestive mucosa, help direct GIT structure and morphology development, modulate the immune responses, protects against luminal

pathogens and aid with nutrient digestion, absorption and utilization (Pickard et al., 2017). Phenolic compounds have bactericidal (Gordon et al., 2010) and bacteriostatic properties (Exteberria et al., 2013) and they minimise the adhesion of pathogenic *E coli* and *Clostridium* bacterial species and inhibit the progression of infections in the digestive tract resulting in improved nutrient utilisation and concomitantly improved poultry and livestock growth performance (Viveros et al., 2011; Duenas et al., 2015; Brenes et al., 2016). In the current study, there were no significant differences in the GIT viscera and GIT accessory viscera organ macromorphometry of quail across dietary treatments.

It has however, to be noted that both the aqueous and alcoholic extracts derived from the *H. sabdariffa* calyces meal used as a feed additive in the current study had high polyphenolic content (Table 3.3). Importantly, the layer Japanese quail fed *H. sabdariffa* based diets, despite having lower total FI, had similar growth performance, feed utilisation efficiency in terms of body mass gain and number of eggs produced compared to counterparts fed the control diet (Table 6.1). It can be speculated that the polyphenols in the meal, though not affecting the viscera macromorphometry, might have altered the microarchitecture of the GIT absorptive mucosa and also suppressed the proliferation of harmful gut bacteria, translating to possible increased nutrient availability and absorption hence the observed similar performance despite a reduced total feed intake.

6.7.3 Haematocrit, serum clinical markers and liver lipid content

Pravda et al., (1996) and Agina et al., (2017) report the haematocrit of Japanese quail to range from 29% to 58% and 25% to 66%, respectively. Findings from the current study show that supplemental *H. sabdariffa* calyces meal did not affect the haematocrit of the pullet Japanese quail. My findings show that the haematocrit of the birds ranged from 42% to 48% and are congruent with those reported by Pravda et al., (1996) and Agina et al., (2017). In addition to closely mirror the haematocrit of Japanese quail reported by other researchers, it can be inferred that *H. sabdariffa* calyces meal can be used as a dietary supplement in pullet/layer Japanese quail diets with no risk eliciting deleterious effects on the haematocrit of pullet Japanese quail.

Evaluation of the serum biochemistry profile of poultry and livestock provides useful information about their physical condition and can be deployed as a useful tool in screening healthy from sick (Lu et al., 2016) as well as to determine the effects of various interventions (Wang et al., 2013). The liver is a central metabolic organ which performs numerous metabolic functions including glucose and lipid metabolism as well as detoxification of drugs and xenobiotics. Alcohol, drugs, xenobiotics, and metabolites can damage liver cells and release aspartate aminotransferase (AST) and alanine aminotransferase (ALT) into blood circulation (Chang, 2014). Furthermore, liver diseases impair liver metabolic functions and progress to non-alcoholic fatty liver disease (NAFLD). High concentrations of ALT and AST in the serum indicate liver diseases and injury (Chang, 2014). The kidney is an excretion organ which also plays a critical role in maintaining homeostasis of glucose, amino acids, urea, sodium and calcium. Kidney function is estimated through measuring pH, blood urea nitrogen, creatinine and basic electrolytes including sodium, potassium, chloride and bicarbonate (Yousafzai et al., 2011). Therefore, any derangement in the concentration of these metabolites and electrolytes could suggest renal impairment since the kidney is the organ responsible for controlling these values. Previous studies reported that the Japanese laying quail serum ALP and ALT activities as well as its albumin, cholesterol and uric acid to range between 407.79U/L - 421.12U/L, 14.78U/L -25.21U/L, 2.92g/L, 3.72mmol/L- 5.04mmol/L and 196.40µmol/L – 255.70µmol/L respectively (Saki et al., 2017; Tang et al., 2017). Findings from the current study show similarities in the serum activities of ALP as well as serum uric acid, albumin and cholesterol concentration of the Japanese quail across dietary treatments. The serum ALT activity of Japanese quail fed the 5% and 10% *H. sabdariffa* calyces meal supplemented diets could not be detected. However, when taken together, my findings suggest that *H. sabdariffa* can used as a dietary supplement in pullet Japanese quail diets with no risk of eliciting damage of hepatocytes and without compromising the synthetic and detoxification of the liver as well as the excretory role of the kidneys.

The liver of many animals including avian species is the main site of fat generation with a capacity twenty (20) times greater than adipose tissue of equal weight (Steven, 1996). Importantly during the egg laying period, formation of triglycerides, phospholipids and cholesterol in the liver increases (Hermier, 1997) and these lipids make up the lipoproteins

that are channelled for incorporation oocytes of the ovaries (Chen et al., 1995). The metabolic flux that mediate the channelling of lipids from the liver to the ovaries in laying hens accounts for the higher circulating concentrations of triglycerides and cholesterol when compared to non-layers (Whitehead, 2004). The liver lipid content of laying Japanese quail is reported to range from 42.28% - 51.21% (Onel et al., 2017). My study findings show that the Japanese quail fed *H. sabdariffa* calyces meal based diets had a lower liver lipid content compared to that of counterparts fed the control diet. It has to be noted that the birds fed *H. sabdariffa* based diets came into lay much later and produced significantly fewer egg per week during the duration of the feeding trial (Figure 5.1). Thus it could be argued that due to metabolic activity in their livers, necessary to supply metabolites for egg production, was relatively less upregulated compared to their counterparts fed the control diet, hence the lower liver lipid content. Additionally, *H. sabdariffa* has hypolipidaemic activity mediated by polyphenols in it that reduce the activity of HMG-CoA reductase, a key enzyme in the synthesis of lipids (Yang et al., 2009). I therefore speculate that perhaps the lower egg production by the Japanese quail fed *H. sabdariffa* based diets could have been due to a likely subnormal supply of metabolites, including lipids, from the liver to the ovaries.

6.8 Conclusion

Hibiscus sabdariffa calyces meal can be used as a supplement in pullet Japanese quail finisher diets without compromising their growth performance because it results in improved feed utilisation efficiency. Additionally, it can be used without compromising liver and kidney function of the birds. However, despite it resulting in improved feed utilisation efficiency, it decreased egg production thus is likely to cause losses where eggs are the key product.

7. CHAPTER SEVEN - CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

7.1 Conclusions

Currently,there is a high engagement in research on alternative feed supplements that can increase poultry performance, improve welfare, and to warrant high-quality and safe meat and eggs. In this study *H. sabdariffa* calyces meal was explored as an alternative feed supplement in broiler and pullet quail.

Supplemental *H. sabdariffa* calyces meal did not affect the body weight gain, GIT visceral morphometry, bone (tibiae and femora) indices, haematocrit and surrogate markers of health in Japanese quail kept for meat and eggs. Supplementing the standard Japanese quail finisher diet with *H. sabdariffa* calyces meal did not affect meat quality parameters (colour, water holding capacity, pH and tensile strength). Importantly, the inclusion of *H. sabdariffa* calyces meal both at 5% and 10% in Japanese quail finisher diets reduced fat deposition in Japanese quail meat and increased protein content in breast meat of Japanese quail kept for meat.

Supplemental *H. sabdariffa* calyces meal at both 5% and 10% in standard Japanese quail finisher diets did not cause any deleterious effects on any of the meat quality parameters measured in this study, instead achieved the goal of reducing fat content in meat, a characteristic desired by health conscious consumers nowadays. Thus I suggest that in Japanese quail kept for meat as demonstrated in this study, *H. sabdariffa* calyces meal can be used up to 10% to produce lean meat with higher protein content. Meat with a high fat content (saturated) is detrimental to the health of the consumer and renders the meat more susceptible to lipid oxidation (case of high PUFAs) and ultimately shortened shelf life. Therefore, these findings suggest that *H. sabdariffa* calyces meal is an ideal supplement in Japanese quail kept for meat. Our results are relevant to to the meat industry as well as the agro-food industry since they could collaborate in using *H. sabdariffa* calyces meal as a natural feed additive in Japanese quail production.

Supplemental *H. sabdariffa* calyces meal at 10% in standard Japanese quail layer feed reduced feed intake of Japanese quail kept for egg production, delayed the onset of laying by one week and reduced laying capacity of Japanese quail. The external and internal quality of eggs were not adversely affected by supplementing standard Japanese quail layer diets with *H. sabdariffa*

calyces meal, however, yolk fat content was reduced in Japanese quail fed diet with 10% *H. sabdariffa* calyces meal. As much as *H. sabdariffa* calyces meal in Japanese quail layer feed does not interfere with weight gain, general health and egg quality parameters of Japanese quail, it delays the onset of laying and reduces number of eggs produced by quail. This is not desirable for the egg producers as it results in production losses. The optimum dose of inclusion of polyphenols in animal diets is difficult to define due to the different composition of phenolic compounds present in these products. Our study revealed that, at 5% dietary inclusion *H. sabdariffa* calyces meal can be used to supplement Japanese quail layer diet without compromising feed intake and egg production. Caution must be exercised as *H. sabdariffa* calyces meal resulted in the production of eggs with a higher lipid and saturated fatty acid content which can compromise consumer health.

7.2 Limitations and recommendations

Although I measured the fat content and profiled the fatty acids on the meat and egg yolk, due to technical complications, I did not measure the amount of cholesterol in these in order to get a clearer picture of the fat composition of the Japanese quail meat and eggs. I recommend determining of cholesterol content in the meat and eggs of Japanese quail in future studies.

The role of the gut microbiome in animal health is an important consideration, I did not measure the gut microflora to see impact of *H. sabdariffa* calyces meal on feed properties, this can be an interesting area of focus in future. Group housing the Japanese quail kept for eggs was a limitation in this study as it becomes difficult to track performance of individual birds, however I considered the fact that Japanese quail are social birds and may perform better when housed in groups.

Although I measured egg production of Japanese quail, I did not look into the possible impact of supplemental *H. sabdariffa* calyces on egg fertility. I recommend measuring of the egg fertility in future studies.

Antioxidant status of meat and eggs were not measured, the serum malonaldehyde levels are a good approximation of the other tissues. Although I measured feed intake and computed FCR, I recommend measuring of digestibility of the feed in order to clearly understand and explain

the observed improved feed efficiency vs a decrease in feed intake. Future research can also consider histology of key organs such as small intestines to dissect possibility of the intervention mediating micro architectural changes in the small intestines that may have resulted in possible increased nutrient absorption.

Future studies can consider other ways of incorporating *H. sabdariffa* to the diet without impacting the proximate composition of the diet. Histology of the liver would help bring a quantitative view of the distribution of the fat in liver for qualifying that the meal is likely to protect against development of fatty livers. Another limitation of our study was the lack of sensory evaluation in the meat as it is part of quality measurement in order to ascertain preference of consumers, I recommend future researchers to consider evaluating the sensory quality of meat and eggs.

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APPENDICES

APPENDIX 1: IDENTIFICATION OF *HIBISCUS SABDARIFFA* PLANT

Department
of PHARMACOGNOSY & ETHNOPHARMACY

Faculty of Pharmaceutical Sciences
USMANU DANFODIYO UNIVERSITY, SOKOTO

VICE CHANCELLOR: Prof. R. A. Shehu, OON, B.Sc (Unisok), Ph.D (Essex)
HEAD OF DEPARTMENT: H. E. Mshelia B.Sc. PGDE, M.Sc.
Email: pharmacognosyunit@yahoo.com
Phone: +2348059221840

Date: 31/10/2013

PLANT IDENTIFICATION

The plant ***Hibiscus sabdariffa*** (common name: Roselle) has been identified in our Department via Taxonomic means by: HALILU, Emmanuel Mshelia. The plant belongs to the family: **Malvaceae** and has been given Voucher number: **PCG/UDUS/Malv/0001**. The voucher specimen has been deposited in the Departmental Herbarium for reference purpose.

Halilu E. Mshelia

HEAD OF DEPT.
DEPT. OF PHARMACOGNOSY &
ETHNOMEDICINE
FACULTY OF PHARMACEUTICAL SCIENCES
U.D.U. SOKOTO

APPENDIX 2: ETHICAL CLEARANCE CERTIFICATE

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2014/46/D

APPLICANT:	Ms N Ndlovu
SCHOOL:	Physiology
LOCATION:	Faculty of Health Sciences
PROJECT TITLE:	*Effects of dietry supplementation with Hibiscus sadbariffa calyces meal in broiler and egg laying quail (Coturnix coturnix japonica)*

Number and Species

330 Coturnix coturnix

Approval was given for to the use of animals for the project described above at an AESC meeting held on 26 August 2014. This approval remains valid until 25 August 2016.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

None.

Signed: _____ (Chairperson, AESC) Date: 12th Sept. 2014

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ (Registered Veterinarian) Date: 11th SEPTEMBER 201

cc: Supervisor: Prof Prof K Erlwanger & Dr E Chivandi
Director: CAS

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