Utilisation of Provitamin A Biofortified Maize in Ovambo Chickens to Improve Food and Nutrition Security

by

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DECLARATION

I, Feyisayo A. Odunitan-wayas declare that the thesis hereby submitted by me for the Philosophiae Doctorate degree in Food Security at the University of KwaZulu-Natal is my own original and independent research work. The thesis was carried out under the supervision of Prof. Michael Chimonyo, Dr. Unathi Kolanisi and Dr. Muthulisi Siwela. This thesis or any part of it has not been previously submitted by me for any degree or examination to another faculty or University. The research work reported in this thesis does not contain any person's data, pictures, graphs or other information unless specifically acknowledged as being sourced from those persons.

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ABSTRACT

The broad objective of the study was to determine the effect of provitamin A biofortified maize (PABM) inclusion, sex and age on growth performance, carcass traits, blood composition, meat quality, vitamin A content, nutritional value and consumers' acceptability of Ovambo chickens. The aim of the study was to evaluate the potential of delivering provitamin A in yellow-orange biofortified maize to vitamin A deficient -vulnerable population groups who prefer provitamin A-devoid white maize. This was achieved through feeding indigenous chickens on the PABM with the expectation that the chicken carcass would have increased concentrations of vitamin A. A total of 102 Ovambo chickens, indigenous to southern Africa, were reared and fed two dietary treatments; the control, white maize (WM) and a PABM-based diet for nine weeks. There was no significant effect of diet on the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). Male birds had higher ($P<0.05$) ADFI and ADG than the female birds. There was also an interaction of diet, age and sex on ADG and ADFI.

Male birds fed on the PABM had higher ($P<0.05$) ADFI at 15 weeks and 21 weeks of age than the WM fed male birds. Male birds had higher ($P<0.05$) ADG than the female birds at 15- 17 weeks of age. Male birds fed on the PABM diet had a significantly higher ADG at 16 weeks of age. The PABM fed female birds had a higher ADG than the WM fed females at all weeks except 21 weeks of age and was significantly higher at 19 weeks of age. The PABM fed female birds had significantly lower FCR at 20 weeks than the WM fed female birds. The male birds had higher ($P<0.05$) FCR at 21 weeks than the female birds.

The PABM diet had a significant effect on the dressed carcass (DC) and leg weight of the birds. Female birds had higher ($P<0.05$) relative breast and back weights, whilst the male birds had higher body weights (BW) and leg weights ($P<0.05$). The DC, leg (thigh and drumstick) and back relative weight of the birds significantly increased as the ages at slaughter increased. The PABM fed birds had a significantly higher relative weight of gizzard and liver than the WM fed birds ($P<0.05$). The female birds had higher gizzard and liver weights than the male birds ($P<0.05$). The gizzard and liver weights of the birds decreased with increasing age. The relative heart weight of the male birds increased with age and was higher than that of the female birds ($P<0.05$).

The PABM diet increased the packed cell volume (PCV) of the birds and the leucocytes (WBC) of the PABM fed female birds were within the normal range but significantly higher than the WM fed female birds. The mean corpuscular haemoglobin (MCH) of females fed on PABM was lower than that of the PABM fed male birds and the WM fed female birds. All the mean values of the haematological parameters were within the normal range regardless of the age, sex and diet of the birds. Age had an effect ($P<0.05$) on alanine transaminase (ALT), total protein (TP), GLOB, triglycerides (TRI) and uric acid (UA). The sex of the birds significantly influenced the TP, GLOB, ALB, CREAT and TRI concentrations. The interaction of diet and age had a significant influence on the ALP, ALT and GLOB concentrations. The PABM diet, sex and age of the birds or their interactions had no significant overall effect on the pH, drip loss (%), cooking loss (%) and shear force of meat ($P>0.05$). The meat and skin of PABM fed chickens had higher Hunter a* (redness) and b* (yellowness) and lower Hunter L* (lightness) values. The skin of the female birds had higher Hunter L* (lightness) values than that of the males ($P<0.05$).

As the age of the birds increased, the skin of the WM fed females became lighter (Hunter L* values increased), whilst the intensity of the yellow colour of the skin of the PABM fed male birds increased (a* values increased) ($P<0.05$). The PABM improved the vitamin A concentration in the Ovambo breast meat. The WM fed chicken meat had an average of 40mcg/100g while the PABM diet increased the vitamin A concentration to 55mcg/100g. Sex and the interaction of diet and sex did not significantly affect the vitamin A concentration in the Ovambo meat. The PABM and sex had no significant effect on the moisture, crude protein and fat content of the meat. The ash content of the meat of the female birds was higher ($P<0.05$) than that of the male birds. The sex of the bird and diet had no effect on the mineral composition of the muscle. The interaction of sex and PABM diet impacted on the copper concentration of the Ovambo chicken meat. The sensory characteristics of the meat of the PABM and WM fed chickens were not significantly different. Age and gender of the consumers and their interactions had no significant effect on the acceptability of all the sensory attributes of the Ovambo chicken meat evaluated. The findings of this study concluded that indigenous chickens fed PABM can be a tool for curbing VAD and improving the meat quality of indigenous chickens in southern Africa regions.

Keywords: Food and nutrition security, growth performance, meat quality, Ovambo chicken, provitamin A biofortified maize, vitamin A deficiency

DEDICATION

This thesis is dedicated to God Almighty, who gave me the strength, grace, favour and the opportunity to complete this work and to my wonderful husband, Stevie Odunitan Wayas and our children, Ayomide and Oluwajomiloju.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADFI	Average daily feed intake
ADG	Average daily gain
AI	Adequate Intake
ALB	Albumin
ALP	Alkaline phosphate
ALT	Alanine transaminase
AOAC	Association of Official Analytical Chemists
AST	Aspartate transaminanse
BW	Body weight
CHOLES	Cholesterol
CIE	International Commission on Illumination
CREAT	Creatinine
CUT	Central Local Testing
DAFF	Department of Agriculture, Forestry and Fisheries
DC	Dressed carcass
FCR	Feed conversion ratio
GLM	General linear model
GLOB	Globulin
Hb	Haemoglobin
HUT	Home Use Test

MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MUFA	Monounsaturated fatty acids
NIH	National Institutes of Health
NVASPGSA	National Vitamin A Supplementation Policy Guidelines for South Africa
PABM	Provitamin A biofortified maize
WM	White maize
PCV	Packed Cell Volume
PUFA	Polyunsaturated fatty acids
RBC	Erythrocytes count
RBP	Retinol Binding Protein
RDAs	Recommended Dietary Allowances
SAS	Statistical analysis systems
SEM	Standard error of means
SFA	Saturated fatty acids
SSA	sub- Saharan Africa
TP	Total protein
TRI	Triglycerides
UA	Uric acid
UNSCN	United Nations Standing Committee on Nutrition

VA	Vitamin A
VAD	Vitamin A deficiency
WBC	Leucocytes count
WHO	World Health Organizational of United Nations
SADC	Southern Africa Development Community

CHAPTER 1

The Problem and its Setting

1.1 Introduction to the research problem

Vitamin A deficiency (VAD) is a major health issue worldwide; its highest prevalence is in the rural areas of sub-Saharan Africa (SSA) and South-East Asian countries (Saltzman *et al*., 2014*)*. Twenty five percent of the countries with the highest rate of VAD are SADC (Southern Africa Development Community) countries (Muthayya *et al*., 2013). Vitamin A deficiency affects an estimated average of 63.6 % of children between the age of one and nine years old and 17 % of pregnant women in SADC countries [Labadarios, 2007; World Health Organisation (WHO), 2009]. About 43.6 % of children in South Africa have VAD (Shisana *et al.,* 2014). Vitamin A deficiency has been found to be most prevalent in South Africa rural areas, particularly in KwaZulu-Natal province (Labadarios, 2007).

Vitamin A deficiency in SSA occurs largely due to imbalanced diet (Haskell, 2013). In the sub-Saharan region, the most common staple crop, maize is generally low in protein and other micronutrients, including vitamin A (Bouis, 1996). In SSA including South Africa, various strategies such as supplementation, food fortification, dietary diversity and the recent biofortification of staple crops have been introduced and implemented over the years to curb VAD. Yet, VAD persists as a challenge. Emphasis on the use of biofortification as a major tool for curbing VAD, especially in the rural communities has been suggested by various researchers as it seems to be the most suitable strategy that will address most of the challenges encountered by the other strategies such as affordability, accessibility, stability and availability (Mayer *et al.,* 2008).

Due to its wide and high utilisation as food by the VAD-prone communities in SSA, maize has been selected for provitamin A biofortification (HarvestPlus Brief, 2006). However, the provitamin A biofortified maize (PABM), with high amount of β carotene is yellow orange in colour, has a unique flavour and aroma which makes it distinctly different from the white coloured maize commonly consumed in southern Africa (Stevens & Winter-Nelson, 2008; Pillay *et al.*, 2011). Moreover, there are negative perceptions about yellow maize, which is similar in colour to the PABM due to its past use as food aid for the poor (Stevens & Winter-Nelson, 2008; Pillay *et al*., 2011). The negative perceptions of yellow maize have negatively influenced consumers' acceptability of PABM. Thus, other methods are being evaluated, such as possible indirect consumption of PABM to curb VAD in southern Africa.

Vitamin A deficiency affects mostly those in the rural areas, where majority breed indigenous chickens as a major source of livelihood and protein diet; and yellow maize (non-biofortified yellow maize) is already being used and accepted as poultry feed [Department of Agriculture, Forestry and Fisheries (DAFF) 2012]. Against this background, a possible solution would be to feed indigenous chickens with PABM as a means of decreasing VAD rate in rural areas. Vitamin A plays an important role in boosting the immunity, vision and growth and development of chickens (Goetz *et al.,* 2014). Carotenoids have also been reported to give the skin of chickens a yellowish colour which is preferred by consumers (Tarique *et al*., 2013).

Karadas *et al*. (2005) have reported that carotenoids fed to pullets are passed into the egg yolk, liver and other tissues of the chicken embryo. The use of PABM as feed for indigenous chickens in southern Africa, therefore, should improve the productivity of the birds in terms of improved growth performances, meat quality and nutritive value. The response of Ovambo chicken, a popular South African indigenous chicken to the effect of the PABM, however, has not been investigated. More so, the consumers' acceptability of the chicken meat of the PABM fed chickens is not known.

1.2 Importance of the study

VAD has to be reduced significantly in southern Africa for improved food and nutrition security. As opposed to the other methods developed to curb VAD, biofortification of maize with provitamin A seems to be the most cost effective method for the resource-poor as the cost of biofortification is once-off and borne by the breeder not the farmer. Biofortification also has the added advantage of being agricultural-based, which makes the intervention method readily available and accessible to the resource poor (Mayer *et al*., 2008). However, the reported low acceptability of PABM-based foods due to its colour (yellow-orange), flavour, taste, aroma and consumer’s perceptions require alternative methods to enhance the utilization of PABM by humans. This study introduces an alternative method under investigation which is feeding PABM to chickens. Although, indigenous birds get some amount of vitamin A from scavenging, these chickens are often deficient in various nutrients, including vitamin A (Yemane *et al*., 2014). It is also difficult to determine whether it is sufficient or insufficient as the birds consume varying diets depending on their environment. The PABM offers a reliable cheap organic source of vitamin A which will be beneficial to both the chicken and humans. The expectation is that the diet will improve the indigenous chickens' value and potentials in terms of higher nutritional value of the chicken meat especially vitamin A, growth performances and meat quality and the consumers' preferences would still be met.

1.3 Overall aim and objectives

The broad objective of the study was to determine if the Ovambo chicken breed could be a potential tool for curbing VAD and improving the livelihood among the rural households in southern Africa by feeding them a PABM diet.

The specific objectives were to:

1. Determine the effect of provitamin A biofortified maize on the growth performances of male and female Ovambo chickens (South African indigenous chicken strains);
2. Assess the influence of provitamin A biofortified maize on the carcass traits and edible offals of female and male Ovambo chickens at different ages;
3. Determine the haematological and biochemical responses of female and male Ovambo chickens to PABM at different ages;
4. Assess the influence of provitamin A biofortified maize on the meat quality of the female and male Ovambo chicken at different ages ;
5. Determine the effect of provitamin A biofortified maize on the vitamin A concentration of the meat of the female and male Ovambo chickens;

6. Determine if the provitamin A biofortified maize diet fed to the chickens will affect the consumers' acceptability of their meat.

1.4 Hypotheses

This study tested the hypotheses that:

1. Provitamin A biofortified maize and age of birds has no effect on the growth performance of the female and male Ovambo chickens;
2. There is no significant increase or difference in the concentration of vitamin A and muscles of the male and female Ovambo chickens fed provitamin A biofortified maize diets;
3. Provitamin A biofortified maize, age and sex of the birds have no effect on the carcass traits and edible offals of the Ovambo chicken;
4. Provitamin A biofortified maize, age and sex of the birds have no effect on the blood composition of the Ovambo chickens;
5. Provitamin A biofortified maize, sex and age of the birds have no effect on the meat quality of the Ovambo chickens; and
6. Provitamin A biofortified maize has no effect on the consumer acceptability of meat of the Ovambo chickens.

1.5 Study assumptions

It was assumed that the rural poultry farmers will be willing to feed their indigenous chickens with PABM. It was assumed that indigenous chicken form a major part of the diet of the resource-poor people as the meat is readily available to them at minimal cost. It was also assumed that the consumer panellists used in the study provided honest and accurate information. Given that the researcher is not conversant with the local language, it was also assumed that translation of the questionnaires were accurate.

1.6 Study's limitation

The research study is restricted to only one strain of indigenous chicken breed common in southern Africa. It evaluates the effect of PABM on chickens fed from 13 weeks old of age and not from day old. It evaluates the effects of PABM as source of vitamin A in chicken feed on growth performance of chickens, carcass traits, and blood composition, and meat provitamin A content and quality. It does not evaluate the effect of PABM on the eggs produced.

1.7 Structure of the dissertation

The current chapter outlines the introduction to the study, statement of the research problem, importance of the study, assumptions and study's limitations. Chapter 2 presents a review of related literature. Chapter 3 presents the study on the effects of PABM on the growth performances of Ovambo chicken. Chapter 4 presents the study of the influence of PABM on the carcass characteristics and organ weights of the Ovambo chicken. Chapter 5 presents the study on haematological and serum biochemical responses of the Ovambo chicken to PABM. Chapter 6 presents the study of the meat quality of Ovambo chicken breed fed PABM diet. Chapter 7 is on the study of the effect of e diet on the vitamin A level and proximate and mineral composition of the Ovambo chicken. Chapter 8 presents the study on the effect of PABM on the sensory characteristics of Ovambo chicken and their influence on consumers' acceptability. Chapter 9 gives the study's general discussion, conclusion and recommendations.

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CHAPTER 2

Literature Review

(Accepted by *African Journal for Physical, Health Education, Recreation and Dance*)

2.1 Introduction

The review discusses the importance of vitamin A in humans and the different forms of vitamin A with emphasis on provitamin A carotenoids. It also discusses the status of VAD in SSA and describes the strategies that have been developed over the years to curb VAD, highlighting the biofortification of maize with provitamin A and the acceptability of provitamin A biofortified maize for human consumption. This review also describes the effects of vitamin A on growth performance, chicken's health, meat quality and nutritive value. The importance and utilization of indigenous chicken to improve food and nutrition security are also reviewed.

2.2 Vitamin A

Vitamin A (VA) is the term used for a number of related compounds with biological activity of retinol (Harrison, 2015). Vitamin A is an isoprenoid compound with a six membered ring and eleven carbon side chain (Edem, 2009). It was discovered in 1913 by McCollum and Davis of the University of Winconsin and also by Osbourne and Mendel of the Yale University (Fawzi, 2006). The discovery was based on their findings that, adding ether extract of yolk or butterfat to the diet fed to their experimental rats corrected the eye condition and retarded growth which they had when they were fed on diets in which only lard was added. As opposed to the water soluble vitamins that are absorbed directly into the bloodstream when ingested, vitamin A is absorbed through the intestinal wall and the lymph system into the bloodstream where it is utilized or stored in the liver and body fat (O'Byrne & Blaner, 2013).

Vitamin A is an essential micronutrient which cannot be synthesized by the body (Mullin, 2011). Vitamin A can be found in foods in two types, as the preformed vitamin A which is usually found as retinyl esters or retinol in animal products such as in egg yolk, milk and liver, and as provitamin A carotenoids in green, yellow and orange vegetables and fruits such as carrots, tomatoes and spinach (Harrison, 2012). There are over 600 carotenoids, but only a few are provitamin A carotenoids (Parker, 1996; Maziya-Dixon *et al*., 2015). Provitamin A carotenoids have the ability to convert into vitamin A when ingested due to the presence of one or more unsubstituted β-ionine ring and unsaturated hydrocarbon chain which enables it to convert to vitamin A (Harrison, 2015). The major provitamin A carotenoids are β-carotene, α-carotene and β-cryptoxanthin (Harrison, 2015). Beta carotene (Figure 2.1) is the most important provitamin A carotenoids as it has higher conversion rate into vitamin A due to the presence of double β-ionine rings which are not present in other provitamin A carotenoids (Harrison, 2015). Beta-carotene is oxidatively cleaved by the β-carotene 15 150 -monooxygenase 1 (BCMO1) into two molecules of all-trans retinal, which subsequently can be oxidised irreversibly to retinoic acid by retinal dehydrogenase or reduced reversibly to retinol by a retinal reductase (Edem,2009) (Figure 2.2).

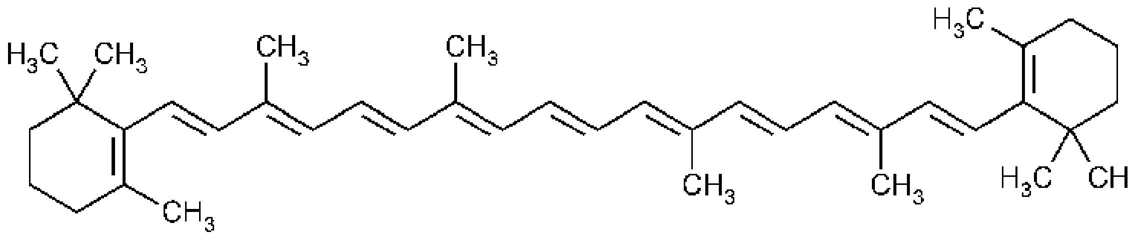

Figure 2.1: β- carotene structure

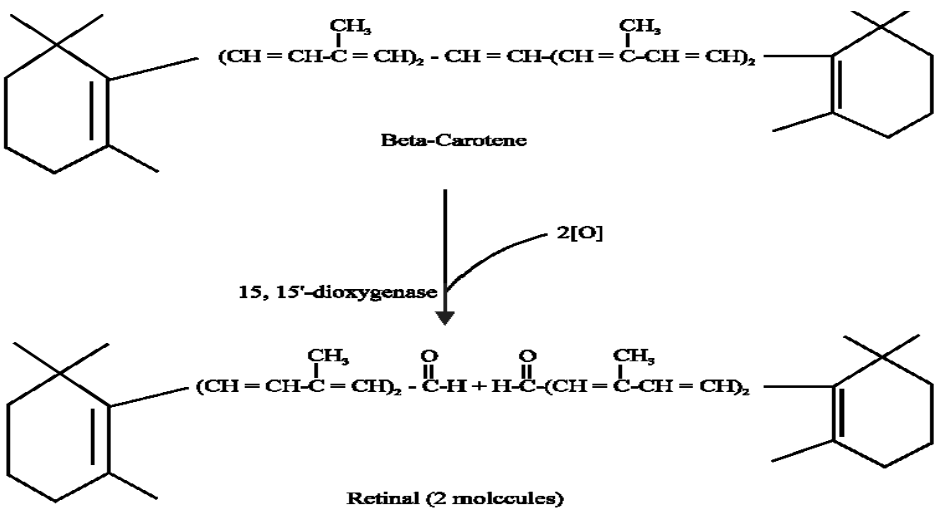

Figure 2.2: Conversion of β carotene to retinal

Source: Edem, 2009

2.3 Importance of vitamin A in humans

Vitamin A is required for normal organogenesis, immune response, tissue and cell differentiation and vision in humans (Sommer & Vyas, 2012). Some common active forms of vitamin A are retinol, retinal and retinoic acid (O'Byrne & Blaner, 2013) Due to the multi-functionality of vitamin A, even mild to moderate deficiencies of VAD has detrimental effects on normal body functions and productivity (Muthayya *et al*., 2013). Although the human body's requirement of vitamin A is minimal, between 400- 1 200 mcg retinol activity equivalents (RAE) daily, depending on the sex, age and status (Table 2.1), insufficient amount can have significant effects in the human body. The recommendation for vitamin A intake is expressed as micrograms (mcg) of RAE. Retinol activity equivalents take into account the fact that the body converts only a portion of β-carotene to retinol. One RAE equals 1 mcg of retinol or 12 mcg of beta-carotene (Davidsson & Haskell, 2011).

2.3.1 Immunity

Vitamin A is essential for normal immune system and regulation (Ross, 2012). Even mild cases of VAD reduce the immunity response to diseases and infections (Harrison, 2015). Vitamin A in the form of retinoic acid is important for good immune response. For example, the retinoic acid is important in sustaining the intestinal functions as the retinoic acid enhances the functions of T lymphocytes which are the major cells that aids to maintain the intestinal mucosal by creating inflammatory/anti-inflammatory balance in the gut (Ross, 2012). Deficiency in adequate intestinal function leads to diarrheal infections which can cause morbidity and mortality (Bhutta *et al*., 2008).

Table 2.1: Recommended dietary allowances (RDAs) for vitamin A

Age	Male(RAE)	Female(RAE)	Pregnancy(RAE)	Lactation(RAE)
0–6 months‡	400 mcg	400 mcg		
7–12 months‡	500 mcg	500 mcg		
1–3 years	300 mcg	300 mcg		
4–8 years	400 mcg	400 mcg		
9–13 years	600 mcg	600 mcg		
14–18 years	900 mcg	700 mcg	750 mcg	1 200 mcg
19–50 years	900 mcg	700 mcg	770 mcg	1 300 mcg
51+ years	900 mcg	700 mcg		

‡ Adequate intake (AI), equivalent to the mean intake of vitamin A in healthy, breastfed infants.

RAE: retinol activity equivalents. Source: National institutes of health (NIH), 2013.

2.3.2 Vision

Adequate vitamin A helps to maintain and have good vision. Vitamin A deficiency is one of the major causes of childhood blindness in developing countries (West, 2002). Vitamin A in the form of retinal is important for good vision and prevention of night blindness (nyctalopia), xerophthalmia, keratomalacia and total blindness (Semba, 2007). The scientific name of vitamin A "retinoid" was actually coined out of the word "retina" which is the light-sensitive layer of tissue at the back of the inner eye that changes images to electric signals that are sent to the brain by the optic nerve (Gollisch & Meister, 2010). The retinal in the retina is needed for colour vision and vision in low levels of light (scotopic vision) (Sherwin *et al*., 2012). During the vision cycle, some amounts of retinal are lost, and as the body does not synthesize vitamin A, the retinal lost in the retina is replaced through the diet of vitamin A enriched food or supplements (Smith & Steinemann, 2000).

2.3.3 Reproduction

Vitamin A is essential for development of both female and male reproductive systems. Vitamin A deficiency prevents spermatogenesis which results in reduced fertility or sterility (Clagett-Dame & Knutson, 2011). In females, vitamin A is essential for normal fertilization, implantation and embryonic development (Clagett-Dame & Knutson, 2011). When there is a severe form of VAD, implantation is prevented. In the case of moderate VAD, embryonic death may occur (Clagett-Dame & Knutson, 2011).

2.3.4 Growth and development of cells and tissues

Vitamin A, as retinyl-esters is important for lung development in the late phase of gestation and the beginning of lung maturation (Biesalski & Nohr, 2003). Vitamin A also aids in regulating stem cell differentiation and maintaining epithelial integrity (Kawaguchi *et al*., 2015). Vitamin A deficiency in the human body can cause abnormality in the anterior spinal cord and hind brain which affects normal growth and development (Maden, 2007).

The importance of vitamin A is many and its deficiency can have adverse effect, especially in regions that majorly consume staple crops which are low in vitamin A.

2.4 Vitamin A deficiency

Vitamin A deficiency occurs when there is insufficient amount of VA for use by the body. The deficiency could either be due to insufficient intake of vitamin A enriched foods or inability of the body to utilize the VA that is being consumed as a result of diseases and infections (Stephensen, 2001). As over 90 % of vitamin A is stored in the liver, VAD is diagnosed when vitamin A in the liver is below 0.7 µmol/L (Sommer & Davidson, 2002).

2.4.1 Status of VAD in Africa

Despite all the nutritional interventions that have been in place over the decades, such as supplementation, food fortification and dietary diversification, many sub-Saharan and South Asian countries still have high prevalence of VAD. The recent global hidden hunger index has indicated an estimate of 95 % VAD prevalence in children in some countries (Saltzman *et al*., 2014). The top 20 countries most affected by VAD consist of 90 % sub-Saharan countries and 10 % Asian countries (Table 2.2). Clearly, the situations in these countries need to be addressed. Death of an estimate of 3 000 children of less than four years of age and an estimated 519 maternal deaths were attributed to VAD in South Africa in the year 2000 (Nojilana *et al*., 2007).

Vitamin A deficiency usually occurs when the day to day diet contains little or no bioavailable vitamin A to meet physiological needs (Underwood, 2000). Even though the prevalence of VAD also affects the high and middle class income earners, a large percentage of the prevalence of VAD is peculiar to the poor due to factors such as inaccessibility to VA supplements, unavailability of VA enriched food due to poverty, political unrest, disease prevalence, ignorance of value of micronutrients, religious beliefs and poor diet (Siddique *et al*., 2015). Table 2.3 gives details on percentages of VAD in SADC countries.

Consequently, the majority of those vulnerable to VAD are children who are more susceptible to infections such as measles, diarrhoea, respiratory infections and are also living in sub-standard conditions (WHO, 2009). Vulnerable groups also include non-breastfed infants, pregnant and lactating women who are in a phase of high nutrient demand, especially those in the rural areas (WHO, 2009; Darnton-Hill & Ahmed, 2010).

Table 2.2: Countries with the highest micronutrient deficiencies

Country	Vitamin A (Low serum retinol (0.7µmol/L) (%)	Global Hidden hunger index (%)
Sao Tome and Principe	95.6	47.7
Kenya	84.4	51.7
Ghana	75.8	47.7
Sierra Leone	74.8	50.0
Benin	70.7	51.3
Mozambique	68.8	51.0
Central African Republic	68.2	51.0
Niger	67.0	52.0
Afghanistan	64.5	47.7
Gambia	64.0	43.7
India	62.0	48.3
Democratic Republic of Congo	61.1	47.7
Malawi	59.2	49.7
Mali	58.6	46.0
Cote d'Ivoire	57.3	44.0
Burkina Faso	54.3	48.3
Zambia	54.1	42.0
Liberia	52.9	45.3
Chad	50.1	43.3
Madagascar	42.1	43.0
Average	**64.3**	**47.6**

Muthayya *et al.* (2013)

Table 2.3: Vitamin A deficiency of 6-60 months old children in SADC countries

	WHO (1995-2005)	**UNSCN (2007)**
Country	**Percentage**	**Percentage**
Mozambique	68.6	33.4
Angola	64.3	43.8
Democratic Republic of the Congo	61.1	42.2
Malawi	59.2	47.1
Zambia	54.1	40.2
Swaziland	44.6	30.1
Madagascar	42.1	33.1
Zimbabwe	35.8	27.3
Lesotho	32.7	28.5
Botswana	26.1	23.1
Tanzania	24.2	33.8
Namibia	17.5	25.1
South Africa	16.9	24.8
Mauritius	9.2	18.8

Adapted from: United Nations Standing Committee on Nutrition (UNSCN), 6th edition, on the world nutrition situation

2.5 Challenges of strategies for VAD intervention

Several programmes and solutions have been, and are being, implemented to eliminate VAD (WHO, 2009). These include dietary supplementation, dietary diversification, food fortification and recently biofortification. These interventions have various challenges and benefits.

2.5.1 Dietary supplementation

Dietary supplementation involves the provision of high-dose vitamin A capsules to children and breastfeeding mothers to boost their vitamin A level (Ross, 2002). The supplements are, however, more available in the urban areas than rural areas where VAD is more prevalent due to various factors that include political unrest, inaccessible roads and mismanagement of funds (Vijayaraghavan, 2002). For example, Sao Tome and Principe with the highest prevalence of VAD in the world had only 34 % vitamin A supplementation coverage in 2012, Mozambique and Liberia had 20 and 13 % coverage respectively in 2012 (World Bank, 2014).

The cost of dietary supplementation is high. For example, vitamin A supplements cost South Africa R16.4 million annually (Saitowitz *et al.,* 2001). Another challenge of supplementation is that many women do not take their children that are older than the range of 12-18 months to the clinic for vitamin A supplements, as they think they are no longer prone to VAD [National Vitamin A Supplementation Policy Guidelines for South Africa (NVASPGSA), 2012].

2.5.2 Food fortification

Fortification is the addition of essential vitamins and minerals to processed foods to improve their nutritional value (Sablah *et al.,* 2013). Accessibility, willingness to buy and affordability of these commercially fortified foods to poor people, however, is a challenge (Nestel *et al*., 2006). Most of the countries with high prevalence of VAD are low income countries with less than $1,035 per capita (World Bank, 2013). Many of the countries that have high prevalence of VAD also have no law mandating the fortification of processed foods (Sablah *et al.,* 2013). Rural people are known to grow a large portion of what they consume and are not likely to purchase and consume the commercially available fortified processed foods (Dary & Mora, 2002).

2.5.3 Diet diversification

The diversification of diets involves the availability of bioavailable rich foods of animal origin, coupled with the continued promotion of nutritious fruit and vegetables (Miller & Welch, 2013). The challenges associated with this intervention are that, there is a lack of diversification of crop systems, as farmers plant the same type of crops all the time, limited nutritional knowledge and poverty also affects the willingness of majority of the rural population to diversify their diets (Ma *et al*., 2008).

2.5.4 Biofortification

Biofortification involves breeding of staple crops for increased vitamin and mineral content using a mix of traditional breeding knowledge and modern biotechnology (Nestel *et al*., 2006). The utilization of the nutrients is dependent on the amount of staple food consumed and how it is processed (Bouis *et al.,* 2011). The timeframe for the complete cycle from development of the biofortified crops to the dissemination and delivery of the seeds to all target areas might, however take a while (Bouis *et al.,* 2011).

Biofortification seems to meet most of the challenges of other interventions such as affordability, accessibility, availability safety and nutritional level. The cost of biofortification is significantly lower than the cost of supplementation as the cost is upfront and not continuous and it is borne by the breeder and initiator and not the consumer. For example, it has been estimated that the cost of biofortifying crops in 10 years is about 0.2 % of the cost of worldwide vitamin A supplementation (Mayer *et al*., 2008). The main issues with biofortification, however, are the acceptability of crops biofortified with provitamin A, the effective cultivation and distribution of the seed to the farmers and the ability of crops to retain the provitamin A after undergoing cooking and other types of processing (Meenakski *et al*., 2010). For example, maize, the most consumed staple crop in SSA undergoes several preparation processes depending on the country in which it is being consumed. In Kenya, preparation of their local maize meal ugali with the PABM has about 50 % retention of provitamin A (Meenakski *et al*., 2010).

2.6 Provitamin A biofortified maize and its potential

Maize is the most common cereal consumed by children between the ages of one and nine years (Labadarios *et al*., 2000). Children consume more staple crops and less of fruits and vegetables in SSA (Chagomoka *et al*., 2015). Maize, as opposed to most of the other staple crops, naturally accumulates carotenoids in its edible seed endosperm, and its germplasm has a wide genetic variation as well as variation in carotenoid compositions (Liu *et al*., 2003; Harjes *et al.,* 2008).

The presence of carotenoids in the seed gives the endosperm a yellow-orange colour which makes the presence of carotenoid easily identifiable (Wurtzel *et al.,* 2012).

The main carotenoids found in maize are lutein, zeaxanthin, α-carotene, β-carotene, and β-cryptoxanthin (Wurtzel *et al.,* 2012). Xanthophylls such lutein and zeaxanthin are abundant in maize, but cannot be converted to vitamin A (Wurtzel *et al.,* 2012). They however, distinct yellow-orange and yellow-red colouration indicates the presence of carotenoid regardless of whether they have provitamin A abilities or not (Davis *et al*., 2008). Chickens absorb lutein well and lutein has been used as supplements fed to chickens to give egg yolk and skin the yellowish colour preferred by consumers (Jang *et al*., 2014). Digested carotenoids are absorbed in different parts of the intestine and then utilized or deposited in broiler adipose tissues; breast, shank, skin and toe web (Tarique *et al*., 2013). Carotenoids that have provitamin A activity are α-carotene, β-carotene and β –cryptoxanthin (Harjes *et al*., 2008).

The traditional yellow maize that is not biofortified with provitamin A has minute amount of provitamin A carotenoids ranging from 0.5 to 1.5 mcg/g β-carotene (Harjes *et al*., 2008). The biofortification of maize with increased amounts of provitamin A carotenoids (>1.5 mcg/g) resulted in provitamin A-biofortified maize (PABM). Potential advantages of the PABM, other than its high provitamin A, are that it is drought and disease resistant, has better milling and kernel quality, higher starch (66.7/100g), fat (4.7g/100g) and protein (12.8/100g) content in comparison to the white maize which had 59.4g, 4.0g and 10.7g of starch, fat and protein, respectively (Bouis *et al*., 2013; Pillay *et al*., 2013). Optimal utilization of PABM is expected to improve the vitamin A status of the consumers and increase income of the producers as a result of higher productivity.

2.7 Retention of β-carotene in PABM after undergoing various processing

The biofortification of maize with provitamin A carotenoids have been successful (Menkir *et al.,* 2015) however, there is the need to know the retention of β-carotene in the maize after it has undergone various processing for human consumption. Milling of PABM into mealie meal resulted in higher retention of β carotene (105.6 – 134.3 %) due to the breakdown of the maize which made the β carotene more bioavailable (Pillay *et al*., 2014). Cooking methods and temperature affect the retention of β carotene in PABM. For example, making thin porridge that requires a high temperature with the PABM resulted in approximately 25 % loss in β carotene (Pillay *et al*., 2014). Boiling and steaming retains 80% provitamin A carotenoids in food in comparison to baking (30%) and frying (18 %) (De Moura *et al*., 2015). There was 79– 90 % retention of β-carotene after PABM was fermented in amahewu, a popular southern African lactic acid fermented non-alcoholic maize-based beverage (Awobusuyi *et al*., 2015). Storage of PABM for longer than 3 months causes high loss ranging from 45- 93 % of provitamin A carotenoids (De Moura *et al*., 2015). Mugode et *al*. (2014), however, reported that a large amount of β carotene was lost within 15 days of storage (44-48 %) and then 76-70 % after six months of storage. Exposure to heat and light also degrades provitamin A carotenoids (Bengtsson *et al*., 2008).

2.8 Consumers' acceptance of provitamin A biofortified maize

The process of biofortification of maize with provitamin A has already been successfully carried out (Bouis *et al.*, 2013). Several consumers' surveys were carried out to determine the acceptability of PABM. White maize is the white coloured maize popular in SADC regions that is devoid of vitamin A and yellow maize is the non- provitamin A biofortified yellow coloured maize with insignificant amount of vitamin A that is not desirable for consumption.

Pillay *et al*. (2011) reported that in KwaZulu-Natal, South Africa, the consumers' survey indicated that adults, primary and secondary school children preferred Phutu, a local meal made with white maize, rather than with the PABM. This preference was vice versa for the pre-schoolers. Pre-school learners, however, will consume what is prepared by the adults, who prefer the white maize. The general reasons for the dislike of PABM were attributed to its aroma, colour, flavour and their association of yellow maize with animal feed. The adults, however, showed significant willingness to consume PABM if it was cheaper than white maize and readily available in local grocery stores. However, willingness does not necessarily mean they will eventually like the PABM, as the disliked aroma, colour and flavour will still be present despite availability and lower prices.

In Zimbabwe, yellow maize is disliked based on its organoleptic properties and the perception that it symbolises suffering and poverty as yellow maize was imported into the country during times of drought and famine (Muzhingi *et al*., 2008). Muzhingi *et al*. (2008) stated that about 94 % of the farmers grow white maize only and only 25 % of the nation's population consumes yellow maize meal in one form or another. However, they reported that about 90 % of farmers will readily grow PABM if it is confirmed that it has better yield than white maize and is more resistant to drought and disease. At least a third of the population in Zimbabwe was also reported to dislike the taste of PABM. Taste is a strong determinant of consumers' choice of food, irrespective of the price. Lowering the price of PABM compared to white maize might lower the acceptability of PABM as it could be perceived as inferior. Poor people might purchase yellow or PABM more during hard times. Once they have higher income levels, they are, however, likely to revert to white maize, as it creates a sense of wealth for them. Acceptability of PABM is also gender related, as more male than females are likely to change from white to yellow maize if it is cheaper. This is due to the fact that females are more taste and quality oriented (Chen, 2013).

At least a third of children's deaths in Mozambique is VAD related and about 2.3 million children are vitamin A deficient (Aguayo *et al*., 2005). Their high rate of VAD is majorly diet related. According to the national agricultural survey of Mozambique in 2008, 78 % of the population in Mozambique produces maize (Cairns, 2012). In 2006, research was conducted using a locally prepared maize meal called xhima, to assess the acceptability of PABM (Stevens & Winter-Nelson, 2008). Xhima was prepared using three different maize lines, namely the PABM, locally produced white maize and isogenic (hybrid) white maize in Mozambique. The survey was carried out in the market and the people were informed about the nutritional benefits of the PABM before they were given the various porridges to consume. The outcome of the survey was that majority significantly preferred their local white maize to the others, based on appearance, texture and taste. However, they preferred the aroma of the PABM over the other maize lines (Stevens & Winter-Nelson, 2008).

In Zambia, more than 50 % of pre-school children have VAD (Micronutrient Initiative, 2009). To determine the acceptability of PABM in rural areas of Zambia with high poverty rates, the Home Use Test (HUT) and Central Local Testing (CUT) were used for the survey (Meenakski *et al*., 2010b). Home Use Test involved giving participants different types of maize meals (PABM, yellow and white) to cook with whatever preferred method and to sample at their homes. The CUT was achieved by giving the participants cooked maize meal, using PABM, yellow maize and white maize in the market place. Prior to these, nutrition information on the PABM was disseminated to the participants via radio and through community leaders. The survey indicated that the influence of information dissemination of the nutrient benefits of the PABM was significant as the PABM was well liked by the majority and they indicated willingness to pay for PABM, even though there was no significant difference between the two methods of dissemination that were used. However, the participants of both HUT and CLT did not like the yellow maize. This indicates that they do not associate PABM with yellow maize (Meenakski *et al*., 2010b) and that PABM is likely to be acceptable to the Zambian population.

Based on the summary of the consumer's acceptability survey, PABM is not well accepted in SADC countries, thus making it difficult to achieve the aim for which the PABM was produced. As maize is a major ingredient in livestock feed with its utilization as chicken feed having the largest percentage. (Ranum *et al*., 2014), it is suggested that PABM could be fed to indigenous chickens to boost the vitamin A in their meat to curb VAD when consumed.

2.9 Indigenous chicken production in Africa

Poultry and crop farming are very important activities in all rural households in SSA for poverty alleviation and curbing protein-energy malnutrition (Mekonnen *et al.,* 2010). Almost every rural household in SSA keep indigenous chickens (Meless*e* 2014). Indigenous chicken meat and eggs are important for reducing malnutrition in children in SSA (Rosegrant & Cline, 2003) as chicken meat and eggs are preferred for consumption by children than provitamin A rich fruits and vegetables. Poultry and eggs are also readily available and affordable for consumption. The importance of poultry is attributed to ease of management, its minimum capital and land requirement compared to other livestock species. Chickens do not have taboos for consumption like other meat products such as beef and pork (Farrell, 2013). In addition, chicken products contain almost all the essential nutrients (Table 2.4) required by humans. They also have the added advantages of being delicious and can be consumed and used in different ways and forms and are used as ingredient in making many other foods or pastries. Indigenous chicken meat and eggs account for 55 and 47 %, respectively of the total production of poultry in Africa (King'ori *et al*., 2010). Majority of the chicken are consumed by the household while others are sold (King'ori *et al*., 2010). Chickens are usually managed by the women and children who are vulnerable to VAD (Ahlers *et al.,* 2009).

As indigenous chickens have been identified as a tool to curb malnutrition, various methods are being investigated on how to improve their growth performance, meat quality and nutritive value. One of such investigated methods is crossbreeding of indigenous chickens with commercial birds to improve the growth performance of the indigenous chickens while still retaining its unique adaptation to harsh environment (Odunitan-wayas et *al.,* 2015). Another strategy is the enhancement of the nutritional value, growth performances and meat quality of chicken products by what is fed to the chickens (Lopez-Ferrer *et al.,* 2001). The diets of an average village chicken in SSA consist of leftovers, edibles from scavenging and whole grains (King'ori *et al*., 2010). Feeding indigenous chickens with PABM should improve the utilization of the provitamin A carotenoids which can be converted to higher amount of vitamin A in the chicken meat. There have been positive evidences that the consumption of PABM could increase vitamin A in meat muscles and the liver and it is a possibility that indigenous chicken can be an alternative medium to utilization of PABM to curb VAD. Little has, however, being done on the effect of PABM with respect to increase in vitamin A in chicken meat when consumed by indigenous chickens.

Higher amount of vitamin A can be obtained from animal origin than plant origin. (Penniston & Tanumihardjo, 2006). The majority of the rural poor, however, consume more of staple crops than meat. This is due to the cost of purchasing meat. However, chickens are reared by majority in the rural community, thus minimizing the issues of affordability and availability. Possible challenges are the frequency of consumption of chicken meat and eggs, the chicken production management systems, low production and growth rate of the indigenous chickens (King'ori *et al*., 2010). Poor nutrition is a factor that contributes to the low growth rate and egg production in free- ranging chickens (King'ori *et al*., 2009). Indigenous chickens reach sexual maturity between 16 to 22 weeks of age and they have low egg production (Van Marle-Köster & Casey, 2001). This results in the consumption of chicken meat and eggs being lower than expected in rural areas. Chicken production alone accounts for about 58 % of the total meat production in South Africa (Table 2.5).

Table 2.4: Nutrient composition of an egg and chicken breast (100g)

Nutrients	Units	Whole egg	Egg white	Egg yolk	Chicken breast (raw)
Energy	Calories	72	17	55	104.65(kcal)
Protein	Grams	6.3	3.6	2.7	22.25
Carbohydrates	Grams	0.4	0.2	0.6	
Total fat	Grams	4.8	0	4.5	1.6
Monounsaturated fat	Grams	1.8	0	2	0.75
Polyunsaturated fat	Grams	1	0	0.7	0.32
Saturated fat	Grams	1.6	0	1.6	0.52
Cholesterol	Milligrams	186	0	184	59
Choline	Milligram	126	0.4	116	
Riboflavin	Milligrams	0.2	0.2	0.1	0.19
Vitamin B12	Milligrams	0.5	0	0.3	0.38
Folate	Milligrams	24	1	25	
Vitamin D	IU	41	0	37	
Vitamin A	IU	270	0	245	15.5
Vitamin C					0.8
Vitamin B6	Milligrams	0.1	0	0.1	
Vitamin B3(niacin)					11
Thaimin	Milligrams	0	0	0	0.11
Vitamin E	Milligrams	0.5	0	0.4	2.2
Selenium	Milligrams	15.4	6.6	9.5	21.4
Phosphorus	Milligrams	99	5	66	231
Iron	Milligrams	0.9	0.	0.5	0.4
Zinc	Milligrams	0.7	0	0.4	0.7
Calcium	Milligrams	28	2	22	12
Sodium	Milligrams	71	55	8	41
Potassium	Milligrams	69	54	19	300
Magnesium	Milligrams	6	4	1	28
Manganese					1.64
Copper					0.03

Abbreviations: SFA- Saturated fatty acids, MUFA- Monounsaturated fatty acids, PUFA- Polyunsaturated fatty acids

Probst (2009)

Discrepancies between nutrient levels in the white+yolk vs. the whole egg are due to sampling error.

Chicken production accounts for approximately 25 % of the total meat production in SADC countries (FAOSTAT, 2014). The production of chicken meat and eggs is expected to increase, as demand is increasing. The common chicken breeds in South Africa are the Potchefstroom Koekoek, Venda, Naked neck and the Ovambo. Ovambo is the most popular indigenous chicken breed in KwaZulu-Natal (Tarwireyi & Fanadzo, 2013). The Ovambo chicken originated from the northern part of Namibia and Ovamboland (Van Marle-Köster & Nel, 2000). The Ovambo's plumage consists of mostly dark red, brown and black which aids to camouflage the bird and protect it from its predators. The chicken is also suitable and preferred for cultural ritual rites because of its dark feather colouring (Van Marle-Koster & Nel, 2000; Tarwireyi & Fanadzo, 2013). In addition, the Ovambo is aggressive and agile; it can fly and roosts in the top of trees to avoid predators (Van Marle-Köster & Casey, 2001). This self-defence mechanism is also another reason for its popularity among rural poultry farmers as their management systems are mainly free-range, extensive and semi-intensive.

2.10 Indigenous chicken production and utilization in VAD-prone rural communities

Indigenous chicken meat and egg are preferred by the majority in Africa, despite its low growth rate because of the texture, colour, taste and aroma of the meat and egg (Bett *et al*., 2013) They are also equally liked by high income earners for nutritional and health reasons, as poultry are white meat which is healthier than red meat such as beef (Farrell, 2013). Consumers also think that indigenous chicken meat and eggs are free from chemical contaminants, unlike products from commercial birds that may contain residual from synthetic supplements and growth promotants (Miao *et al*., 2005).

Table 2.5: Meat production (tonnes) compared with chicken production in SADC countries in 2012

Country	Total meat production	Chicken meat	% of chicken meat
Angola	225 560	23 400	10.4
Botswana	87 336	65 00	7.4
DRC	255 000	11 500	4.5
Lesotho	29 330	1 600	5.5
Madagascar	314 587	38 400	12.2
Malawi	131 800	22 400	17.0
Mauritius	51 822	47 200	91.1
Mozambique	176 476	23 400	13.3
Namibia	79 440	12 400	15.6
Seychelles	1 507	875	58.1
South Africa	2 776 152	1 488 662	53.6
Swaziland	26 696	5 600	21.0
Tanzania	440 755	56 500	12.8
Zambia	183 053	44 000	24.0
Zimbabwe	248 849	63 825	25.6
Average			**24.8**

DRC: Democratic Republic of Congo.
Source: FAOSTAT, 2014

Indigenous chickens provide eggs and meat for the rural household's own consumption, they are also sold to earn income, used as insurance and inheritance and are used for making traditional medicine (Magothe *et al.,* 2012; Melesse, 2014). Attributes such as the body weight, sex, plumage colour and health status of the indigenous birds are factors that affect their selling price (Moges *et al.,* 2010). Chickens are usually sold as live birds in the rural communities, however, the demand for dressed carcass of indigenous chickens, especially; in the urban where it can be sold at higher prices is increasing (Moges *et al*., 2010). Packaged whole dressed or cut parts of indigenous chickens are now available for purchase in supermarkets to meet the demands of the consumers. This increases the income of the resource- poor households (King'ori *et al*., 2010). It is, therefore, important to improve the productivity and quality of the indigenous chickens for improved food security and nutrition.

2.11 Impact of vitamin A on growth performance of chickens

Maize is one of the major ingredients in the feed of the chicken; therefore, the utilization of the provitamin A biofortified maize as feed for chickens is suggested. Vitamin A deficiency in chickens affects growth rate, feed utilization, development of bone, coordination, vision, reproduction, resistance against diseases, and mortality (Sklan *et al*., 1994; Weber, 2009). Vitamin A is needed by chicks for growth and by adult birds to maintain good health (Weber, 2009). Chickens have a fast response to vitamin A deficient dietary intake, as deficiency symptoms occur usually within 3 to 4 weeks (Uni *et al.,* 1998). The utilisation of carotenoids in the chicken's feed and conversion to vitamin A are influenced by many factors, and the efficiency of dispersion of carotenoids is affected by the presence or absence of other components in the diet, as well as by the general health status of the animal (Tanumihardjo, 2002). The conversion of carotene to vitamin A is tightly regulated and dependent on VA status and the amount administered in the dose or meal (van Lieshout *et al.,* 2001). It is important for food security practitioners to determine whether PABM can be a tool to improve feed utilization and growth performance of Ovambo chickens.

Compared to females, males significantly deposit more carotenoids in their when fed a carotenoid-supplemented diet (McGraw *et al*., 2002). These results indicate that sex-specific expression of carotenoid pigmentation in birds may be affected by the way males and females physiologically utilize (absorb, transport, metabolize, deposit) carotenoid pigments available to them in the diet (McGraw *et al*., 2002). Karadas *et al*. (2005) reputed that carotenoids fed to pullets are passed into the egg yolk, liver and other tissues. Increased vitamin A concentration in broiler's thigh and breast muscles were achieved when the chickens' diets were supplementation with retinol and β-carotene (Jensen *et al*., 1998).

Vitamin A deficiency causes loss of appetite which reduces feed intake (Uni et al., 1998). Reduced feed intake causes a reduction in the growth rate, followed by general weakness, staggering gait, and ruffled plumage (Portsmouth, 2013). The feed conversion ratio which is the efficiency with which feed consumed is converted into live weight gain has a major impact on productivity to the farmer (Losinger, 1998; Barbut *et al*., 2008). Age of birds, diet and sex affect the feed intake, body weight and daily weight gain in birds (Carrasco *et al*., 2014).

2.12 Influence of vitamin A on metabolism of indigenous chickens

Knowledge about the haematological parameters such as packed cell volume (PCV), erythrocyte concentration (RBC), leucocytes concentration (WBC), haemoglobin concentration (Hb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and serum biochemistry are important for assessing the nutritional, clinical and physiological responsiveness of birds (Khan & Zafar, 2005).

Haematological parameters of indigenous birds are influenced by various factors that include the sex, age and diet (Elagib & Ahmed, 2011). Serum biochemical constituents are positively correlated with the quality of the diet (Etuk, 2014; Rubio *et al*., 2014). Vitamin A increases the immune response in chickens and the serum biochemical constituents of chickens (Moghaddam & Emadi, 2014).

2.13 Effects of carotenoids and vitamin A diet in indigenous chickens on meat quality

Meat quality includes colour, tenderness (shear force), pH, cooking loss and drip loss (Bavelaar & Beynen, 2003). These qualities influence the acceptability and preference of meat by consumers (Ponsano *et al.*, 2004). Other factors that have been reported to have an influence on meat quality are breed, age and sex of the chicken (Musa *et al*., 2006; Baéza *et al*., 2012). The pH of meat is one of the important determining factors of what other meat attributes will be (Dadgar *et al*., 2011). Ultimate pH of the muscle directly influences the capacity of myoglobin to express the red colour in meat and its ability to bind water (Souza *et al*., 2011). The diet consumed by poultry has an impact on the meat quality produced. For example, yellow maize and other feeds containing carotenoids have been known to give yellowish colour to chicken's skin due to the presence of carotenoids (Tarique *et al*., 2013).

2.13.1 Nutritional composition of meat

Meat is rich in protein consist of essential micronutrient including vitamin A. Chicken meat, especially the breast part has high protein content (Pereira & Vicente., 2013). The meat from poultry tends to vary in chemical and nutritional composition between species and between different parts of the carcass. Chickens have both light and dark meats (caused by different muscle fibres) which differ in nutrient profile, contributing to differences in nutrient profile within species (Bogosavljevic-Boskovic *et al.*, 2015). Various factors such as breed, diet, environment and processing may affect the chemical composition of poultry (Bogosavljevic-Boskovic *et al.*, 2015). The nutritional composition of chicken meat has been successfully manipulated through the diet of the chickens (Jensen *et al*., 1998).

2.13.2 Sensory evaluation

Sensory characteristics such as taste, texture, colour and aroma are important quality features in meat (Bukala & Kedzior, 2001). Meat purchasing decisions are influenced by color more than any other quality factor because the colour of the meat is used as a physical indicator of the meat's freshness (Mancini & Hunt, 2005).

Raw meat has little or no aroma and a blood-like taste. Cooking plays a vital role in flavour development and it affects the acceptability and volatile flavour components of poultry meat

(Jayasena *et al*., 2013). Meat flavours are primarily contributed heat induced complex reactions between non-volatile components of lean and fatty tissues during cooking (Mottram, 1998; Jayasena *et al.,* 2013). Flavour comprises mainly of taste and aroma. It determines to a large extent a consumer's preference (Shahidi, 1998; Sitz *et al*., 2005). Flavour of chicken meat is also affected by various factors such as breed, diet presence of free amino acids and nucleotides, irradiation, high pressure treatment, cooking, antioxidants and ageing (Fanatico *et al*., 2007; Jayasena *et al*., 2013). Diet can either positively or negatively influence the flavour of chicken meat. The reported effects of sex on chicken meat flavour vary. Some researchers have reported no significant difference between the flavours based on sex (Ramaswamy & Richards, 1982; Farmer, 1999).

The effect of vitamin A supplement and some other natural sources of carotenoids inclusion on the growth performances, carcass characteristics, meat quality, haematology and sensory characteristics in the diet of bird, especially commercial birds, have been researched on to an extent, however, the effect of PABM diet inclusion as a natural source of vitamin A in the growth performances, carcass characteristics, meat quality, haematology and sensory characteristics of indigenous chicken have not yet been investigated. It is thus, important to cover this knowledge gap for the improvement of food security and nutrition among the resource- poor households.

2.14 Conclusion of literature review

There is a high rate of VAD in SSA. Provitamin A biofortified maize (PABM) was developed as an intervention to curb VAD among the resource-poor communities who majorly grow and consume staple crops. The acceptability of the consumption of PABM as a tool to curb VAD in Africa has, however, been low due to its unique organoleptic properties, cultural beliefs and African pride. It is thus suggested that as indigenous chickens have already been identified as a tool to curbing malnutrition in Africa and various means of improving their productivity, meat quality, nutritive value and health status have been researched on. There is, therefore, the need to identify the effect of PABM inclusion in the diet of the female and male indigenous chicken at several stages. The broad objective of the current study was, therefore, to determine the effect of PABM diet, sex and age of the Ovambo chicken on their growth, physiological parameters, meat quality and consumers' acceptability .

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CHAPTER 3

Effects of Provitamin A Biofortified maize Inclusion on Growth Performance of Ovambo Chickens

Abstract

The objective of the study was to determine the effect of provitamin A biofortified maize diet as a natural source of vitamin A on growth performance of male and female Ovambo chickens. A total of 102 female and male Ovambo chickens were reared and fed on two dietary treatments; the white maize (WM), the control, a low vitamin A diet and PABM diet for nine weeks. Each dietary treatment had four replicates. Average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were estimated. There was no significant effect of diet on the ADG, ADFI and FCR. There was an interaction ($P<0.05$) of diet, age and sex on ADG and ADFI. Male birds fed on the PABM had higher ($P<0.05$) ADFI at 15 weeks and 21 weeks of age than the WM fed male birds. Male birds had higher ($P<0.05$) ADG than the female birds at 15- 17 weeks of age. Male birds fed on the PABM diet had a significantly higher ADG at 16 weeks of age. The PABM fed female birds had a higher ADG than the WM fed females at all weeks except 21 weeks of age and was significantly higher at 19 weeks of age. The PABM fed female birds had significantly lower FCR at 20 weeks than the WM fed female birds. The male birds had higher ($P<0.05$) FCR at 21 weeks than the female birds. It can be concluded that the influence of PABM diet depends on the age and sex of the bird.

Keywords: age, average daily gain, average daily feed intake, feed conversion ratio, sex

3.1 Introduction

A large percentage of the population of southern Africa is poor. More than 33% of the population survive on less than R24 a day (Hoogeveen & Özler, 2006; Tarwireyi & Fanadzo, 2013). Indigenous chickens are important to the sustainability of rural livelihoods as they provide income, self-worth, and nutrients (Magothe *et al*., 2012). These chickens have a potential to improve food security and nutrition in the resource-poor households due to their wide availability, affordability and ease of slaughtering. Indigenous chickens are reported to be largely owned and used as a source of income by women and children who are the groups more vulnerable to malnutrition (Islam *et al.,* 2014).

Indigenous chickens require little inputs, are tolerant to high temperatures and adapt reasonably well to harsh environmental conditions. Their growth performance is, however, low (Tarwireyi & Fanadzo, 2013). The diet of indigenous chickens consists of food got from scavenging, leftovers and whole or crushed white or yellow maize grains that are low in vitamin A. This diet is a contributing factor to their low performance (Magothe *et al*., 2012). The demand and preference for indigenous chickens is on the increase in Africa due to distinct taste and flavour of the meat and the fact that their meat is free from contamination by antibiotics and other feed additives (Laroche Dupraz *et al*., 2008; Sodjinou *et al.,* 2014). The increase in consumption demand of indigenous chickens will improve the food security and nutrition status of the resource-poor households who rear indigenous chickens. However, growth performances of the indigenous chickens need to be improved to meet demand.

In chickens, vitamin A is important for reproduction, immunity, growth and development (Weber, 2009). Higher average daily gain (ADG) and feed conversion ratio (FCR) were reported in chickens supplemented with vitamin A at specific ages indicating an interaction of diet and age of birds (Bhuiyan *et al*. 2004). The average daily feed intake (ADFI) of chickens was also influenced by the supplemented vitamin A (Bhuiyan *et al.,* 2004). Reduced feed intake compromises growth rate, followed by general weakness, staggering gait, and ruffled plumage (Portsmouth, 2013). Due to the importance of vitamin A in the diet of birds, intensively managed broilers are supplemented with synthetic vitamin A. Although, indigenous birds get some amount of vitamin A from scavenging, it is difficult to determine whether it is sufficient or insufficient as the birds consume varying diets as they are kept on free range management system. It is, therefore, important to evaluate if the available natural sources of vitamin A intake improves the growth performances of the indigenous chickens. This is important for enhanced livelihood and food security and nutrition of the rural poor with minimal financial implications using methods that are simple and appropriate for the smallholder farmers.

Provitamin A biofortified maize is a natural source of β-carotene, a provitamin A carotenoid which is converted to vitamin A when consumed. Natural sources of carotenoids such as broccoli stem and leaf meal have been used to replace various nutrients including β carotene in the diet of chickens (Hu *et al*., 2012). It is, however, not known if the use of PABM will improve the growth performance of indigenous chickens. It is also not clear whether the male and female indigenous chickens have similar capabilities to utilize PABM.

The Ovambo chicken is one of the common indigenous chicken breeds in southern Africa (Van Marle-Köster & Nel, 2000). The average mature weight of Ovambo male and female are 2.16 and 1.54 kg, respectively (Ramsey *et al.,* 2000). The objective of the current study was, therefore, to compare the effect of PABM on the growth performance of the male and female Ovambo chickens. It was hypothesized that the utilization of the PABM on growth performance of the Ovambo would not be affected by the age and sex of the bird.

3.2 Materials and methods

3.2.1 Ethical considerations of the study

The management and care of the chickens were in accordance with internationally accepted standards for the welfare and ethics of chickens (Austin *et al*., 2004). Management and use adopted for the study were approved by the University of KwaZulu-Natal Animal Ethics Research Committee (019/14/Animal: Appendix 1).

3.2.2 Study site

The study was conducted between September 2014 and February 2015 at Cedara College of Agriculture. The site is located in the upland savannah zone on 29.53^{o}S and 30.27^{o}E. The average environmental temperature was 21.3^{o}C and the relative humidity an average of 63.2 % (Table 3.1).

Table 3.1: Average minimum and maximum temperature and average relative humidity from 13 to 21 weeks of age of the trial

		Temperature (^{0}C)		Relative humidity (%)
Weeks	Mean	Minimum	Maximum	
13	22.4	16.4	28.3	76.6
14	23.5	18.0	29.0	71.8
15	22.9	18.6	27.1	75.6
16	24.4	19.3	29.4	61.3
17	25.4	18.1	32.6	61.8
18	21.8	16.1	27.4	60.5
19	20.5	14.3	26.7	63.0
20	17.9	12.1	23.7	56.3
21	16.5	13.6	25.4	61.1

3.2.3 Birds, treatments and their management

A total of 200 unsexed Ovambo chicks that were hatched from parent stock by the Agricultural Research Council (ARC), Irene, Pretoria, were raised together in a well-ventilated floor area under a deep litter management system at Cedara College of Agriculture where the research was conducted. Floors were adequately covered with wood shavings. A broiler starter meal was given *ad libitum* to the chickens from Day 1 to Day 49 and grower diet was given from Day 50 to 91. The chemical compositions of the starter and grower diets are given in Table 3.2. Water was offered *ad libitum* in 4L plastic founts and feed was provided in tube feeder made of standard gutter materials. Light and heat using infra-red were provided continuously. The birds were vaccinated against Newcastle disease at 14 days of age. Gumboro vaccine was also given at 6 weeks of age. The vaccinations were administered orally by putting the vaccines in the drinking water of the chickens.

Two dietary treatments were used. The control, with low level of vitamin A (WM), was formulated with 100 % white maize. The high vitamin A (PABM) was formulated with 100 % PABM maize variety HP326-6 (Table 3.3). The PABM maize was obtained from the Makhathini Research Station, Jozini, KwaZulu-Natal, where it was planted in April, 2014. The aim of biofortification of maize with provitamin A was to increase the concentration of β carotene in the endosperm of the maize. Temperate maize has more β- carotene than the tropical maize (Yan *et al*., 2010). Therefore, the high provitamin A temperate maize sources was tropicalized to convert it to the popular African open pollinated varieties and inbred lines. The inbred line was planted at a spacing of 0.75 m between rows and 0.25 m between plants within a row

Table 3.2: Chemical composition of basal starter and grower diet

Composition	Starter	Grower
Crude protein (g/kg)	200	180
Metabolisable energy (MJ)	12.8	13.0
Fat (g/kg)	25.0	25.0
Crude fibre (g/kg)	50.0	60.0
Dry matter (g/kg)	880.0	880.0
Calcium (g/kg)	12.0	12.0
Lysine (g/kg)	12.0	10.0

Supplied by Smith Animal Feeds. Pietermaritzburg, South Africa
ME/CP= Metabolisable energy/ crude protein

Table 3.3: Feed composition of experimental diets

Ingredients	(Control-WM) kg	PABM
Provitamin A biofortified maize	0.0	417.7
White maize	417.7	0
Soyameal	175.4	175.4
Vegetable oil	23.8	23.8
Limestone	12.3	12.3
Dicalcium phosphate	6.9	6.9
Salt	1.9	1.9
DL-Methionine	1.2	1.2
L-Lysine	0.1	0.1
Vit.-min. premix (excluding vit A)	3.2	3.2
Nutrient composition		
Metabolisable energy (MJ/kg)	12.56	13.01
Crude protein (g/kg)	199	198
Fat (%)	3.52	5.09
Ash (%)	11.04	9.73
Calcium (%)	1	1
Phosphorus (%)	0.74	0.81
Provitamin A carotenoids(mg/kg)	0.1	0.5

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

Fertilizer and field management practices recommended for optimum maize production were used (Azmach *et al*., 2013). The maize was grown in a secluded area to prevent cross pollination from other maize varieties. After the maize were harvested, it was dried sufficiently, shelled, bagged in vacuum sealed bags and stored in a cool, pest free and dark place to preserve the quantity and quality of the pro-vitamin A carotenoids.

The PABM and WM maize were milled separately into flour using a hammer mill (Glen Creston, Stanmore, England) fitted with a 1.0 mm sieve. The fat content of dry milled maize flour was determined using Soxhlet extraction method (Association of Official Analytical Chemists (AOAC), 1984) About 2 g maize flour was weighed into the Soxhlet cellulose extraction thimbles. The thimbles were plugged with a cotton wool to avoid loss of sample. The thimbles were transferred into the Soxhlet extractor. Sufficient petroleum ether was poured into the Soxhlet extractor beakers. The beakers were clamped into the Soxhlet machine and the electric heating plates were lifted to the base of the beakers. The samples were subjected to extraction for 3 hours. Recovered solvent was transferred into an air oven (100^oC) for 1 hour and then cooled in desiccators and weighed. The amount of oil extracted was calculated and expressed as a percentage of the original sample.

To determine the crude protein, the total nitrogen content was determined by Kjeldahl nitrogen analysis, according to AOAC (1995). The percentage of crude protein was estimated by multiplying the total nitrogen content by a factor of 6.25. To determine the ash/ mineral content, dry samples of milled maize was weighed in to a dry porcelain dish and heated in a muffle furnace at 600^0C for 12 hours after which it was placed in desiccators for cooling before it was weighed. The percentage ash content was calculated as: % ash = weight of ash x 100/ weight of sample (AOAC, 1980). The gross energy values were estimated by multiplying the crude protein, fat and carbohydrate by their water values of 4, 9 and 4 kcal/g, respectively. Each sample was analyzed in triplicate. Calcium and phosphorus were determined by atomic absorption spectrophotometer method, and colorimetrically respectively according to AOAC (1984). Carotenoid analysis was carried out using a HewlettPackard 1100 HPLC (Agilent Technologies Incorporated, Loveland, CO, USA) consisting of a binary pump, autosampler, column thermostat, diode array detector and ChemStation software (Revision B.03 02, Agilent Technologies Incorporated, Loveland, CO, USA).

The birds were acclimatised to their experimental pen environment for 14 days prior to the commencement of the trial. During this period, the birds were fed a common proprietary grower diet (Table 3.2). The pens were placed in open sided houses with cement floor on a deep litter system. The wood shavings were changed fortnightly or whenever there was water spillage.

At 13 weeks of age, 44 male and 58 female birds of similar body weight were selected from the 200 birds raised from day one, weighed and randomly placed in 16 pens (eight pens were allocated for each diet, four pens for males and four pens for the females). The initial individual weights of the birds were 1.5 ± 0.5 and 1.0 ± 0.5 kg for males and females, respectively. The pens were 230 cm long, 13cm wide and 120 cm high. Each experimental unit, represented by a pen, contained a minimum of five birds. A minimum of 15h light was provided daily throughout the experimental period. No antibiotic or growth promotant was administered. Water and feed were given *ad libitum*. Water was provided in 4L plastic founts and the feed was given in a 10L plastic hanging feeders.

3.2.4 Data collection

Body weights were obtained by weighing the chickens weekly from day 1 of the experiment (13 weeks of age) using a Model MEASURETEK OCS-L digital electronic hanging scale (Scale Tronic Services, South Africa). Weekly body weights were collected early in the morning before 08:00 am at the end of each experimental week for uniformity and accuracy. The data from the weekly body weights were used to calculate the average daily gain per bird (ADG). Feed intake was measured weekly to determine the mean daily feed intake per bird (ADFI). Feed consumption was measured every week to establish the mean daily feed intake per bird (ADFI). The ADFI was calculated as the amount of feed consumed weekly minus spillage divided by the number of chickens in a pen. Feed conversion ratio (FCR) was calculated as the amount of feed consumed per unit of body weight gain per bird. Data were collected and calculated weekly from 13 weeks of age to 21 weeks of age.

3.2.5 Statistical analyses

The data were analyzed using the GLM (General linear model) procedure of Statistical Analysis System (SAS) 2010 with repeated measures and. The initial weights of the measures were used as covariates. The model used included diet, age and sex as fixed variables. Least square means were compared using the PDIFF procedure of SAS (2010). Statistical significance was considered at the 5% level of probability.

The model used was:

$Y_{ijkl} = \mu + D_i + S_j + A_k + (D \times S)_{ij} + (D \times A)_{ik} + (D \times S \times A)_{ijk} + E_{ijkl}$

Where: Y_{ijkl} = response variable (ADG, ADFI, FCR);

μ= the overall mean

D_i= effect of the ith diet with i = PABM and WM diet;

S_j= effect of the jth sex with j= male and female;

A_k= effect of the kth age with k= 13, 14…..21 weeks;

$(D \times S)_{ij}$= interaction of the ith diet and the jth sex;

$(D \times A)_{ik}$= interaction of the ith diet and the kth age of bird;

$(D \times S \times A)_{ijk}$ = interaction of the ith diet, jth sex of bird and the kth age of bird;

E_{ijkl}= random error term assumed to be normally and independently distributed with mean 0 and variance equal to ð$^{2.}$

3.3 Results

No chick deaths were recorded during the entire experimental period.

3.3.1 Effect of diet, sex and age on the average daily feed intake of Ovambo chickens

There was no significant difference of diet on ADFI. There was significant difference of the influence of sex on the ADFI. The age of the birds influenced (P<0.05) ADFI. Significant interaction of diet, age of bird and sex on ADFI was observed. There was no interaction of diet and age (P>0.05) on ADFI (Table 3.4). Across the age groups, the ADFI of male birds on the PABM diet were significantly higher than female birds fed on the PABM diet. Male birds fed on the PABM had higher (P<0.05) ADFI at 15 weeks (95.48 ±3.42g) and 21 weeks(97.42 ± 3.42g) of age than the WM fed male birds (88.38± 3.42 and 88.58 ±3.42 respectively) (Figure 3.1).

3.3.2 Effect of diet, sex and age on the average daily gain of Ovambo chickens

There was no significant effect of diet on ADG, although a significant influence of sex on the ADG was noticed. The age of the birds affected (P<0.05) ADG. There was a significant interaction of diet, age and sex on ADG (Table 3.4). The ADG of male birds fed on the PABM diet was higher than those fed on the WM diet during all the weeks, except at 18 weeks, where the WM fed male birds had a significantly higher ADG. Male birds fed on the PABM diet had a significantly higher ADG at 16 weeks of age (23.53 ± 3.04g) than the WM fed males (14.48 ±3.04g) The PABM fed female birds had a higher ADG than the WM fed female at all weeks except 21 weeks of age and was significantly higher at 19 weeks of age (11.18 ± 3.04g and 5.72 ± 3.04g for PABM and WM fed females respectively). The highest ADG for the PABM fed male and female birds were at 15 weeks of age (28.3 ± 3.04 g) and 14 weeks of age (22.6 ± 3.04 g), respectively. Regardless of their diets and sex, the ADG of the birds increased from week 14 to week 17. Male birds had higher (P<0.05) ADG than the female birds at 15- 17 weeks of age (Figure 3.2).

Table 3.4: Levels of significance of effects of diet, sex and age of bird on growth performance of Ovambo chickens

Source of variation	ADFI	ADG	FCR
Diet	NS	NS	NS
Sex of bird	***	***	NS
Age of bird	**	***	***
Diet × sex	**	NS	*
Diet × age	NS	NS	NS
Diet × age × sex	*	***	NS

ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio
NS: not significant; * P< 0.05; ** P < 0.01; *** P < 0.001

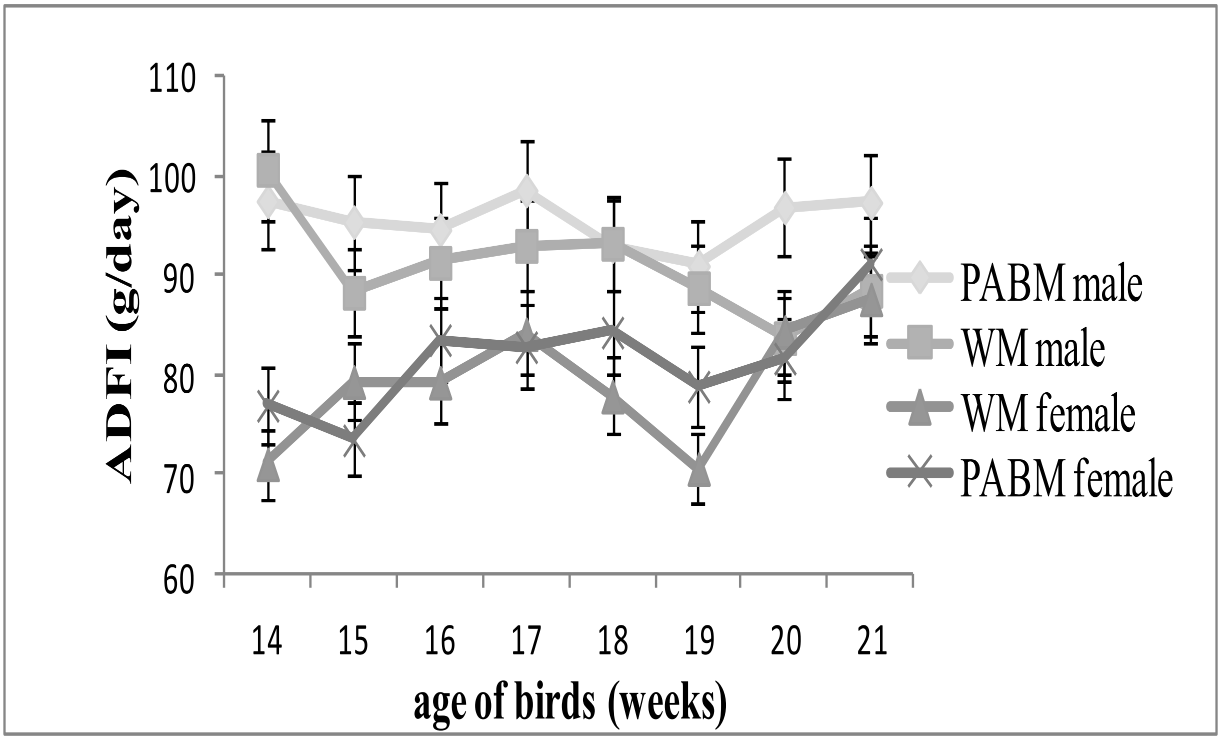

Figure 3.1: Effect of diet, sex and age of bird on ADFI in growing Ovambo chickens

WM: white maize; PABM: provitamin A biofortified maize; ADFI: average daily feed intake; g/day: grams per day

3.3.3 Effect of diet, sex and age on the feed conversion ratio of Ovambo chickens

There was no significant difference (P>0.05) of diet on the FCR and the influence of sex on the FCR. The age of the birds had an influence (P<0.05) on all the FCR, and there was influence of age and sex (P<0.05) on FCR. The interaction of diet, age and sex on FCR was not significant (Table 3.4). There was an overall increase in FCR of all the birds regardless of their diets as they increased in age until week 19 when the FCR started to decrease. The PABM fed female birds had significantly lower FCR at 20 weeks (8.36 ±2.46) than the WM fed female birds (12.86 ±2.46).. The male birds had higher (P<0.05) FCR at 21 weeks (14.99 ±2.46 for PABM fed males and 13.39 ±2.46 for WM fed males) than the female birds (9.74 ±2.64 for the PABM fed females and 9.06 ±2.64 for the WM fed female birds) (Figure 3.3).

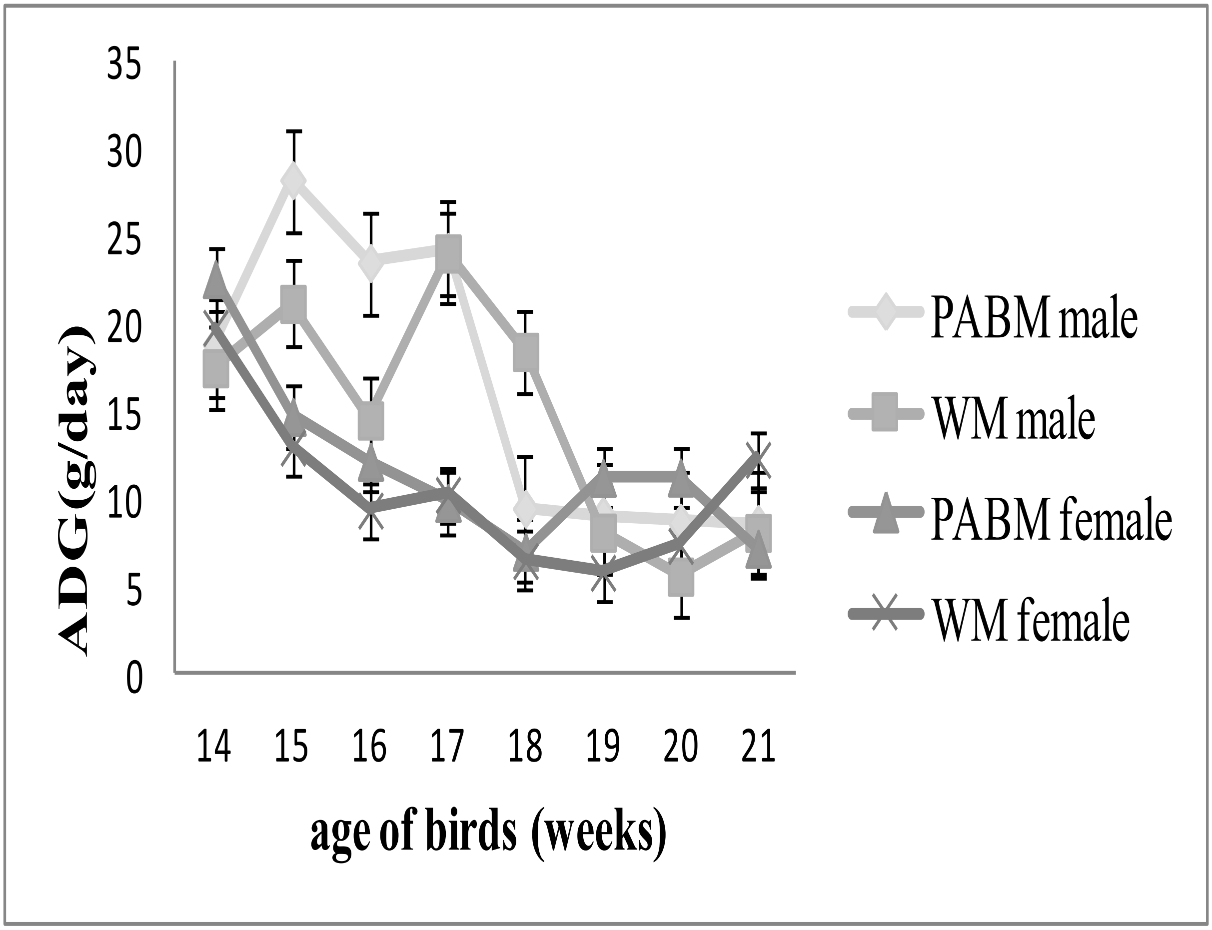

Figure 3.2: Effect of diet, sex and age of bird on the ADG of Ovambo chickens

WM: white maize; PABM: provitamin A biofortified maize, ADG: average daily gain; g/day: grams per day

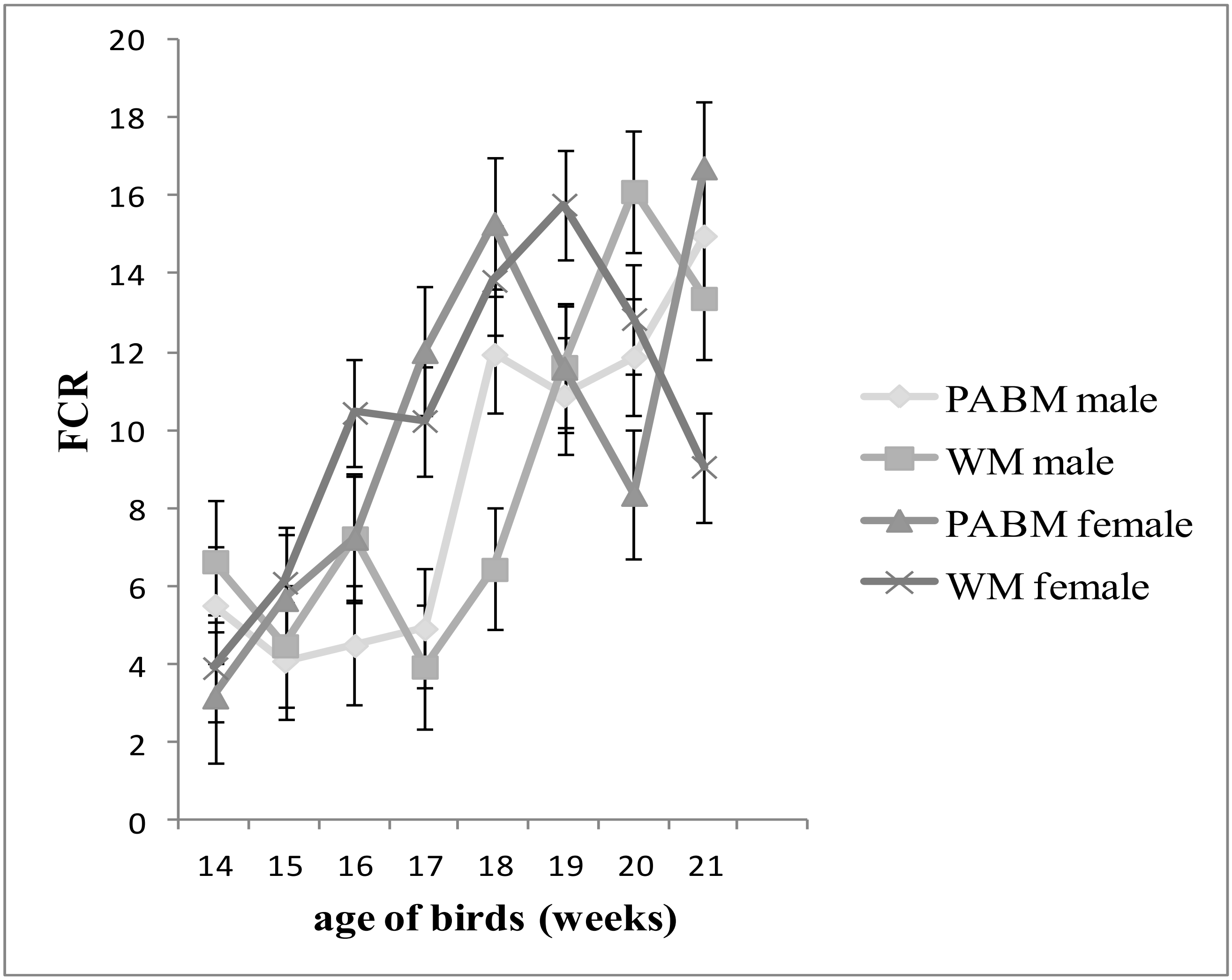

Figure 3.3: Effect of diet, sex and age of bird on the mean of FCR of the Ovambo chicken

WM: white maize, PABM: provitamin A biofortified maize, FCR: feed conversion ratio

3.4 Discussion

The observation that vitamin A had no effect on the ADFI, ADG and FCR is similar to earlier reports (Moghaddam *et al*., 2010; Ogbamgba & George, 2015). Hu *et al*. (2012) reported that the inclusion of broccoli stem and leaf, high in β-carotene in the diet of broilers did not influence their growth performances. The findings of the current study on the non- significant effect of PABM diet on the ADFI, ADG and FCR, however, are in contrast to those from Nikodémusz *et al*. (2010) who reported that birds fed dietary Spirulina, a natural ingredient that is high in carotenoids, increased the productive performance of birds. Sirri *et al*. (2007) also reported improved FCR when broilers were supplemented with marigold extract, a natural ingredient also high in carotenoids. The difference in the present study's findings on ADFI, ADG and FCR to these reports could be due to the presence and amount of nutrients and other carotenoids different from β-carotene. These could have been present in the experimental diets used by Sirri *et al*. (2007) and Nikodémusz *et al*. (2010), which were different forms of natural sources of vitamin A. The strains and ages of the birds used were also different from the current study.

Age is a determining factor of how much feed a bird consumes. As chickens grow, their nutrient requirements increase causing them to increase their feed intake (Sohail *et al*., 2013). Male birds consume more than female birds (Taha *et al*., 2010), probably because bigger birds consume more feed to meet the maintenance requirements and male birds are bigger than female birds of the same age (El- Hossary & Dorgham, 1992). The difference in ADFI based on diets of the female birds was not significant except at the 19th week. The PABM male birds' ADFI was significantly higher at 15 and 20 weeks of age than the WM fed male birds.

The non-significant effect of PABM diet on ADG of birds is similar to the findings of Beckford and Bartlett (2015) who fed sweet potato meal which is high in β carotene to broilers. The observation in the current study that male birds had a higher ADG than the female birds is similar to earlier findings (Tadelle *et al*., 2003; Moghaddam *et al.,* 2010; Osei-Amponsah *et al*., 2012; Salim *et al*., 2012). The higher ADG in males could be due to the higher ADFI and due to the growth hormones in male birds that are more active in comparison to the females and sexual sex dimorphism (Szekely *et al*., 2007). The ADG of indigenous chickens has been reported to start declining from the 14th week (Magothe *et al*., 2010). The decline in the present study which started at 15 weeks in the female birds and at 16 weeks in the male birds with variations in the growth rate corresponds to earlier report of Raach-Moujahed *et al.* (2011). The significant interaction of PABM diet and age on ADG agrees with Bhuiyan *et al*. (2004). The significant effect of the PABM diet and age on ADG could be as a result of the onset of vitamin A deficiency which reduced the appetite in those fed WM diet and became evident after 4 weeks of the experiment period. The higher ADG in male chickens fed on the PABM diet in comparison to WM male birds agrees with earlier report that supplemental vitamin A significantly increased the ADG in supplemented broilers (Kucuk *et al*., 2003).

The FCR of birds fed on vitamin A supplements were not significantly better than those with no vitamin A supplements. This agrees with earlier reports (Raza *et al*., 1997; Moghaddam *et al*., 2010). The finding that FCR is not affected by sex of the birds agrees with Tadelle *et al*. (2003). The interaction of diet and sex is however, significant. Higher FCR were reported in vitamin A supplemented chickens at specific ages in comparison to diet that were not supplemented with vitamin A (Bhuiyan *et al.,* 2004). The effect of age where the male had higher FCR than the females until 20 weeks of age is supported by the findings of Osei-Amponsah *et al.* (2012). The FCR of indigenous breeds fed a standard commercial diet in Ethiopia from day 1 to 22 weeks of age reared under intensive management system ranged from 11.0 to 18.9 (Hassen *et al.,* 2006), while the current study's FCR ranged from 3.2 to 16.7. The FCR of the current study is thus lower and better.

3.5 Conclusions

There was a significant interaction of diet, age and sex on the ADFI and ADG. The PABM diet increased the ADFI and ADG of the birds depending on their age and sex. The PABM diet improved the FCR of the females at 20 weeks of age. The ADG and FCR improved at 17 weeks in male birds and at 20 weeks in female birds. Thus, use of provitamin A biofortified maize as a source of PABM diet depends on age and sex of the bird, especially the growth performances of male Ovambo chickens at 14 , 17 and 20 weeks of age. It is, however, important to also assess the effect of PABM diet, sex and age not only the growth performance of the Ovambo, but also on the most preferred and consumed chicken parts and edible offals.

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CHAPTER 4

The Effects of Provitamin A Biofortified Maize Diet Inclusion on Carcass characteristics and Organ Weights of Ovambo Chickens

Abstract

The study objective was to determine the effect of PABM diet, age and sex of Ovambo chickens on carcass and organ weights. A total of 102 male and female Ovambo chickens were randomly distributed and fed on WM, a low vitamin A diet as the control, and PABM diet. Each treatment had 4 replicates. Birds fed on the PABM diet had a higher dressed carcass (DC) and relative leg weights. The sex of the birds significantly affected all the carcass measurements, except the DC. Female birds had higher ($P<0.05$) relative breast and back weights, while male birds had higher body weight (BW) and leg weights ($P<0.05$). Male birds fed on the PABM diet had a significantly higher relative breast weight than those fed on the WM diet. The DC, leg (comprises of both the thigh and drumstick) and back relative weights of the birds were significantly higher as the age at slaughter increased. The relative leg weights of the birds were significantly lower as the age at slaughter increased. Birds fed on the PABM had a higher ($P<0.05$) relative weight of gizzard and liver than birds fed on the WM diet. Female birds had higher ($P<0.05$) relative weights of gizzard and liver than male birds. Male birds had a higher relative heart weight ($P<0.05$) than female birds. The relative heart weight of the male birds increased with age while the weight of the gizzard and liver in female birds decreased with age ($P<0.05$).

Keywords: carcass weights, edible offals, sex, age, provitamin A biofortified maize, white maize

4.1 Introduction

Indigenous chickens are one of the major sources of protein and income for resource-poor households (Mtileni *et al.,* 2009). They are kept more for their meat than for egg production (Tarwireyi & Fanadzo, 2013). Chickens are sold as live birds, dressed whole carcass or in cuts. Consumers' preference for whole chicken is shifting to preferences for specific chicken cuts (Mehaffey *et al.,* 2006; Abdullah *et al.,* 2010). Indigenous chicken are usually sold as whole dressed chickens, however, a large number of consumers also have preference for specific chicken cuts such as the breast and leg thigh and drumstick parts (Cevger *et al.,* 2004; Khosravinia *et al.,* 2006). The market price for indigenous chicken meat, apart from health and sensory preferences, also depends on the size of the bird, and the size of the desired carcass parts and sex of the bird (Kennedy *et al*., 2004). The type of chicken cut, sex and age of the birds are some of the criteria that influence the purchasing decision of the consumer (Pirvutoiu & Popescu, 2013). The offals (heart, liver and gizzard) are also widely consumed in Africa, particularly by children. They are cooked as delicacies in many developing countries (Omojola *et al.,* 2004). Although, the purchasing price of the male birds is higher, there is also a ready market for female birds.

Diet, age and sex of a bird affect productivity of chickens (Chapter 3; Brickett *et al.,* 2007). Therefore, replacement of yellow and white maize with PABM, should improve productivity of the chickens as vitamin A is important for growth, development, and immunity. Indigenous chicken attain table weight (of about 1.2 kg) between 16 and 21 weeks of age (Wattanachant, 2008). It is important to determine the extent to which age at slaughter and sex will affect the carcass, especially the preferred chicken parts and the weight of the offals. Sexual dimorphism affects the growth and carcass characteristics of birds (Fanatico *et al*., 2005; Zhao *et al*., 2012). The aim of the poultry farmer is to have enough meat to feed his household and meet consumers' demands and preferences. The desire of the resource poor farmers is, therefore, to get heavier birds or bigger cuts of chickens at an earlier age to meet consumers' demand for improved livelihood (Grobbelaar *et al*., 2010). There is, however, no information on the effect of the interaction of PABM diet, sex and age on the carcass and organ characteristics of the South African indigenous chickens and on the utilization of PABM diet.

The objective of the current study was to determine the effect of the PABM diet, age and sex of bird on the carcass and offals of Ovambo chickens. It was hypothesized that there is no effect of PABM, age and sex bird or their interactions on the weight of the carcass cuts and offals from Ovambo chickens.

4.2 Materials and methods

4.2.1 Study site and the ethical aspect of the study

The detailed description of the study site, average ambient temperature and relative humidity, and the ethics approval for management and use of birds are given in sections 3.2.1 and 3.2.2.

4.2.2 Birds, treatments and their management

The details of the type of strain, number of birds, dietary treatments, management of the birds and the agronomic practices used for planting the PABM are provided in section 3.2.3.

4.2.3 Data collection

Body weights were obtained by weighing the chickens weekly from day 1 of the experiment (13 weeks of age) using a Model MEASURETEK OCS-L digital electronic hanging scale (Scale Tronic Services, South Africa). At 18 weeks and 21 weeks (6 weeks and 9 weeks after the beginning of the experimental period, respectively), 16 birds from each dietary treatment, i.e. eight female and eight males, two birds from each replicate were randomly selected for the measurement of carcass characteristics and internal organ weights. Feed was withdrawn 12h before slaughter. The birds were slaughtered in the early morning by decapitating the head from the neck with a sharp knife. The carcasses were scalded in hot water (at about 70^{o}C) and the feathers plucked manually. The carcasses were eviscerated to separate the portions, as described by Kleczek *et al.* (2006). The leg was obtained by cutting through the hip joint (from the pubic process, through the groin towards the back, and then along the backbone, starting from the anterior border of the pelvis). The breast was obtained by a double cut through the cartilaginous junctures of the ribs, from the inferior border of the backbone towards the coracoids. The back was defined as the dorsal–lumbar quarter. The liver, heart and gizzard (without contents) were also collected, cleaned and weighed. A digital electronic scale (Jadever JPS-1050, Micro Preciso Calibraton Inc, USA) was used to measure the weights. The carcass yields were expressed as ratio of the carcass weight (dressed carcass) and the weight of the internal organs were expressed as a proportion of body weight.

4.2.4 Statistical analyses

The data were analyzed using GLM procedure of SAS (2010). The model used incorporated the diet, age and sex of bird as fixed variables. Least square means were compared using the PDIFF procedure (SAS, 2010). Statistical significance was considered at the 5 % level of probability.

The model used was:

$Y_{ijkl} = \mu + D_i + S_j + A_k + (D \times S)_{ij} + (D \times A)_{ik} + (D \times S \times A)_{ijk} + E_{ijkl}$

Where: Y_{ijkl} = response variable (carcass weights and internal organ weights)

μ= the overall mean

D_i= effect of the i^{th} diet with i = PABM and WM diet;

S_j= effect of the j^{th} sex with j= male and female;

A_k= effect of the k^{th} age with k= 18 and 21 weeks;

$(D \times S)_{ij}$= interaction of the i^{th} diet and the j^{th} sex;

$(D \times A)_{ik}$= interaction of the i^{th} diet and the k^{th} age of bird;

$(D \times S \times A)_{ijk}$ = interaction of the i^{th} diet, j^{th} sex of bird and the k^{th} age of bird;

E_{ijkl}= random error term assumed to be normally and independently distributed with mean 0 and variance equal to $ð^{2.}$

4.3 Results

4.3.1 Effect of diet, sex and age on the body weight, relative dressed carcass and leg weight of Ovambo chickens

The PABM diet had a significant effect on the dressed carcass (DC) and leg weight, Sex significantly affected the body weight (BW), DC and leg weight. Age of slaughter of the birds significantly affected the DC and leg. The interaction of diet and sex had an effect ($P<0.05$) on the DC. The interaction of age and sex significantly influenced the body weight (BW) and leg weight (Table 4.1).

Male birds significantly had higher BW than the female at both ages at slaughter. Female birds fed on the PABM diet for 21 weeks had a significantly higher body weight than at 18 weeks (Table 4.2). Male fed on the WM for 18 weeks of slaughter had a significantly higher DC than the female fed WM at the same age of slaughter. Females fed on the PABM diet had higher DC at 18 and 21 weeks of slaughter than females fed on the WM diets (Table 4.2). The relative leg weights of the males were significantly higher than the females at 18 and 21 weeks when slaughtered. The relative leg weight of both the male and female birds were higher ($P<0.05$) at 18 weeks than at 21 weeks of age. Female birds fed on the PABM diet for 18 weeks had higher ($P<0.05$) leg weights than those fed on the WM diet (Table 4.2).

Table 4.1: Levels of significance of effects of diet, sex and age of bird on carcass characteristics of Ovambo chickens

Source of variation	BW	DC	Leg	Back	Breast
Diet	NS	*	*	NS	NS
Sex of bird	**	NS	**	**	**
Age of bird	NS	**	**	**	NS
Diet × sex	NS	**	NS	**	NS
Age × sex	***	NS	*	*	*
Diet × age	NS	NS	NS	NS	NS
Diet × age × sex	NS	NS	NS	NS	NS

BW: body weight, DC: dressed carcass (expressed as ratio of body weight.), leg (expressed as ratio of dressed carcass weight),), back (expressed as ratio of dressed carcass weight), breast (expressed as ratio of dressed carcass weight).
NS: not significant; *P< 0.05; ** P < 0.01; *** P < 0.001

Table 4.2: Effect of diet, sex and age of slaughter of birds on the body weight, dressed carcass and leg of Ovambo chickens

Age at slaughter	Diet	Sex	BW (kg)	DC	Leg
		Male	2.25^{c}	0.69^{b} (1.61)	0.17^{d}(0.27)
	WM	Female	1.63^{a}	0.67^{a} (1.14)	0.14^{c} (0.16)
18 weeks					
		Male	2.35^{cd}	0.69^{b} (1.69)	0.17^{d} (0.28)
	PABM	Female	1.58^{a}	0.70^{b} (1.05)	0.16^{d} (0.16)
		Male	2.35^{cd}	0.72^{c} (1.59)	0.13^{b} (0.25)
21 weeks	WM	Female	1.76^{ab}	0.70^{bc} (1.27)	0.10^{a} (0.16)
		Male	2.45^{d}	0.71^{c} (1.69)	0.13^{bc} (0.28)
	PABM	Female	1.89^{b}	0.72^{c} (1.33)	0.11^{a} (0.18)
Pooled SEM			0.068	0.006	0.003

Values with different superscripts in the same column differ (P<0.05).
WM: white maize (control); PABM: provitamin A biofortified maize.
BW: body weight, dressed carcass (expressed as ratio of body weight); leg (expressed as ratio of dressed carcass weight).Figures in parentheses are the actual means of carcass traits in kg.
SEM: standard error of means

4.3.2 Effect of diet, sex and age on the relative breast and back weight of Ovambo chickens

The sex of the bird significantly influenced the breast and back yields while the age at slaughter significantly affected the relative back weight. There was an interaction of diet and sex on ($P<0.05$) relative back weights. The interaction of age and sex significantly influenced the back and breast weight (Table 4.1). Male birds fed on the PABM diet had a significantly higher relative breast weight than those fed on the WM diet. The relative breast weights of females were higher than those for male chickens in both diets but it was only significant in the WM fed females at both 18 and 21 weeks (Table 4.3). The PABM fed male and female chickens had a significantly higher relative back weight than the WM fed chickens. The female birds slaughtered after 21 weeks had a higher back weight compared to the male birds at 21weeks of age. The birds at 21 weeks of age had a higher ($P<0.05$) back weight than at 18 weeks of age, except for male birds fed on the WM diet (Table 4.3).

Table 4.3: Effect of diet, sex and age of slaughter of birds on the breast and back weight of Ovambo chickens

Age at slaughter	Diet	Sex	Relative breast weight	Relative back weight
18 weeks	WM	Male	0.23^{a} (0.41)	0.22^{a} (0.35)
		Female	0.27^{c} (0.31)	0.24^{a} (0.27)
	PABM	Male	0.25^{bc}(0.39)	0.23^{a} (0.39)
		Female	0.27^{c} (0.28)	0.22^{a} (0.24)
21 weeks	WM	Male	0.24^{ab} (0.41)	0.23^{a} (0.36)
		Female	0.28^{c} (0.34)	0.26^{b} (0.36)
	PABM	Male	0.26^{bc}(0.40)	0.26^{b} (0.43)
		Female	0.27^{bc} (0.37)	0.28^{c}(0.34)
Pooled SEM			0.008	0.008

Values with different superscripts in the same column differ ($P<0.05$).
WM: white maize (control); PABM: provitamin A biofortified maize.
Relative breast weight (expressed as ratio of body weight; relative back weight (expressed as ratio of dressed carcass weight). Figures in bracket are actual means of carcass traits in kg.
SEM: standard error of means

Table 4.4: Levels of significance of effects of diet, sex and age at slaughter of birds on offal weights of Ovambo chickens

Source of variation	**Gizzard**	**Liver**	**Heart**
Diet	*	*	NS
Sex of bird	**	**	**
Age of bird	**	NS	NS
Diet× sex	**	NS	NS
Age × sex	NS	NS	*
Diet × age	NS	NS	**
Diet × age × sex	NS	**	NS

*P< 0.05; ** P < 0.01; NS: not significant.

Table 4.5: Effect of diet, sex and age of slaughter of birds on the relative gizzard, liver and heart of the Ovambo chickens

Age at slaughter	Diet	Sex	Gizzard	Liver	Heart
18 weeks	WM	Male	0.021^{b}(0.049)	0.013^{a} (0.032)	0.006^{cd} (0.014)
		Female	0.026^{c} (0.042)	0.018^{bc} (0.029)	0.005^{bcd} (0.008)
	PABM	Male	0.021^{b} (0.051)	0.016^{b} (0.041)	0.006^{cd} (0.014)
		Female	0.031^{d} (0.049)	0.017^{b} (0.026)	0.004^{ab} (0.007)
21 weeks	WM	Male	0.019^{ab}(0.043)	0.014^{ab} (0.043)	0.006^{d} (0.013)
		Female	0.022^{b}(0.038)	0.016^{ab} (0.037)	0.004^{a} (0.007)
	PABM	Male	0.017^{a} (0.040)	0.015^{ab}(0.047)	0.007^{e} (0.016)
		Female	0.026^{c} (0.047)	0.019^{c} (0.048)	0.005^{bc} (0.010)
Pooled SEM			0.001	0.001	0.0004

Values with different superscripts in the same column differ (P<0.05)
WM: white maize (control); PABM: provitamin A biofortified maize.
Relative gizzard, liver and heart weight (expressed as ratio of body weight). Figures in parentheses are actual means of edible offals in kg.
SEM: standard error of means.

4.3.3 Effect of diet, sex and age on the relative gizzard weight of the Ovambo chicken

The PABM diet had a significant effect on the relative weights of the gizzard. The age at slaughter of the bird and the sex of the birds had an influence ($P<0.05$) on the gizzard's weights. The interaction of diet and sex was significant on gizzard weights (Table 4.4). Female birds fed on the PABM diet had a higher ($P<0.05$) relative weight of gizzard than those fed on the WM diet. Female birds had a significantly higher gizzard weight than male birds. There was a significant decrease in the gizzard relative weight of the male and female birds fed both diets as the birds increased in age (Table 4.5).

4.3.4 Effect of diet, sex and age on the relative liver weight of Ovambo chickens

Diet had a significant effect on the relative weights of the liver. The age at slaughter of the bird was not significant) on all the liver relative weights. There was an interaction of diet, age and sex on the liver weight (Table 4.4). The relative weights of the liver of male birds fed PABM at 18 weeks at slaughter was significantly higher the male fed WM diet at the same age of slaughter. The weights of the female chickens fed on the PABM at 21 weeks was higher ($P<0.05$) than the female birds fed on the WM diet at the same age of slaughter. Females had a significantly higher relative weight of liver than the males. The heart weight of the PABM fed male birds at 21 weeks was significantly lower than at 18 weeks of age at slaughter, while the weight of female fed on the PABM diet at 21 weeks was significantly higher (Table 4.5).

4.3.5 Effect of diet, sex and age on the relative heart weight of Ovambo chickens

The age at slaughter of the bird did not influence ($P<0.05$) the heart's relative weights. The interaction of diet and age and the interaction of age and sex significantly influenced the weights of the heart (Table 4.4). Birds fed on the PABM diet had a higher ($P<0.05$) relative heart weight than those fed on the WM diet for 21 weeks. The weight of the hearts of the male at 21 weeks was significantly higher than the female birds of the same slaughter age. The weight of the PABM fed male birds at 18 weeks was higher ($P<0.05$) than the PABM fed female birds of the same slaughter age. The heart weight of the PABM fed male birds at 21 weeks of age at slaughter was significantly higher than at 18 weeks. The heart weight of the PABM fed female birds at 21 weeks of age at slaughter was higher ($P<0.05$) than the WM fed female birds of the same age (Table 4.5).

4.4 Discussion

The higher BW and DC of male birds than female birds were expected based on earlier reports due to their difference in metabolic differences (Shafey *et al*., 2001; Haitook, 2009; Shafey *et al*., 2013). Haitook (2009) reported that male indigenous chickens have higher proportion of total carcass weight and higher carcass traits. The higher yield of DC in PABM fed chickens agrees with Kucuk *et al*. (2003) who reported higher yields in vitamin A supplement birds than non-supplemented birds. The higher yield of legs and body weight in the male birds than the females is similar to earlier reports which showed higher yields and weight of carcasses, thighs and drumsticks in males than female birds (Rondelli *et al*., 2003; Negesse & Tera, 2010; Almasi *et al*., 2012). The higher breast and back yields in females than male have also been reported earlier (Lopez *et al*., 2011; Sola-Ojo *et al.,* 2011). The higher breast and back yields is as a result of the larger deposition of abdominal fat in females, especially during the stage of sexual maturity. Young *et al.* (2001) reported higher yield in the breast cut as age increases. The higher breast percentage of the female birds at 21 weeks is due to the attainment of sexual maturity which is at an average of 20 weeks (Van Marle-Köster & Nel, 2000). The finding showed that age of bird affected all the carcass measurements except the BW, wings, breast and head are similar to earlier reports (Isidahomen *et al.,* 2012; Tougan *et al.,* 2013). Carcass yield is influenced by various factors, including diet, strain body weight, conditions at slaughter and sex (Havenstein *et al.,* 2003; Brickett *et al.,* 2007). Higher BW, back, leg and breast relative weights in chickens fed on PABM were observed in the current study.

The higher yield of liver and gizzard in chickens fed on the PABM diets agree with Kucuk *et al*. (2003) who also reported a higher yield in birds supplemented with vitamin A. Onifade *et al*. (1997), Okeudo *et al*. (2005) and Soltan (2009) also reported that chickens fed on palm kernel meal, which is high in β-carotene had higher gizzard weight. The higher weight of the female bird's gizzard is similar to the findings of Zhao *et al.* (2012). The female bird's higher relative weights in gizzard and liver are attributed to the deposition of fat in the liver in preparation for egg production (Ridell, 2011). The functions of the liver include absorption and storage of vitamin A and fat metabolism (Bechmann *et al*., 2012; O'Byrne & Blaner, 2013). As the PABM maize has higher vitamin A, it could have caused the increase in liver weights of the female chickens fed on the PABM diet. Vitamin A increased the carcass yields and internal organ weights (Kucuk *et al*., 2003). Sola-ojo *et al.* (2011) reported no significant differences in the weight of the liver and gizzard between sexes. The findings of higher relative weight of the male chicken's heart than females are similar to earlier reports (Sola-ojo *et al*., 2011; Zhao *et al.,* 2012). The higher relative weight of the heart in the males can be attributed to their higher growth rate which leads to a higher oxygen demand. Sex hormones are a possible independent factor in cardiac metabolism (Pasanen *et al.,* 1997; Wittnich *et al*., 2013). The findings in the current study that the relative weight of liver and gizzard were significantly influenced by slaughter age as it decreased with age corresponds with Tougan *et al.* (2013). Edible offals are earlier maturing organs and they either decrease with age or age has no effect on them once fully matured (Oluyemi & Roberts, 2000).

4.5. Conclusions

The PABM diet improved some of the carcass traits and edible offal weights, however, the significant improvement of diet was more effective in their interaction with either/both age and sex depending on the carcass cuts being evaluated. Female had better relative weights in the breast, back, gizzard and liver, while the male birds had better relative weights in the BW, heart and legs (thigh and drumstick). The relative leg and gizzard weights however decreased with increase of age of slaughter. Generally, the WM fed diet did not significantly improve the carcass and organ parameters measured. It can be concluded that PABM diet can be a source of natural vitamin A diet to chickens to improve carcass and edible offal weights without any detrimental effects. The age and sex of the birds influenced the effect of the PABM diet on carcass and edible offals characteristics in birds. There is a sexual dimorphism in the carcass characteristics of Ovambo chicken which is enhanced by the PABM diet and the age of the birds. It can be concluded that overall, PABM diet, sex and age has a positive effect on the carcass characteristics and edible offals.

The evaluation of the health status and well-being of the birds fed PABM is also important to assess the utilization of PABM by Ovambo chickens to improve food nutrition security among the resources-poor households in southern Africa.

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CHAPTER 5

Haematological and Serum Biochemical Responses of Ovambo Chickens Fed on Provitamin A Biofortified Maize

Abstract

The current study was conducted to investigate the influence of PABM diet, sex and age of birds on the haematological and serum biochemistry parameters of Ovambo chickens. A total of 102 male and female Ovambo chickens were reared and fed on either a low vitamin A diet (WM) or a PABM based diet for nine weeks. Packed cell volume (PCV), haemoglobin (Hb), erythrocyte concentration (RBC), leucocytes concentration (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured. Albumin (ALB), globulin (GLOB), total proteins (TP), triglycerides (TRI), cholesterol (CHOLES), uric acid (UA) and creatinine (CREAT) concentrations were assessed. The activity of alanine transaminase (ALT) alkaline phosphate (ALP) and aspartate transaminanse (AST) were also assayed. The PABM diet increased ($P<0.05$) PCV and WBC of female birds. All mean values for the haematological parameters regardless of the age, sex and diets were within the normal range. Age had an effect ($P<0.05$) on ALT, TP, GLOB, TRI and UA. Sex of the birds significantly influenced the TP, GLOB, ALB, CREAT and TRI levels. There was an interaction of diet and age of bird ($P<0.05$) on ALP, ALT, and GLOB concentrations. The cholesterol level in male birds fed on the WM diet for 18 weeks was above the normal range. In conclusion, feeding PABM diet to female and male Ovambo chickens will not negatively impact on the health status of Ovambo chickens.

Keywords: age, blood profiles, low vitamin A, provitamin A biofortified maize, sex

5.1 Introduction

The indigenous chicken is important to southern Africa rural households as it is a major source of protein and livelihood. Indigenous chickens are preferred by consumers because of their distinct organoleptic properties and lower fat content (Mtileni *et al*., 2009; Farrell, 2013). Indigenous chickens could be a cost effective intervention to curbing malnutrition in sub-Saharan, which is prevalent in most rural areas (Alders & Pym, 2009). The chickens are abundant in the rural communities and require minimal inputs to survive. The Ovambo is an important chicken breed in communal areas of Southern Africa due to its dark colouring, which helps to camouflage it from predators, its adaptability, and ability to fly to roost on trees (Van Marle-Köster & Nel, 2000).

Vitamin A deficiency, a micronutrient deficiency which inhibits growth and development, vision and immune system is prevalent among rural households. Five countries in southern Africa are among the top 20 countries with VAD in the world (Muthayya *et al*., 2013). The PABM, because of its high β-carotene concentration, could be fed to indigenous chickens with the aim of increasing vitamin A concentration in the chicken meat. The chicken meat can then be consumed by the VAD-vulnerable groups, which are the infants, children, pregnant and lactating women and the elderly.

β-carotenes are antioxidants which protect body cells from damage (Bárdos *et al*., 2011). The effects of the interaction of PABM, age and sex of the Ovambo chicken have been discussed in Chapters 3 and 4. There is, however, little information on the role and influence of PABM as a natural source of high vitamin A on the nutritional, clinical and physiological responsiveness and well-being of indigenous chickens. Haematological and serum biochemical parameters are influenced by age of the birds, sex, feed, medication, toxic compounds and infections (Agbede & Aletor, 2003; Huff *et al*., 2008; dos Santos Schmidt *et al.,* 2009).

There is need to assess the interaction between high vitamin A diet, age and sex of the birds on their blood profiles. The objective of the study was to compare the haematological and biochemical responses of female and male Ovambo chickens to PABM at different ages. It was hypothesized that there was no difference in haematological and biochemical responses to PABM between the male and female Ovambo chicken at different age groups.

5.2 Materials and methods

5.2.1 Study site and the ethical aspects of the study

The detailed description of the study site, average ambient temperature and relative humidity, and the ethics approval for management and use of birds are given in sections 3.2.1 and 3.2.2.

5.2.2 Birds, treatments and management

The details of the type of strain, number of birds, dietary treatments, management of the birds and the agronomic practices used for planting the provitamin A biofortified maize are provided explicitly in section 3.2.3.

5.2.3 Data collection

At 18 and 21 weeks (6 and 9 weeks of feeding on experimental diets), 16 birds from each dietary treatment, that is, eight female and eight male birds, two birds from each replicate were randomly selected. Two sets of blood samples were collected via the jugular vein. One set of blood samples were collected into a 5mL purple top vacutainer tubes containing the anticoagulant, ethylene diaminetetra-acetic acid (EDTA) for determining the haematological parameters. The other set of blood samples were collected into a 5mL vacutainer tubes that did not contain any anti-coagulant. The coagulated blood samples were centrifuged for 15 minutes at 3000 rpm to collect serum before they were stored at -20^{0}C until they were analysed.

5.2.3.1 Haematological parameters

The packed cell volume (PCV) was measured by a microhaematocrit capillary tube using a Hemocrit reader. Erythrocyte concentration (RBC), haemoglobin (Hb) and leucocytes concentration (WBC) counts were measured using an automated cell counter within 24 hours after collection of blood The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) was calculated as described by Jain (1986). The formulae used were:

MCV in femtolitres (fL) = 10 x PCV (%)/RBC counts (millions/µl).

MCH in pg/cell= haemoglobin (g/ 100 mL)/ RBC counts (millions/µl).

MCHC in g/dl= haemoglobin (g/ 100 mL) x 100/PCV (%).

5.2.3.2 Biochemical parameters

The albumin, serum lipid profile, total protein, uric acid, creatinine, cholesterol, triglycerides and activities of the liver: alanine transaminase, aspartate transaminase and alkaline phosphate were measured at the School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Westville Campus, Durban, South Africa using an automated chemistry analyser (LabmasPlenno, Labtest, Lagoa-Santa Brazil). Globulin was calculated as the difference between total protein and albumin.

5.2.4 Statistical analyses

The data were analyzed using the GLM procedure of SAS (2010) software. The model used included diet, age and sex as fixed variables. Least square means were compared using the PDIFF (SAS, 2010). Statistical significance was considered at the 5% level of probability.

The model used was: $Y_{ijkl} = \mu + D_i + S_j + A_k + (D \times S)_{ij} + (D \times A)_{ik} + (D \times S \times A)_{ijk} + E_{ijkl}$

Where: Y_{ijkl} = response variable (haematological and serum biochemistry parameters)

μ = the overall mean

D_i = effect of the i^{th} diet with i = PABM and WM diet;

S_j = effect of the j^{th} sex with j= male and female;

A_k = effect of the k^{th} age with k= 18 and 21 weeks;

$(D \times S)_{ij}$ = interaction of the i^{th} diet and the j^{th} sex;

$(D \times A)_{ik}$ = interaction of the i^{th} diet and the k^{th} age of bird;

$(D \times S \times A)_{ijk}$ = interaction of the i^{th} diet, j^{th} sex of bird and the k^{th} age of bird;

E_{ijkl}= random error term assumed to be normally and independently distributed with mean 0 and variance equal to $ð^{2.}$

5.3 Results

5.3.1 Effect of diet, sex and age on haematological parameters of the Ovambo chicken

All the values obtained for the haematological parameters in this study were within their normal range. The diet of the birds affected WBC. The effect of diet, sex of birds and the interaction of diet and sex of the birds were not significant in the RBC and MCHC. The effect of the sex of the birds influenced ($P<0.05$) Hb, PCV, WBC, MCV and MCH. Age of the birds had no significant influence ($P>0.05$) on any of the parameters assessed. There was no significant interaction of diet, sex and age of the birds in all the haematological parameters assessed (Table 5.1). Female birds fed on the PABM diet had a significantly higher RBC than the female birds fed on the WM diet. The Hb and PCV of male birds fed on the WM diet were higher ($P<0.05$) than the female fed on the WM diet. The PABM fed birds had significantly higher WBC than the WM fed birds. The WBC of the female birds was higher than for males. The MCV and MCH of the PABM male birds were significantly higher than for the PABM female birds. The MCH of the PABM fed female birds was also lower than the WM fed female birds. There was no effect ($P>0.05$) of sex and diet on the MCHC.

Table 5.1: Levels of significance of effects of diet, sex and age of bird on haematological parameters of Ovambo chickens

Source of variation	RBC	Hb	PCV	WBC	MCV	MCH	MCHC
Diet	NS	NS	NS	***	NS	NS	NS
Sex of bird	NS	*	**	**	**	**	NS
Age	NS	NS	NS	NS	NS	NS	NS
Diet × sex	NS	NS	NS	NS	NS	NS	NS
Diet × age	NS	NS	NS	NS	NS	NS	NS
Sex × age	NS	NS	NS	NS	NS	NS	NS
Diet × sex × age	NS	NS	NS	NS	NS	NS	NS

RBC: erythrocyte concentration; Hb: haemoglobin; PCV: packed cell volume; WBC: leucocytes concentration; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration.

NS: not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 5.2: Effect of diet, sex and age of bird on the haematological parameters of Ovambo chickens

	18 weeks				21 weeks				
Diet	WM		PABM		WM		PABM		
	Male	Female	Male	Female	Male	Female	Male	Female	SEM
RBC (x106μ)	2.79^{ab}	2.57^{a}	2.85^{ab}	2.88^{b}	2.84^{ab}	2.45^{a}	2.77^{ab}	2.86^{b}	0.11
Hb (g/dl)	11.28^{b}	10.16^{a}	11.45^{b}	10.80^{ab}	11.00^{ab}	10.23^{a}	11.48^{b}	11.01^{ab}	0.36
PCV (%)	38.00^{b}	33.75^{a}	38.25^{b}	36.50^{ab}	38.20^{b}	34.34^{a}	37.67^{b}	35.94^{ab}	1.07
WBC (g/dl)	15.95^{a}	20.65^{b}	23.20^{bc}	26.20^{c}	15.15^{a}	20.50^{b}	22.16^{bc}	23.45^{bc}	1.29
MCV (fL)	136.50^{b}	131.61^{ab}	134.96^{b}	127.41^{a}	131.20^{b}	133.02^{ab}	135.99^{b}	125.66^{a}	2.17
MCH (pg/cell)	40.43^{b}	39.61^{b}	40.37^{b}	29.64^{a}	39.21^{b}	40.18^{b}	41.44^{b}	38.50^{b}	0.57
MCHC (g/dl)	29.64^{a}	30.11^{a}	29.94^{a}	29.58^{a}	29.10^{a}	30.00^{a}	30.48^{b}	30.63^{a}	0.29

Values of superscripts that differ in rows are significant ($P<0.05$).
WM: white maize (control); PABM: provitamin A biofortified maize.
RBC: erythrocyte concentration; HGB: haemoglobin; PCV: packed cell volume; WBC: leucocytes concentration; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration.
SEM: standard error of means.

5.3.2 Effect of diet, sex and age on serum biochemistry of Ovambo chickens

All the birds regardless of their diet, age at slaughter and sex were within the normal range of TP and ALB. Sex, the interaction of diet and age and the interaction of diet, age and sex of the birds have a significant effect on the ALB of the birds (Table 5.3). The PABM fed male birds at 21 weeks of age had a significantly higher ALB than the WM fed male of the same age. The ALB of the WM fed male birds at 18 weeks of age was significantly higher than the WM male birds at 21 weeks of age. The WM fed male birds at 18 weeks had a higher ALB than the WM fed female birds at 18 weeks of age (Table 5.4).

Sex, age and the interaction of sex and age significantly influenced TP concentrations. All the sources of variation significantly influenced the globulin in the serum (Table 5.3). There was a significant difference of the TP in the blood between the male and female birds at 21 weeks of age. The female birds at 21 weeks of age had significantly higher globulin than the male birds of the same age. The PABM fed female at 21 weeks of age had higher ($P<0.05$) globulin than the WM fed female of the same age. The female birds at 21 weeks of age had a higher ($P<0.05$) globulin amount than the female birds at 18 weeks of age (Table 5.4).

Diet, the interaction of diet and age influenced the ALP. Age of the birds, diet and age, and diet and sex influenced ($P<0.05$) ALT concentrations. There was no influence of the diet, sex, sex and age of the birds and their interactions at all levels in the AST in the serum (Table 5.3). The WM fed male birds has a significantly higher ALT than the PABM fed males at 18 weeks of age. The AST of the WM fed females at 21 weeks of age was significantly higher than the males of the same age and the PABM fed females of the same age (Table 5.4).

There was significant influence of the interaction of sex and age on the creatinine concentrations. There was no effect ($P>0.05$) of diet, sex and age and their interactions on CHOLES. There was interaction of age and sex on triglyceride concentration was significant (Table 5.3). The triglycerides of the female birds regardless of the diet and age were higher than the male birds; however it was only significant between the female and male birds at 21 weeks of age. The triglycerides of the female birds at 21 weeks of age were also significantly higher than the female birds at 18 weeks of age. The WM fed males at 21 weeks of age had a significantly lower creatinine than the WM fed females of the same age (Table 5.4).

The age of birds influenced ($P<0.05$) the amount of uric acid. The interaction of diet and sex was significant on the uric acid in the serum of the Ovambo chickens (Table 5.3). The PABM fed male birds at 21 weeks of age had a significantly lower uric acid concentration than the PABM fed female birds and the WM male birds of the same age (Table 5.4).

Table 5.3: Levels of significance of effects of diet, sex and age of bird on serum biochemistry of Ovambo chickens

Source of variation	ALB	TP	GLOB	ALP	ALT	AST	CHOLES	CR	TRI	UA
Diet	NS	NS	*	*	NS	NS	NS	NS	NS	NS
Sex	*	***	***	NS	NS	NS	NS	NS	**	NS
Age	NS	*	***	NS	**	NS	NS	NS	*	*
Diet × age	NS	NS	*	*	*	NS	NS	NS	NS	NS
Diet × sex	NS	NS	*	NS	*	NS	NS	NS	NS	*
Sex × age	**	***	***	NS	NS	NS	NS	*	*	NS
Diet × sex × age	*	NS	*	*	NS	NS	NS	NS	NS	NS

ALB: albumin; TP: total protein; GLOB: globulin; ALP: alkaline phosphate; ALT: alanine transaminanse; AST: aspartate transaminanse; CHOLES: cholesterol; CREAT: creatinine; TRI: triglycerides. NS: not significant * P< 0.05; ** P < 0.01' *** P < 0.001.

Table 5.4: Effect of diet, sex and age of bird on the serum biochemistry of Ovambo chickens

	18 weeks				21 weeks				
Diet	**WM**		**PABM**		**WM**		**PABM**		**SEM**
Gender	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	
ALB (mg/dl)	2.08^{bc}	2.12^{bc}	2.06^{bc}	2.17^{bc}	1.50^{a}	2.36^{c}	1.97^{b}	2.15^{bc}	0.11
ALP (U/L)	986.50^{c}	703.75^{ab}	558.50^{a}	647.00^{ab}	686.00^{ab}	791.00^{bc}	714.25^{ab}	713.50^{ab}	76.43
ALT (U/L)	6.25^{bc}	3.00^{ab}	1.13^{a}	4.25^{ab}	3.50^{ab}	4.50^{bc}	6.50^{cd}	8.75^{d}	1.19
AST (U/L)	276.38^{a}	241.88^{a}	325.00^{a}	276.13^{a}	263.75^{a}	855.00^{b}	279.50^{a}	240.25^{a}	146.26
CHOLES (mg/dl)	230.63^{c}	113.63^{ab}	115.50^{ab}	106.25^{ab}	119.25^{ab}	141.50^{ab}	100.75^{a}	129.00^{ab}	46.08
CREAT(mg/dl)	1.69^{a}	0.62^{a}	1.40^{a}	0.77^{a}	1.02^{a}	5.54^{b}	1.00^{a}	3.43^{ab}	1.42
TP (g/dl)	4.14^{b}	4.17^{b}	4.10^{b}	4.24^{b}	3.55^{a}	4.85^{c}	4.00^{ab}	5.22^{c}	0.17
TRI (mg/dl)	66.25^{a}	96.63^{a}	65.75^{a}	81.88^{a}	56.25^{a}	512.25^{b}	51.75^{a}	441.50^{b}	120.97
Uric acid (mg/dl)	10.92^{b}	10.94^{b}	9.17^{b}	10.71^{b}	8.29^{b}	8.96^{b}	5.26^{a}	9.06^{b}	0.96
Globulin	2.06^{a}	2.04^{a}	2.04^{a}	2.06^{a}	2.05^{a}	2.49^{b}	2.03^{a}	3.06^{c}	0.10

Values with different superscripts in the same row differ ($P<0.05$).
WM: white maize; PABM: provitamin A biofortified maize. ALB: albumin; ALP: alkaline phosphate; ALT: alanine transaminanse; AST: aspartate transaminanse; CHOLES: cholesterol; CREAT: creatinine; TP: total protein; TRI: triglycerides. SEM: standard error of means.

5.4 Discussion

The normal ranges of the haematological parameters are RBC (2.5-3.5) x10^6 µl, PCV (22-35) %, Hb (7-13) g/dl and WBC (12-30) x 10^3 µl (Bounous & Stedman, 2000). All the values obtained for the haematological parameters in this study were within their normal range. The MCV is used to calculate the average red blood cell size, the MCH to measure haemoglobin amount per blood cell and the MCHC to know the amount of haemoglobin relative to the size of the cell per red blood cell. Their normal ranges are MCV (90-140) fL, MCH (33-47) pg/cell and MCHC (26-35) g/dl (Bounous & Stedman, 2000).

Erythrocytes function is to transport oxygen from the lungs to tissues and remove carbon dioxide from the tissues to the lung in the body via haemoglobin. The WBC aids to protect the body from pathogen and carotenoids build up immunity (Saladin, 2003; Osman *et al*., 2004). PABM diet increased the WBC of the birds in the current study. Vitamin A increases the immune response in chickens (Moghaddam & Emadi, 2014**).** This is similar to earlier report that supplementation of vitamin A increased the WBC concentration (Kermanshahi *et al*., 2008). The higher WBC of the female birds than the male birds is similar to the earlier findings (Sharmin & Myenuddin, 2004; Addass *et al*., 2012). They also reported that there was no difference in the Hb and PCV when supplemented with vitamin A (Kermanshahi *et al.* 2008), however, did not report any effect of vitamin A supplementation on the RBC. The variation could, however, be because the current study's evaluation includes the influence of sex and diet and their interaction on haematological parameters as only the female PABM fed birds had significantly higher RBC. The RBC of the chickens is affected by the strain, sex and diet of the birds (Kaminski *et al*., 2014).

The significantly higher Hb and PCV of the male birds fed WM diet than the WM fed female birds is supported by earlier findings on indigenous chickens (Sharmin & Myenuddin, 2004; Pavlak *et al.*, 2005; Elagib & Ahmed, 2011). RBC and Hb are influenced by reproductive hormones. The higher amount of Hb and MCV in the male birds can be attributed to the androgen hormone present in the male birds, as increased number of RBC and PCV in cocks correspond with the time of androgen production (Cecil & Murray, 1990; Adedibu *et al*., 2014) The increase in PCV can be used to envisage sexual maturity and the start of semen production. In general, haematological parameters of indigenous birds are influenced by various factors that include the sex and diet (Elajib & Ahmed, 2011).

Total protein is made up of ALB and globulin. Globulin is calculated as the difference between TP and ALB. The normal ranges of the TP and ALB in bird's blood are 3.0-4.9 mg/dl and 1.17-2.74 g/dl, respectively (Clinical Diagnostic Division, 1990; Meluzzi *et al*., 1992). All the birds regardless of their diet, age at slaughter and sex were within the normal range of TP and ALB. Albumin, a serum protein is synthesized in the liver. It is responsible for transporting insoluble substance in the blood and aids to maintain oncontic pressure (Fischbach, 2003). A higher concentration might be as a result of dehydration while a lower concentration may be due to the liver not functioning adequately due to factors such as malnutrition and infection (Esubonteng *et al*., 2011). Total protein in the female birds at both ages of slaughter was higher than the male birds. This could be attributed to estrogen induced increase in globulin in preparation of the female body for egg laying (Simaraks *et al.,* 2004).

Liver enzymes, namely the alanine transaminase albumin (ALT), alkaline phosphate (ALP) and aspartate transaminase (AST) are important in the determination of the proper functioning of the liver (Ambrosy *et al*., 2015). These enzymes are present in negligible concentration. An increase in the concentration of these enzymes may be as a result of damaged or diseased cells which denote the status of the liver function. The normal range of the concentration of liver enzymes activities are: AST (70-220) U/L, ALP (568-8831) U/L (Meluzzi *et al*., 1992). The high concentration of AST in the WM fed female birds at 21 weeks of age could be an indication of damage to the liver function as vitamin A deficiency increases the level of AST and ALT (Roodenburg *et al*., 1996).

Creatinine is used to determine the status of the kidney**.** The functions of the kidney include excretion of waste products resulting from protein metabolism and muscle contraction (Ileke *et al*., 2014). Creatinine is excreted by the kidney as a by-product of creatinine phosphate metabolism which is produced as a result of energy production by the skeletal muscles (Esubonteng *et al*., 2011). The high level of creatinine in the female birds at 21 weeks is due to the hormones and metabolic changes as a result of sexual maturity (Menon *et al*., 2013). A high amount of creatinine indicates that the kidney is not functioning optimally thus, the significant high level in the WM fed female birds at 21 weeks of age indicates a lower than optimal functioning of the kidney. Vitamin A and its active metabolites have been reported to affect the development of kidney which affects the proper functioning of kidney resulting in the high level of creatinine (Gilbert, 2002). This could also be attributed to the high level of AST which could be a sign of vitamin A deficiency (Roodenburg *et al.,* 1996).

High protein intake, increased protein metabolism, stress and dehydration influence the concentration of uric acid in the blood as it is a produced as a result of protein metabolism (Chernecky & Berger, 2008). The normal range of uric acid is 1.9-12.5 mg/dl (Clinical diagnostic division, 1990). Age, sex and diet of birds influence the amount of uric acid. A high level of uric acid (hyperuricemia) is usually evident in female birds due to ovulatory activities (Ibrahim *et al*., 2012). The amount of uric acid in females was higher than the male birds at both stages of slaughter; however it was only significant at 21 weeks of age in the PABM fed birds. This may be due to the fact that, at 18 weeks of age, the female birds had not started laying eggs. The average maturity age for egg laying is 21 weeks (Van Marle-Koster & Nel, 2000).

Cholesterol concentrations in the PABM fed birds were lower than the WM fed birds at 18 and 21 weeks of age. The WM fed male birds at 18 weeks of age had a significantly higher cholesterol level than the WM fed females at 18 weeks and the PABM fed male birds at 21 weeks. The concentration of cholesterol of the WM fed male birds at 18 weeks was above the normal range (87-192) mg/dl (Meluzzi *et al.,* 1992) of cholesterol in birds. Cholesterol is synthesized from fats consumed and endogenously synthesized within the cells. A high level of cholesterol is an indication of a high risk to cardiovascular disease. Triglycerides are synthesized in the liver from fatty acids and from protein and glucose when these substrates are above the body's current needs and then stored in adipose tissue (Esubonteng *et al.,* 2011). The TRI of the WM fed female birds at 21 weeks of age was above the normal range (45.7-172) mg/ml (Meluzzi *et al.,* 1992) and higher than the PABM fed females and the male birds. Furthermore, the absence or presence of cholesterolaemic effects of dietary components in an animal depends on various factors such as breed, gender and age, and also on the composition of the feed (Toghyani *et al.,* 2010). Serum biochemical constituents are positively correlated with the quality of the diet (Adeyemi *et al.,* 2000; Etuk *et al*., 2014).

5.5 Conclusions

PABM diet increased the WBC of the chickens. The PABM also increased the RBC of females fed on the PABM diet than the WM fed females. The MCH of the PABM fed female was lower than the PABM fed male birds and the WM fed female birds. However, all the mean values for the haematological parameters regardless of the age, sex and diets are all within the normal range. High vitamin A diet also increased the ALP and globulin. Age had an effect on ALT which was higher in the PABM fed birds at 21 weeks of age than at 18 weeks of age. The serum biochemistry of the birds regardless of their diet, sex and age were all within the normal range except the CHOLES, TRI and AST. The cholesterol level in the WM fed male birds at 18 weeks was above the normal range. The TRI of the WM fed female at 21 weeks was above the normal range and the AST of WM fed male at 18 weeks was above the normal range. Use of PABM, as feed and source of vitamin A on female and male Ovambo chickens within the age of 13 to 21 weeks will not negatively impact on the health status of the Ovambo chicken. Thus, the use of indigenous chicken can be potential tool to curb VAD in southern Africa without negatively impacting the clinical and physiological responsiveness of Ovambo chickens. The quality of meat is a physical determinant of the health of the chicken and this is important to the consumers and influences their preferences. It is therefore important to assess the impact of PABM on the meat quality of the female and male Ovambo at different ages.

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CHAPTER 6

Effect of Provitamin A Biofortified Maize Inclusion on Quality of Meat from Ovambo Chickens

Abstract

The objective of the current study was to determine the effect of PABM inclusion on meat quality in male and female Ovambo chickens at 18 and 21 weeks of age. A total of 102, 13 weeks-old Ovambo chickens were randomly distributed into two treatment groups with four replicates each and fed on two dietary treatments; the WM diet, the control and PABM diet, a vitamin A maize. Breast muscles and skin from eight male and female birds from each treatment were used to determine the pH, drip loss, cooking loss, shear force, meat and skin colour. There was no significant effect of PABM, sex and age on meat pH, drip loss (%), cooking loss (%) and shear force. The PABM fed chickens had higher ($P<0.05$) redness (a*) and yellowness (b*) and lower lightness (L*) in the meat and skin colour. Female birds had higher ($P<0.05$) L* values than males on the skin. As the age of the birds increased, the L* value in the skin of female fed on the WM diet increased. The a* value of the PABM fed male birds increased ($P<0.05$) with age. The male birds had better ($P<0.05$) breast skin and meat quality than the female birds. The PABM diet improved ($P<0.05$) the skin and muscle colour of the Ovambo chicken and will be readily acceptable to the southern Africa consumers.

Keywords: age, breast muscle, meat colour, sex, skin colour, vitamin A

6.1 Introduction

Provitamin A biofortified maize, with high β-carotene level has been developed as a tool to curb VAD in sub-Saharan Africa especially among the rural communities where the deficiency is prevalent (Muthayya *et al*., 2013). Indigenous chickens, due to their availability in the rural communities are an alternative tool to effective curbing of VAD by feeding the PABM to the chickens with the aim of increasing the vitamin A level in the meat which will be consumed. Chapters 3, 4 and 5 have indicated that the PABM maize has not impacted negatively on the growth performances, carcass characteristics and the health status of the Ovambo chicken. The interactions of PABM diet, sex and age of the Ovambo chicken have improved the carcass characteristics and health status of the Ovambo chicken. There is, therefore, the need to evaluate the effect of PABM diet, sex and age on the meat quality of the Ovambo chicken.

Meat quality is a major factor in the attractiveness of meat to consumers regardless of how high the nutritional composition is (Wood *et al*., 1999). Thus, the physical meat quality is important in the acceptability of the PABM fed chicken meat as a tool for curbing VAD. The type of diet consumed by poultry has an impact on the colour, tenderness, pH, cooking loss and drip loss of the meat produced (Bavelaar & Beynen, 2003; Ponsano *et al.*, 2004). For example, yellow maize gives yellowish colour to bird's skin due to the presence of carotenoids (Tarique *et al*., 2013). Natural sources of carotenoids are more efficient at increasing skin yellowness than the synthetic pigments (Pérez-Vendrell *et al.,* 2001; Castañeda *et al.,* 2005). Carotenoids present in maize are lutein, zeaxanthin, β-cryptoxanthin, β- carotene and α-carotene. Lutein and zeaxanthin do not have provitamin A abilities, however, they are often used as colouring agent for chicken egg and skin and they are good anti-oxidants (Tarique *et al.,* 2013). The yellowness in the skin is usually seen as a sign of healthy meat to most consumers in Africa and Asia (Ripoll *et al*., 2015). Other factors that influence meat quality are the breed, age and sex of the chicken. Researchers have reported conflicting results on the effect of these factors including diet and their interactions on chicken meat quality results (Yang & Jiang, 2005; Musa *et al*., 2006). The effect of the PABM on the meat quality of the chicken has, however, not been investigated, neither has the effect of the interaction of PABM diet, age and sex of indigenous chickens.

It is important for food security practitioners to determine whether PABM can be a tool, not only to improve the growth performances and carcass and organ characteristics but, also, to improve the meat quality of the Ovambo chickens. The objective of the study was to determine the effect of provitamin A biofortified maize diet meat quality of the male and female Ovambo chicken at several ages. It was hypothesized that PABM, sex and age of the birds have no effect on the meat quality of the Ovambo chickens

6.2 Materials and methods

6.2.1 Study site and the ethical aspect of the study

The detailed description of the study site, average ambient temperature and relative humidity, and the ethics approval for management and use of birds are given in sections 3.2.1 and 3.2.2.

6.2.2 Birds, treatments and management

The details of the type of strain, number of birds, dietary treatments, management of the birds and the agronomic practices used for planting the provitamin A biofortified maize are provided explicitly in section 3.2.3.

6.2.3 Slaughtering of birds

At 18 weeks and 21 weeks (6 weeks and 9 weeks of feeding on experimental diets), 16 birds from each dietary treatment, that is eight female and eight male birds, two birds from each replicate were randomly selected for meat quality evaluation. The birds were slaughtered conventionally early in the morning by decapitating the head from the neck with a sharp knife. The birds were scalded in hot water (about 90^{o}C) after being bled and plucked manually. The carcasses were then eviscerated and weighed. The breasts muscle and skin were cut and stored in food grade density polyethylene bags at 4^{o}C, pending analyses.

6.2.4 Meat quality measurements

6.2.4.1 pH

The pH of the breast muscles was determined after chilling at 4^{0}C for 24 h. The pH after 24 h was measured by homogenising 5 g of raw breast muscle meat with 25 mL of distilled water. The homogenate was filtered and the pH of each sample was measured with a pH meter at room temperature. The pH meter was calibrated using buffer solution of pH 4 and 7.

6.2.4.2 Drip loss

To determine the drip loss, a slice of breast muscle was hanged for 24 h in double plastic bags at 4^0C (Honikel, 1987). Drip loss was determined by weighing the breast muscle (IW) before placing it in a refrigerator at 4^oC for 25h. The breast muscle was blotted dry using a paper towel and weighed again (WA) after 24h after blotting the breast muscle dry with a paper towel (WA). The drip loss percentage was then calculated as IW-WA/IW x 100 % (Petracci & Baeza, 2011).

6.2.4.3 Cooking loss

The meat samples were weighed and cooked in an oven, preheated to 200°C for 15 min to an internal temperature of 80°C. After cooking the chicken meat samples as described were weighed to determine the cooking loss as: Cooking loss = (weight raw – weight after cooking) /weight raw × 100.

6.2.4.4 Shear force

The force (N), energy (N/mm) and extension(mm) of boiled breast muscle were determined in 5 rectangular strips (1 cm × 3 cm long) per sample using a Warner–Bratzler (WB) shear device attached to an Instron universal testing machine (Model 5565, Instron Ltd., Buckinghamshire, UK). A cross head speed of 200 mm/min and a 5 kN load cell calibrated to read over a range of 0–100 N were applied.

6.2.4.5 Meat and skin colour

After chilling the breast skin and muscle for 24h at 4^0C, the skin and muscle colour of the breast samples were measured using a Hunterlab Colour Flex EZ spectrophotometer (Model45/0^0, Hunter Associates Laboratory, USA). The spectrophotometer was standardized using white and black calibration tiles. A portion of breast muscle measuring approximately 5cm and 1cm in width and thickness respectively was cut from three different locations on each breast sample. The colour was determined based on Hunter colour scales of lightness (L*), redness (a*) and yellowness (b*) (International Commission on Illumination (CIE), 1976).

6.2.5 Statistical analyses

The data were analyzed using the GLM procedure of SAS (2010). The model used included diet, age and sex as fixed variables. Least square means were compared using the PDIFF SAS (2010). Statistical significance was considered at the 5 % level of probability.

The model used was: $Y_{ijkl} = \mu + D_i + S_j + A_k + (D \times S)_{ij} + (D \times A)_{ik} + (D \times S \times A)_{ijk} + E_{ijkl}$

Where: Y_{ijkl} = response variable (meat quality parameters);

μ= the overall mean

D_i= effect of the i^{th} diet with i = PABM and WM diet;

S_j= effect of the j^{th} sex with j= male and female;

A_k= effect of the k^{th} age with k= 18 and 21 weeks;

$(D \times S)_{ij}$= interaction of the i^{th} diet and the j^{th} sex;

$(D \times A)_{ik}$= interaction of the i^{th} diet and the k^{th} age of bird;

$(D \times S \times A)_{ijk}$ = interaction of the i^{th} diet, j^{th} sex of bird and the k^{th} age of bird;

E_{ijkl}= random error term assumed to be normally and independently distributed with mean 0 and variance equal to $ð^{2.}$

6.3 Results

6.3.1 Effect of diet, sex and age on the pH of the breast muscle of Ovambo chickens

There was a significant interaction of age and sex of the birds on the pH of the meat (Table 6.1). Females fed on the PABM diet had a higher ($P<0.05$) pH than the PABM fed male birds at 18 weeks of age. The WM fed male birds at 21 weeks of age had a higher pH ($P<0.05$) than the WM fed female of the same age. The male bird's pH increased with age while the female bird's pH decreased with age although the differences were not significant. All the meat types in the current study irrespective of the diet, sex and age of the birds were within the standard range of pH for standard meat quality which is between 5.7- 6.1 (Lesiow *et al.*, 2009).

6.3.2 Effect of diet, sex and age on the drip loss of the breast muscle of Ovambo chickens

The age of the birds and the interaction of the diet and sex of the birds has an effect ($P<0.05$) on the drip loss of the meat (Table 6.1). The drip loss (%) of the WM fed female birds at 18 weeks of age had a higher ($P<0.05$) drip loss (%) than the PABM fed female at 18 weeks. The WM fed female birds at 18 weeks of age had a higher drip loss (%) ($P<0.05$) than the WM and PABM birds at 21 weeks of age (Table 6.2).

Table 6.1: Levels of significance of effects of diet, sex and age of bird on pH, drip loss, cooking loss and shear force of Ovambo breast muscle

Source of variation	pH	Drip loss	Cooking Loss	Shear force
Diet	NS	NS	NS	NS
Sex of bird	NS	NS	NS	NS
Age of bird	NS	*	NS	NS
Diet × sex	NS	*	NS	NS
Age × sex	**	NS	NS	NS
Diet × age	NS	NS	NS	NS
Diet × age × sex	NS	NS	NS	NS

*P< 0.05; ** P < 0.01; *** P < 0.001; NS: not significant (P>0.05).

Table 6.2: Effect of diet, sex and age of slaughter of birds on the pH, drip loss, cooking loss and shear force of Ovambo chicken meat

Age at slaughter	Diet	Sex	pH	Drip loss (%)	Cooking loss (%)	Shear force
18 weeks	WM	Male	5.8^{abc}	2.5^{a}	43.2	58.4
		Female	5.9^{abc}	6.5^{b}	40.4	59.9
	PABM	Male	5.8^{ab}	4.3^{ab}	39.1	55.4
		Female	6.0^{c}	3.6^{a}	42.1	60.2
21 weeks	WM	Male	6.1^{c}	1.7^{a}	44.6	62.3
		Female	5.7^{a}	1.9^{a}	42.3	57.1
	PABM	Male	6.0^{bc}	3.6^{a}	38.9	57.6
		Female	5.9^{abc}	2.4^{a}	45.9	57.9
Pooled SEM			0.1	1.0	3.5	3.8

Means with different superscripts in the same column differ significantly ($P<0.05$).
WM: white maize (control); PABM: provitamin A maize.
SEM: standard error of means

6.3.3 Effect of diet, sex and age on the cooking loss and shear force of the breast muscle of Ovambo chickens

There was no effect of diet, age and sex of the birds (P>0.05) on the cooking loss percentage and shear force (Table 6.1 and 6.2).

6.3.4 Effect of diet, sex and age on the skin colour of the Ovambo

The diet of birds influenced the L* value (P<0.05) of the breast skin colour. Age of the birds influenced (P<0.0001) the L* value of the breast skin. The interaction of age and sex of the birds has effect (P<0.01) on the L* value. The interaction of diet, age and sex of the bird was significant on the L* value of the breast skin (Table 6.3). The WM fed female birds at 18 weeks of age had a higher (P<0.05) L* value than the PABM fed female birds of the same age at slaughter. The WM fed female birds at 21 weeks had higher L* value (P<0.05) the WM fed female birds at 18 weeks and PABM fed female birds at 21 weeks of age at slaughter. The PABM fed female birds at 21 weeks had a higher L* value than the PABM male birds of the same age. The female birds had higher L* value at 21 weeks of age than 18 weeks of age. The L* value in the birds at 21 weeks of age were higher, though not all significant than at 18 weeks of age (Table 6.4).

Diet of the birds influenced (P<0.01) the a* value of the breast skin colour. The PABM fed birds had higher a* value than the WM fed birds, although, it was only significant in the PABM male birds at 21 weeks old than the PABM fed females of the same age. The a* value of the birds at 21 weeks of age were higher, although not significantly higher than the birds at 18 weeks of age at slaughter (Table 6.4).

Table 6.3: Levels of significance of effects of diet, sex and age of bird on breast's skin and meat colour

Source of variation	Skin colour			Meat colour		
	L*	a*	b*	L*	a*	b*
Diet	*	**	**	**	**	**
Sex of bird	NS	NS	NS	NS	NS	*
Age of bird	****	NS	*	*	NS	NS
Diet × sex	NS	NS	NS	NS	NS	NS
Age× sex	**	NS	**	NS	NS	NS
Diet × age	NS	NS	NS	NS	NS	NS
Diet × age × sex	*	NS	NS	NS	NS	NS

L*: brightness; a*: redness; b*: yellowness.
*P< 0.05; ** P < 0.01; *** P < 0.001; ****P<0.0001; NS: not significant (P>0.05)

Table 6.4: Effect of diet, sex and age of slaughter of birds on the skin colour of Ovambo chicken meat

			Skin colour		
Age at slaughter	**Diet**	**Sex**	**L***	**a***	**b***
18 weeks	WM	Male	64.2^{b}	7.2^{abc}	16.8^{a}
		Female	64.2^{b}	5.2^{c}	15.7^{a}
	PABM	Male	64.6^{b}	8.2^{ab}	22.7^{b}
		Female	59.5^{a}	7.8^{abc}	19.0^{ab}
21 weeks	WM	Male	69.1^{cd}	7.3^{abc}	17.5^{a}
		Female	71.0^{d}	6.7^{ac}	22.1^{b}
	PABM	Male	65.5^{bc}	9.8^{b}	21.0^{b}
		Female	70.6^{d}	8.8^{a}	23.3^{b}
Pooled SEM			1.3	1.1	1.6

Means with different superscripts in the same column differ significantly (P<0.05).
L*: brightness; a*: redness; b*: yellowness.
WM: white maize (control); PABM: provitamin A biofortified maize.
SEM: standard error of means

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The diet of the birds significantly influenced the b* value of the breast skin colour. The age of the birds influenced (P<0.05) the b* value of the breast skin colour. The interaction of age and sex of the birds influenced (P<0.05) the b* value (Table 6.3). The PABM fed male birds had a higher skin (P<0.05) b* value at 18 and 21 weeks of age at slaughter than the WM fed male birds of the same age at slaughter. The WM fed females at 21 weeks of age had a significantly higher b* value than the WM fed male birds of the same age and the WM fed females at 18 weeks of age (Table 6.4).

6.3.5 Effect of diet, sex and age on meat colour of the breast muscle of Ovambo chickens

The diet of the birds had a significant effect on the redness (a*) of the breast's muscle (Table 6.3). The PABM fed male birds at 21 weeks of age at slaughter has a higher a* value than the WM fed male birds of the same age (Table 6.5). The diet of the birds had a significant effect on the brightness (L*) of the breast's muscle. The age of the birds had an effect (P<0.05) on the L* value of the meat (Table 6.3). The WM fed male birds at 21 weeks had a higher L* value (P< 0.05) than at 18 weeks of age. The WM fed female birds had a higher L* value (P<0.05) at 21 weeks of age than the PABM fed female of the same age (Table 6.5).

The diet of the birds had a significant effect on the yellowness (b*) value of the breast's muscle. The sex of the birds influenced (P<0.05) the b* value of the breast muscle (Table 6.3). The PABM fed birds at 18 weeks of age had a higher b* value (P<0.05) than WM birds of the same slaughter age respectively. The PABM fed male birds at 21 weeks of age had a higher b* value (P<0.05) than the WM fed male birds of the same age (Table 6.5).

Table 6.5: Effect of diet, sex and age of slaughtered birds on the breast muscle colour of Ovambo chicken meat

			Breast muscle colour		
Age at slaughter	**Diet**	**Sex**	**L***	**a***	**b***
18 weeks	WM	Male	56.0^{a}	7.7^{ab}	13.6^{ab}
		Female	57.3^{ab}	7.3^{ab}	13.9^{b}
	PABM	Male	55.4^{a}	9.2^{bc}	16.6^{c}
		Female	55.8^{a}	9.1^{bc}	17.8^{c}
21 weeks	WM	Male	62.0^{bc}	6.8^{a}	11.3^{a}
		Female	63.2^{c}	6.8^{a}	15.8^{bc}
	PABM	Male	57.0^{ab}	10.2^{c}	15.7^{bc}
		Female	55.5^{a}	8.9^{abc}	16.1^{bc}
Pooled SEM			1.9	0.8	0.9

Means with different superscripts in the same column differ significantly ($P<0.05$).
L*: brightness; a*: redness; b*: yellowness.
WM: white maize (control); PABM: provitamin A biofortified maize.
SEM: standard error of means.

6.4 Discussion

The pH for standard meat quality is between the range of 5.7 to 6.1 (Lesiow *et al*., 2009). pH values below 5.7 is prone to high Pale Soft Exudative meat (PSE) which has pale colour, soft texture as a result of protein denaturation and high drip loss which makes the quality of the meat poor. A pH of above 6.1 gives Dark Firm and Dry meat (DFD). The DFD meat appears dark, variations in tenderness and easily susceptible to spoilage (Lesiow *et al*., 2009). The PABM diet did not significantly improve the pH of the meat compared to those fed WM diet. This is in agreement with He *et al*. (2010) who reported that β carotene supplement in pig's diet did not improve the pH. The finding of the decreased pH of birds as they increased in age agrees with earlier report by Abdullah *et al.* (2010). Similar higher pH in male birds than the female birds was also reported by Schneider *et al*. (2012).

A high drip loss is an indication of poor meat quality as it increases the loss in meat weight, leading to reduced economic value (Traore *et al.,* 2012). Drip loss reduced as the age at slaughter increases (Baeza *et al*., 2002). This is in agreement with the findings of the current study. These findings could be due to the lower muscle water content in the breast region as the bird ages (Baeza *et al*., 2002). Drip loss is an important factor that affects the shear force (tenderness) and cooking loss (He *et al*., 2010).

Cooking loss is an indication of the water holding capacity of the meat. A high water holding capacity results in a lower cooking loss and vice versa (López *et al*., 2011). Shear force is also termed as tenderness of meat. The findings on the non- significant effect of diet, age and sex and their interactions in cooking loss and shear force agree with earlier reports (Musa *et al.,* 2006;

Fanatico *et al*., 2007; Kruk, *et al*., 2008; López *et al.,* 2011). Wang *et al.* (2013) reported that age increased the cooking loss and reduced the tenderness in broilers. The variation from the current study could be due to the fact that the ages compared were different.

The colour of the meat is used by the consumer to determine its freshness. Colour in meat is influenced by blood and muscle pigment which can be affected by age, sex and diet (Pearson, 2013). The WM fed chickens had a higher L* value in meat muscle and skin which increased with age. The fact that the females have a significant higher L* value as their age increased is supported by the findings of Anadon (2002). There was no uniform difference in the L* value of the male and female birds. These results agree with Daghar *et al*. (2011).

Redness (a*) in the meat is an indication of freshness. The findings of the current study on the effect of PABM on the a* value of breast meat and skin agrees with the findings of Rajput *et al.* (2012). The result in the current study on the effect of age and sex on the a* value in the meat colour agrees with Dadgar *et al.* (2011) as there was no effect of age and sex in the a*value.

The PABM diet had a higher level of carotenoids which gave the chicken's skin and meat a yellowish colour (Castañeda *et al*., 2005; Rajput *et al*., 2012). The intensity of pigmentation in bird's skin mainly relates to the total amount of carotenoids in diets which also determines the amount of its absorption in the skin and subcutaneous fat (Pérez-Vendrell *et al.,* 2001; Castañeda *et al*., 2005). The effect of the PABM diet on b* value of the breast skin and muscle colour in the current study could have however been influenced by the fact that b* value is more visible and evidenced in the leg and shank more than the breast skin muscle (Tougan *et al*., 2013).

Carotenoid-supplemented chickens have greater skin yellowness in all body parts than non-supplemented chickens. Chickens fed a higher concentration of natural carotenoids have a yellowish colour in meat muscle as a result of the carotenoid deposit due to diet (Rajput *et al*., 2012) which is in agreement with the current study's finding. In many African and Asian countries, yellowish skin colour is preferred than white skin colour. The findings of higher b* value in the breast meat and skin of the male birds in comparison to the female birds is supported by the report of Smith *et al*. (2015).

High L* value in the skin and meat quality is an indication of PSE meat which reduces the quality of the meat. The findings that L* value of the breast muscle increased as the age increased is similar to the report by Lawrie and Ledward (2006) which showed that age affects the meat quality of animals. However, there was no uniform differences in the L* value of male and female birds which agrees with Dadgar *et al.* (2011) on broiler's breast meat quality. Meat colour is influenced by various factors that include the age and diet of the birds (Lyon & Lyon, 2001). The concentration of the PABM diet decreased L* value and increased a* and b* values depending on age and sex as contributory factors (Condron *et al.,* 2014).

6.5 Conclusions

The impact of PABM are not evident in the overall pH, drip loss, cooking loss and shear force, however, they are significant in the overall breast skin and muscle by giving it a higher b*, a* and lower L* value. The concentration of the PABM diet decreased L* value and increased a* and b* values depending on age and sex. These qualities are preferred by the African communities as they are believed to be evidence of healthy meat. It can be concluded that PABM diet improves the physical attributes such as skin and muscle colour of Ovambo chicken and will be readily acceptable to the South African consumers. It is, however, important to ascertain if PABM diet inclusion will also improve the nutritive value of the meat, especially the vitamin A. The quality of the skin and meat colour could have been influenced by carotenoids such as lutein and zeaxanthin also present in the maize which do not have provitamin A activities.

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CHAPTER 7

Effect of Provitamin A Biofortified Maize on Nutritive Value of Ovambo Chicken Breast Muscle

Abstract

The objective of the current study was to determine the effect of PABM inclusion on vitamin A content, proximate and mineral composition of the male and female Ovambo chicken meat to improve the food security and nutrition status of resource-poor households. A total of 102, 13 weeks-old Ovambo chickens were randomly distributed into two treatments fed on either a WM diet or PABM diet. They were reared for nine weeks. Breast muscles from eight male and female birds from each treatment were used. There was a significant increase of vitamin A concentration in the breast muscle of the PABM fed chickens. The WM diet had an average of 40mcg/100g while the PABM diet increased the vitamin A concentration to 55mcg/100g. There was no significant effect of PABM and sex on moisture, crude protein and fat content of the meat. The ash content in female birds were higher ($P<0.05$) than the male birds. There were no sex and diet effects ($P>0.05$) on the mineral composition of the muscle, except for copper. The PABM diet can be used to improve the vitamin A concentration in meat which can be consumed to improve vitamin A status of the resource-poor; however, it does not influence the overall proximate and mineral composition of the meat.

Keywords: proximate and mineral composition, resource-poor, sex, vitamin A, white maize

7.1 Introduction

Poultry meat is well liked and consumed all over the world (Henchion *et al*., 2014). Indigenous chickens are a potential tool for curbing malnutrition in southern Africa due to their large number in rural areas where VAD is prevalent, availability for consumption, their nutritive value, contribution to the rural livelihood and ease of management (Melesse, 2014).

Indigenous chicken have been mooted as a potential tool for curbing VAD and also improving the livelihood of the resource-poor household by their utilization of PABM as feed with the aim of increasing vitamin A level in chicken meat as well as other growth performances and meat quality. The effect of PABM has been evaluated on the growth performance, carcass and offal characteristics and blood profiles (Chapters 3 to 5). It was also important to assess the influence of PABM on meat quality, which is critical to the acceptability and preference of the meat by consumers. Apart from the effect of PABM diet on the physical appearance (colour), tenderness, cooking loss, pH and drip loss of the Ovambo meat which has been discussed in chapter 6, another important aspect of meat quality is its nutritive value. Consumers' preference is moving away from exotic birds to indigenous chickens as the indigenous chicken has higher protein and lower fat content than exotic birds (Wattanachant *et al*., 2004; Choe *et al*., 2010; Jayasena *et al.,* 2013). Indigenous chickens are, therefore, regarded as good sources of affordable, healthy, and nutritious meat (Jung *et al*., 2013). An increase in vitamin A presence in the meat of indigenous chicken will make it an effective tool for improving vitamin A status of the vulnerable groups in resource-poor households. It is possible to improve the nutritive value of chicken meat by manipulating the diet of the birds (Owens *et al.,* 2010). The extent to which sex and diet of birds influence the nutritive value of meat from Ovambo chickens is still unknown.

It is suggested that provitamin A biofortified maize diet could improve the nutritive value of the meat, especially vitamin A content in the meat (De Marchi *et al.,* 2005; Souza *et al*., 2011; Jung *et al.,* 2015), as β- carotene has provitamin A activities which could increase vitamin A in the meat. Beta-carotene also works with other micronutrients (notably vitamins E, C, and selenium) in the protection of tissues, in particular nervous tissues, from aggression by free radicals or active forms of oxygen (Bourre, 2006). There have been many reports on the positive effects of carotenoid diets and vitamin A supplements on the carotenoids and vitamin A concentration in the liver of birds (Bhuiyan *et al*., 2004; Pretorius, & Schönfeldt, 2013; Nogareda *et al.,* 2015) . The amount of vitamin A in the liver is used to determine the vitamin A status and absorption efficiency of bird. There is, however, little information on the vitamin A concentration in the meat muscles of birds as a result of various high vitamin A diets. There is no information on the effect of provitamin A maize diet inclusion, as a high vitamin A diet on the vitamin A concentration in the meat of indigenous chicken. Consumers want chickens that provide a high level of nutrition with minimal or no synthetic supplement due to increased fear of diseases such as cancer as a result of ingesting synthetic supplement residuals in food products (Owens *et al*., 2006). The breast part of the chicken has been reported to have high nutritional value, especially protein than other parts of the chicken (Večerek *et al*., 2005).

The objective of the current study was to determine the effect of provitamin A biofortified maize on the nutritional value of the meat of the male and female Ovambo chicken. It was hypothesized that PABM diet and sex does not have any effect on the nutritive value of the Ovambo meat.

7.2 Materials and methods

7.2.1 Study site and the ethical aspects of the study

The detailed description of the study site, average ambient temperature and relative humidity, and the ethics approval for management and use of birds are given in sections 3.2.1 and 3.2.2.

7.2.2 Birds, treatments and management

The details of the type of strain, number of birds, dietary treatments, management of the birds and the agronomic practices used for planting the provitamin A biofortified maize are provided explicitly in section 3.2.3.

7.2.3 Slaughter of the birds

The birds were slaughtered at 21 weeks as described in section 4.2.3.

7.2.4 Chemical and mineral composition of the breast meat samples

The breast meat samples without skin were freeze-dried for six days after which they were ground through a 2 mm screen. The vitamin A, dry matter, crude protein (N × 6.25), ash, fat and mineral content of the breast samples were analysed using the standard methods. The analyses were done in triplicate. To determine the vitamin A (retinol) present in the meat, dried meat samples were centrifuged for 15 min at 5800 rpm. About 10 g of meat sample was extracted with 250 mL hexane, 125 mL acetone and 125 mL ethanol. The sample was then mixed with about 4.5 mL hexane plus 0.5 mL sample and stirred. Retinol extraction and determination was performed by filtering samples through a column of aluminium oxide, packed in a Pasteur pipette, plugged with glass fibre, and then washed with hexane until clear. Two millilitres of sample was diluted to 10 mL with hexane. Total retinol was determined with a Genesys 10 spectrophotometer, at 450nm (ASM 072), (AOAC, 2005).

The fat content of dried meat was determined using Soxhlet extraction method (AOAC, 1984). About 2 g grounded meat sample was weighed into the Soxhlet cellulose extraction thimbles. The thimbles were plugged with a cotton wool to avoid loss of sample. The thimbles were transferred into the Soxhlet extractor. Sufficient petroleum ether was poured into the Soxhlet extractor beakers. The beakers were clamped into the Soxhlet machine and the electric heating plates were lifted to the base of the beakers. The samples were subjected to extraction for 3 h. Recovered solvent was transferred into an air oven (100^{o}C) for 1 h and then cooled in desiccators and weighed. The amount of oil extracted was calculated and expressed as percentage of original sample. % fat-= weight loss of sample (extracted fat)/ weight of sample.

To determine the crude protein, the total nitrogen content was determined by Kjeldahl nitrogen analysis, according to AOAC (1995). The percentage of crude protein was estimated by multiplying the total nitrogen content by a factor of 6.25. To determine the ash/ mineral content, dry samples of meat was weighed into a dry porcelain dish and then heated in a muffle furnace at 600^{0}C for 12 hours after which it was placed in desiccators for cooling before weighing. The percentage ash content was calculated as: % ash = weight of ash x 100/ Weight of sample (AOAC, 1980). Calcium, sodium, magnesium, zinc, copper and iron were determined by atomic absorption spectrophotometer method, and phosphorus was determined colorimetrically according to AOAC, (1984).

7.2.5 Statistical analyses

The data were analyzed using the GLM procedure of SAS (2010). Least square means were compared using the PDIFF (SAS, 2010). Statistical significance was considered at the 5 % level of probability.

The model used was:

$Y_{ijk} = \mu + D_i + S_j + (D \times S)_{ij} + (E_{ijk})$;

Where: Y_{ijk} = response variable (vitamin A, proximate and mineral composition);

μ= the overall mean;

D_i= effect of the i^{th} diet (i = PABM and WM diet);

S_j= effect of the j^{th} sex (j= male and female);

$(D \times S)_{ij}$= interaction of the i^{th} diet and the j^{th} sex;

E_{ijk}= random error term assumed to be normally and independently distributed with mean 0 and variance equal to $ð^2$.

7.3 Results

7.3.1 Effects of diet and sex of bird on the vitamin A concentration of Ovambo chicken breast meat

There was a significant influence of PABM in the vitamin A concentration in the Ovambo chicken breast meat. The vitamin A in the breast meat of the PABM fed chicken was higher than in the WM fed chicken. Sex of the birds and the interaction of diet and sex were not significant (Table 7.1 & 7.2).

7.3.2 Effect of diet and sex of Ovambo chicken on the proximate composition of breast meat

The effect of diet and sex and their interaction was not significant on moisture, crude protein and fat. There was a significant influence of sex of the birds on the ash content of the breast meat (Table 7.1). Females had higher level of ash content than the male birds. Female birds of both the PABM and WM had higher ash content ($P<0.05$) than the WM male birds (Table 7.3).

7.3.3. Effect of diet and sex of Ovambo chicken on the mineral composition of breast meat

The effect of diet was not significant on all the minerals. The effect of sex was not significant on all the minerals. There was no interaction of diet and sex on all the minerals, except copper (Table 7.1) The Cu level in the PABM female birds (6.25 ± 1.54) was higher ($P<0.05$) than the PABM male birds (1.72 ± 1.54) (Table 7.4).

Table 7.1: Levels of significance of effects of diet and sex of bird on vitamin A, proximate and mineral composition of Ovambo chicken breast muscle

Source of variation	Diet	Sex of bird	Diet × sex
Vitamin A	**	NS	NS
Proximate composition			
Moisture	NS	NS	NS
Crude protein	NS	NS	NS
Fat	NS	NS	NS
Ash	NS	*	NS
Mineral composition			
Sodium	NS	NS	NS
Calcium	NS	NS	NS
Magnesium	NS	NS	NS
Phosphorus	NS	NS	NS
Iron	NS	NS	NS
Copper	NS	NS	*
Zinc	NS	NS	NS

NS: not significant;* P< 0.05; ** P < 0.01; *** P < 0.001

Table 7.2: Effects of diet and sex of bird on the vitamin A of Ovambo chicken breast meat

	Vitamin A (mcg/100g)	
	Male	Female
WM	40.0[a]	40.0[a]
PABM	55.0[b]	50.0[b]
Pooled SEM	3.0	3.0

Values with different superscripts within columns and rows are different (P<0.05)
WM: white maize; PABM: provitamin A biofortified maize.
SEM: standard error of means

Table 7.3: Effects of diet and sex of bird on the chemical composition of Ovambo chicken breast meat

Diet	Sex	Moisture (g/kg)	Crude protein (g/kg)	Fat (g/kg)	Ash (g/kg)
	Male	738.1	218.4	38.0	19.8[b]
WM	Female	744.2	207.1	11.5	42.7[a]
	Male	740.1	216.7	19.4	31.7[ab]
PABM	Female	735.8	226.1	54.0	42.7[a]
Pooled SEM		18.3	14.5	11.3	4.2

Values with different superscripts within columns are different (P<0.05).
WM: white maize (control); PABM: provitamin A biofortified maize.
SEM: standard error of means

Table 7.4: Effect of diet and sex of bird on the mineral composition of Ovambo chicken breast meat

Diet	WM		PABM		
	Male	Female	Male	Female	SEM
Sodium (%)	0.19	0.23	0.24	0.24	0.01
Potassium (%)	1.02	1.27	0.93	1.22	0.14
Calcium (%)	0.03	0.04	0.02	0.04	0.01
Magnesium (%)	0.09	0.11	0.11	0.11	0.01
Phosphorus (%)	0.67	0.83	0.79	0.82	0.03
Iron (mg/kg)	25.88	25.77	23.66	20.45	1.59
Copper (mg/kg)	2.16^{ab}	2.26^{ab}	1.72^{b}	4.24^{a}	0.77
Zinc (mg/kg)	17.25	19.33	20.43	19.37	1.16

Within a row, values with different superscripts differ (P<0.05).
WM: white maize; PABM: provitamin A biofortified maize.
SEM: standard error of the means.

7.4 Discussion

One of the major aspects of meat quality is the types and amount of nutrients in the meat (Wattanachant, 2008). Chicken meat contains almost all the essential nutrients needed by the human body. The concentration of vitamins in the chicken meat can be influenced by the diet of the chicken (Sahlin & House, 2006). Beta-carotene in the diet of chicken is not majorly deposited in the meat tissues, but utilized as it is converted to retinol due to its provitamin A activities (Hudon, 1994). Birds are good converters of β-carotene to vitamin A (Surai *et al*., 2000). The findings of the higher amount of vitamin A present in the meat muscle of the PABM fed Ovambo chickens is supported by Jensen *et al*. (1998) who indicated that high vitamin A supplement increased the vitamin A concentration in the meat of chickens. The authors were able to increase the vitamin A in meat muscle from 90 to 180 mcg/kg (Rooke *et al.,* 2010). The current study increased the vitamin A in the meat muscle from 40mcg/100g to 55mcg/100g which is equivalent to an increase from 400 µg/kg to 550 µg/kg. Kang *et al*. (1998) also confirmed the findings of the current study on the increase of vitamin A in the meat of chicken fed natural sources of vitamin A using a palm oil diet rich in vitamin A. Increased dietary vitamin A intake also increased the vitamin A in the meat tissues of pig which has a similar digestive metabolism as chickens (Ayuso *et al*., 2015). Provitamin A carotenoids is absorbed through the intestine and about half of the provitamin A carotenoids are converted to retinol and the rest absorbed majorly by the liver and consequently other tissues in the body depending on the vitamin A status of the body (Harrison, 2012). The use of dietary provitamin A carotenoids is a good source of vitamin A as it does not have the risk of vitamin A toxicity as the cleavage of provitamin A carotenoids to retinal which is converted to retinol is a highly regulated step that is dependent on the body's requirement (Penniston & Tanumihardjo, 2006). Jlali *et al.* (2014)

wrote contrary to the current study, that the diet of supplemented β-carotene did not affect the concentration of vitamin A in the chicken's breast muscle of chickens. The difference in from the current study could be due to due the wheat based diet and used of controlled amount β-carotene supplement in the diet. The PABM is a natural source of β-carotene and other carotenoids with and without provitamin A activities. The average daily vitamin A requirement for human being is 600mcg (National Institute of Health (NIH), 2013). In the current study, the WM diet had an average of 40mcg/100g while the PABM diet increased the vitamin A concentration to 50-55mcg/100g. There is no relevant information on the effect of sex on the vitamin A concentration in the meat of chickens.

Moisture content is one of the main determinants of chicken meat quality (Castellini *et al.,* 2002). The range of the moisture content (736 to 744 g/kg) % reported in the current study is similar to earlier reports on indigenous chickens (Wattanchant *et al*., 2004; Chuaynukool *et al*., 2007). The higher the moisture content in meat, the lower the amount of dry matter which constitutes protein, fat and ash (Večerek *et al*., 2005). No sex differences in moisture content of breast meat have been reported earlier (Intarapichet & Maikhunthod, 2005; Saláková *et al*., 2009; Bogosavljevic-Boskovic *et al.,* 2010; Jung *et al*., 2015). High moisture content and high drip loss will result in high cooking loss (Nikmaram *et al*., 2013). As described in Chapter 6, cooking loss in the breast meat was not influenced by diet and sex of bird.

Protein is the most important nutrient in meat (FAO, 2007). Consumers want chickens that provide a high level of nutrition with minimal synthetic supplement (Owens *et al.,* 2006). The breast muscle has been reported to have higher protein level than other parts of the bird (Večerek *et al*., 2005). The protein level in Ovambo chicken breast meat agrees with several reports (Suchy *et al*., 2002; Wattachant *et al.*, 2004; Jung *et al*., 2015). The findings of protein level in the Ovambo breast meat in the current study is, however, higher than those reported by Van Marle-Koster and Webb (2000) on Ovambo chicken breast meat. The difference is probably because they used a combination of different parts such as the feet, muscle, skin and bone of the chicken to analyse proximate composition. Different parts of chicken have varying level of protein (Večerek *et al*., 2005). The breast muscle has the highest amount of protein in comparison to the other parts of the chicken (Večerek *et al*., 2005; Javaid *et al.,* 2012). The lack of sex effect on protein level in the breast meat agrees with De Marchi *et al*. (2005) and Intarapichet and Maikhunthod (2005) on indigenous chickens.

Fat content in meat affects the taste and texture of meat (Adeyanju *et al*., 2013). The PABM diet did not have any influence on the fat content in the level in the current study. This agrees with Jin *et al.* (2015) who reported that β- carotene did not influence the fat content in meat. The non - significant influence of sex on the fat content of breast meat of the Ovambo chicken agrees with earlier reports (De Marchi *et al*., 2005; Saláková *et al*., 2009).

De Marchi *et al.* (2005) reputed that sex affects the ash content which is a measure of the total amount of minerals present in breast meat of chickens. The higher ash content in the breast meat of female birds than male birds is similar with the current study. The ash content, however, reported by Souza *et al*. (2011) was higher in the male birds than the female birds. This contrast could be due to the difference in the meat part that was used. Souza *et al*. (2011) analysed the thigh meat. Ash constitutes muscle mineral contents that aid in muscle contraction and development. The muscle is more developed in the breast part of the female while the leg muscles are more developed in the male birds.

The interaction of diet and sex on copper of breast meat is unclear. Copper is important to human beings as its deficiency can results in anaemia and hereditary defect to excrete copper which can result in its accumulation and this detrimental to health (Cakci *et al*., 2013). Magnesium helps in supporting the functioning of immune system; assists in preventing dental decay by retaining the calcium in tooth enamel; it has an important role in the synthesis of proteins, fat, nucleic acid, glucose metabolism as well as membrane transport system of cells (Jahnen-Dechent & Ketteler, 2012). The similarity in the protein and fat content in this study based on diet and sex could have also resulted in the non-significant difference in magnesium as it aids in the synthesis of fat and protein.

Iron is the central metal in the haemoglobin molecule for oxygen transport in the blood and is present in myoglobin located in muscles. This important mineral is vital for wound healing and to keep the immune system strong (Moscow & Jothivenkatachalam, 2012). Iron is an essential element for human beings and animals and is an essential component of haemoglobin. It facilitates the oxidation of essential macronutrients to control body weight, which is very important factor in diabetes (Moscow & Jothivenkatachalam, 2012). Calcium in meat is used as an indicator of the amount of bone in chickens. The study shows that vitamin A and sex of birds does not have an influence on the amount of bone present in a chicken. The lack of influence on of PABM diet on the sodium, iron and zinc content level of the breast meat is similar to the report of Zapata *et al*. (1998).

The similarity in mineral composition of breast meat between males and females agrees with Geldenhuys *et al*. (2015) on geese except that there was an influence of sex on copper content in the current study. This difference could be due to difference in specie. The sex effect in the current study on iron also agrees with Intarapichet and Maikhunthod (2005) that sex of the birds does not have an influence on the Fe content of breast meat samples. This could be due to breast meat having low amount of myoglobin which contains iron and high amount of white fibers (Lengerken *et al.,* 2002). Breed, sex, nutrition, management system and meat type influence the chemical and nutritional composition of chicken meat (Bogosavljevic-Boskovic *et al.*, 2010).

7.5 Conclusions

Provitamin A biofortified maize diet inclusion increased the vitamin A in the meat muscle of chicken. Although, there was sex effect in the ash content and copper content of the breast muscle, the PABM diet did not have any overall impact in the proximate and mineral composition of the breast muscle. It can be concluded that feeding of PABM diet to indigenous chicken will increase the vitamin A in the meat of the chicken to provide a substantial proportion of the daily human requirement of vitamin A requirement to improve the vitamin A status of VAD vulnerable groups in the resource-poor households.

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CHAPTER 8

Effect of Provitamin A Biofortified Maize Inclusion on the Consumers' Acceptability of Ovambo Chicken Meat

Abstract

There is low acceptability of human consumption of PABM, in southern Africa to curb VAD. The objective of the current study was to determine whether PABM fed to the Ovambo indigenous chicken influences consumer acceptability of the meat. The leg (thigh and drumstick) of 21 week-old male birds were used for the consumers' acceptability evaluation. A total of 52 consumer panellists evaluated the sensory characteristics using a preference test and pictorial 5-point hedonic scale. There was no significant difference in the consumer preference of the two diets (WM and PABM). There was no significant interaction of diet, age and gender of the consumers on the taste, texture, aroma and acceptability of the meat. Females and youths (less than 30 years old) consumers had low hedonic rating ($P<0.05$) of the colour of the meat of chickens fed on the PABM maize. It was concluded that PABM diet fed to indigenous chickens will not reduce the overall acceptability of the chicken meat by VAD vulnerable consumers. Chickens fed on the PABM diet can be a tool for curbing VAD among the resource-poor households.

Keywords: age of consumer, consumers' acceptability, gender of consumer, Ovambo chicken, vitamin A

8.1 Introduction

Despite the various intervention strategies that have been implemented to curb VAD among resource-poor households, its prevalence is still substantial (Mason *et al*., 2014). Biofortification of maize, which is the major staple food in southern Africa with high β- carotene, a provitamin A carotenoid, is being implemented to achieve a higher reduction of VAD. The generally accepted maize for human consumption is, however, the white maize (WM), a low vitamin A maize. Other coloured maize types, such as yellow maize are primarily used as livestock feed (Shiferaw *et al*., 2011). Consumer acceptability surveys of PABM in various countries of southern Africa have indicated that consumers do not like the colour (yellow orange), aroma and taste of the provitamin A biofortified maize (Muzhingi *et al*., 2008; Pillay *et al*., 2011).

The indigenous chicken is a major source of protein and livelihood to the average rural households of southern Africa (Besbes, 2009). The cocks are usually consumed due to their larger body size and hens are kept for producing eggs for breeding, sale and consumption (Banerjee, 2012). Consumers also have preferences for certain chicken cuts. The leg (thigh and drumsticks) of chickens is a preferred part/cut for consumption (Khashman, 2012).

Quality of food is determined by their nutritional value, safety and sensory qualities of food are major factors for determining the quality of meat (Grunert *et al.,* 2004). A key goal of food security practitioners is to improve the nutritional quality of food without reducing the acceptability of the food as a result of changes in the organoleptic properties (Bou *et al.,* 2005). The effect of PABM and age improved the quality of the Ovambo chicken meat, especially the male birds at 21 weeks of age (Chapter 6), while there was an overall non-significance effect of

PABM and sex on the proximate and mineral composition (Chapter 7). If the PABM improves the quality of the Ovambo meat but negatively impacts its sensory characteristics, the acceptability of the meat will be low. This will make the intention of using the Ovambo chicken as an intervention for alleviating VAD by feeding the chicken with PABM maize futile. It is, however, not known whether a PABM-based diet affects the taste, texture, aroma and flavour of meat produced from the indigenous Ovambo chickens. Consumer preference and acceptability of meat, is based on a number of factors such as sensory attributes (Droval *et al*., 2012). The perceptions of these attributes and acceptability could be influenced by factors such as age and gender of consumers (He *et al*., 2003).

The objective of the current study was to determine the effect of age and gender of the consumers on sensory characteristics of meat from Ovambo male chickens fed on a PABM based diet. The hypothesis tested was that there is no significant difference between the PABM fed chicken meat and the WM fed chicken meat.

8.2 Materials and methods

8.2.1 Birds, treatments and their management

The detailed description of the rearing site of the chickens, average ambient temperature and relative humidity, and the ethics approval for management and use of birds are given in sections 3.2.1 and 3.2.2. The details of the type of strain, number of birds, dietary treatments, management of the birds and the management practices used for planting the provitamin A biofortified maize are provided in section 3.2.3.

8.2.2 Ethical aspects of the study and study site

The consumer acceptability tests were conducted in July, 2015 at Mkhambathini local municipality in KwaZulu-Natal. Mkhambathini local municipality is a peri-urban community, which is in close proximity to urban areas. A large portion of the municipality is rural in nature and generally underdeveloped. The municipality is situated along the south-eastern boundary of Umgungundlovu District Municipality. It covers an area of approximately 917 km^2 and is the second smallest municipality within Umgungundlovu District Municipality.

Ethical approval to conduct the study was obtained from the University of KwaZulu-Natal, Humanities and Social Science Research Ethics Committee (Approval number HSS/0482/015D- Appendix 2). Ethical approval was also obtained from Mkhambathini Municipality with the assistance of the municipality's management (Appendix 3). The sensory evaluation panellists were required to sign a consent form before participating in the study, the consent form was clearly explained in IsiZulu to accommodate illiterate individuals (Appendices 4 & 5).

8.2.3 Slaughter of birds

At the end 21 weeks of age, four male birds per treatment were randomly selected as they have higher boy weights than female birds resulting in their high demands by consumers. They also have better meat quality as stated in chaper 6. The birds were slaughtered conventionally by using a sharp knife to decapitate the heads from the necks. The birds were scalded in water at temperature ranging between 70 and 90^0 C. Feathers were plucked manually, the carcass eviscerated cut into different parts. The legs (thigh and drumsticks) were labelled according to the treatments and stored below -20^{o}C. The meat samples were thawed at -4^{o}C for 24 hours before it was used for sensory characteristics.

8.2.4 Meat sample preparation

Thighs and drumstick samples were cooked according to the traditional way of the Mhkambathini community. Briefly, the meat samples were slightly fried in vegetable oil. After about 10 minutes, water, salt and spices were added and it was cooked for 25 minutes. The skin and bones from the meat samples were removed and cut into small samples of approximately 15g.

8.2.5 Data collection

Consumer panel of 52 people were used for the evaluation of the sensory characteristics and preference test of the thigh and drumstick. The panellists consisted of both female and male youths, middle-aged and the aged that reside in Mkhambathini Community. The panel demographic characteristics are shown in Table 8.1. There were more females than males as the rural areas in South Africa have higher number of females and the females were more willing to participate in the evaluation. The panellists were selected based on the ages, locality, availability and voluntary willingness. The panellists were given a detailed understanding of the meaning of the meat attributes to be assessed (Table 8.2) and how to evaluate and record scores for each of the sensory characteristic. The explanations given to the panellists were adequate for them to participate in the consumer acceptability evaluation.

Table 8.1: Consumer panel demographics for consumer sensory evaluation of Ovambo chicken meat

	Characteristics	**Percentage (%)**
Sex	Male	21.2
	Female	78.8
Age	<30 years old	50.0
	30-50 years old	30.8
	>50 years old	19.2

Table 8.2: Definition of attributes for sensory analysis of chicken thigh and drumstick from Ovambo chicken

Attribute	5 point hedonic rating	Definition
Taste	1-5	Intensity of preferred taste of cooked chicken meat.
Texture	1-5	Intensity required to compress the sample between the teeth.
Aroma	1-5	Intensity of odour perception.
Colour	1-5	Intensity of meat darkness.
Acceptability	1-5	Overall likeness of the cooked chicken meat.

5 point hedonic scale rating: 1 = very bad; 2= bad; 3= average; 4 = good; 5= very good

To reduce bias associated with the labeling of samples, a table of random numbers was used to assign a unique three-digit code for the two meat samples. The samples were tested in randomised order from left to right. The meat samples were served in small white porcelain labelled bowls. To prevent the panellists from influencing each other's responses, the sensory evaluation panellists were made to sit about an arm's length apart. The bowls were labelled with 3-digit numbers according to the sample codes and served in a random order according to a random permutation of two. The panellists were also served with water. Once a sample has been tasted, the panellists would rinse their mouths before tasting the other sample to reduce crossover effects. The sensory evaluation was conducted over a period of three days at different locations in the community. The numbers of panellists for the three days were 15, 17 and 20, respectively.

8.2.5.1 Preference tests

The cooked samples were presented to 52 panellists. The panellists were asked to taste the two coded samples of cooked chicken meat taking into consideration the tenderness, juiciness, colour and flavour of the two coded samples, and then complete the preference evaluation form which was written in English and IsiZulu (Appendices 6 & 7) to select which sample was preferable.

8.2.5.2 Acceptability tests

A 5-point hedonic scale was used (Table 8.2) for the assessment of the sensory characteristics for consumers' acceptability. The evaluations forms were available in English and IsiZulu (Appendices 8 & 9). Fifty-two panellists were asked to indicate their degree of liking for each sample using the scale and indicate how much they liked or disliked each sample.

8.2.6 Statistical analyses

The data were analyzed using GLM procedure of SAS (2010). The model used incorporated the diet, age and sex of the panellists as fixed variables. Least square means were compared using the PDIFF procedure (SAS, 2010). Statistical significance was considered at the 5 % level of probability.

The model used was: $Y_{ijkl}=\mu + D_i + S_j + A_{k} + (D \times S)_{ij} + (D \times A)_{ik} + E_{ijkl}$

Where: Y_{ijkl} = response variable (sensory characteristics)

μ= the overall mean

D_i= effect of the i^{th} diet with i = PABM and WM diet;

S_j= effect of the j^{th} sex with j= male and female consumers;

A_k= effect of the k^{th} age; with k = <30 years old, 30-50 years old and >50 years old

$(D \times S)_{ij}$= interaction of the i^{th} diet and the j^{th} sex of consumer;

$(D \times A)_{ik}$= interaction of the i^{th} diet and the k^{th} age of the panellists;

E_{ijkl}= random error term assumed to be normally and independently distributed with mean 0 and variance equal to $ð^{2.}$

8.3 Results

8.3.1 Effect of diet, age and gender of consumers on the preference of Ovambo chicken meat

Half of the panellists preferred the chicken meat fed the control diet. There was no significant difference in the preference based on gender and age of the panellists. There was no interaction of diet and gender of the consumers ($P > 0.05$) and diet and age of the consumer ($P > 0.05$) on the preference of the meat.

8.3.2 Effect of diet, age and gender of consumers on taste of Ovambo chicken meat

Panellists in the 30-50 years age group gave the highest rating in taste in both diets, however there was only a significant difference in the hedonic ratings of the WM fed chicken meat between the 30-50 years old and the older than 50 years old panellists (Table 8.3). The effect of the PABM diet and age of the panellists on taste was not significant. There was no interaction of diet and the gender of consumers on taste of the meat (Table 8.4).

8.3.3 Effect of diet, gender and age of consumers on the texture of meat

Panellists in the 30-50 years age group gave the highest ratings in texture in both diets. There was a significant difference in the hedonic ratings of the WM fed chicken meat between the 30-50 years old and the older than 50 years old panellists (Table 8.4). The interaction of diet and the gender of consumers on texture of the meat was not significant (Table 8.3).

8.3.4 Effect of diet, gender and age of consumers on the colour of Ovambo chicken

Panellists who were younger than 30 years old preferred the colour of the WM meat ($P<0.05$). Panellists who were older than 50 years old preferred ($P<0.05$) the colour of the PABM meat more than the younger than 30 years old (Table 8.3). There was a higher likeness of the colour of the WM meat by male and female consumers; however, the preference of the colour of WM meat was only significant in the female consumers (4.1 ± 0.21) in comparison to the colour of the PABM meat (3.5 ± 0.21) (Table 8.4).

8.3.5 Effect of diet, gender and age of consumers on the aroma of Ovambo chicken meat

There was no significant influence of the diet that the birds consumed, gender and age of the consumers on the aroma of the meat (Table 8.3). There was no significant interaction of diet and the gender of consumers on aroma of the meat (Table 8.4).

8.3.6 Effect of diet, gender and age of consumers on the acceptability of Ovambo chicken meat

The interaction of diet of the birds and age of the consumers on acceptability of the meat was not significant (Figure 8.4). There was no interaction of the diet of the birds and the gender of consumers on acceptability of the meat (Table 8.3). There was no hedonic rating regardless of diet of the birds, age and gender of the consumers that had a lower rating than 2.8 ± 0.21. There was an overall higher hedonic rating of the females; however it was not significant (Table 8.4).

Table 8.3: Effect of diet and age of consumers on the sensory characteristics of Ovambo chicken meat

Diet	Gender	Taste	Texture	Colour	Aroma	Acceptability
	<30 years old	3.8^{ab}	3.9^{ab}	3.8^{b}	3.5^{ab}	3.9
WM	30-50 years old	4.5^{a}	4.5^{a}	4.0^{b}	3.9^{ab}	4.1
	>50 years old	3.2^{b}	3.2^{b}	3.4^{ab}	4.1^{b}	3.8
PABM	<30 years old	3.7^{ab}	3.6^{ab}	2.9^{a}	2.9^{a}	3.5
	30-50 years old	4.0^{ab}	4.0^{ab}	3.2^{ab}	3.3^{ab}	3.9
	>50 years old	3.2^{ab}	3.9^{ab}	3.9^{b}	3.8^{ab}	4.1
Pooled SEM		0.4	0.4	0.2	0.2	0.4

Values with superscripts that differ in columns are significant (P<0.05).
WM: white maize (control); PABM: provitamin A biofortified maize.
SEM: standard error means.

Table 8.4: Effect of diet and gender of consumers on the sensory characteristics of Ovambo chicken

Diet	Gender	Taste	Texture	Colour	Aroma	Acceptability
	Male	3.5	3.5	3.4[a]	3.8	3.5
WM	Female	4.3	4.2	4.1[b]	3.8	4.3
	Male	3.8	3.8	3.2[a]	3.2	3.8
PABM	Female	3.9	3.9	3.5[a]	3.5	3.9
Pooled SEM		0.4	0.4	0.2	0.2	0.4

Values with superscripts that differ in columns are significant (P<0.05).
WM: white maize (control); PABM: provitamin A biofortified maize.
SEM: standard error mean

8.4 Discussion

The male Ovambo were used in the study on consumer's acceptability as they are consumed more than the females as the female birds are kept for egg laying (Banerjee, 2012). Another reason is the consumption preference of male chickens due to their larger sizes. The male birds were significantly heavier than female birds as discussed in chapter 3. The selection of the age of the male bird was selected based on the fact that the body weight of the male birds was significantly heavier and the skin and meat colour better at 21 weeks at age of slaughter in comparison to 18 weeks at age of slaughter as discussed in chapter 3 and 6 respectively.

The non-significant effect of PABM maize diet inclusion in the preference of the meat regardless of the age and gender of the consumers is similar to the finding of Bartlett and Beckford, (2015) on the sensory characteristics of chicken meat fed sweet potato meal which has high level of β carotene. The major five factors that are important to meat consumers are taste, texture, juiciness, colour and aroma, texture and colour (Fletcher, 2002). Based on the results of the preference test, it is evident that these factors in the meat were not influenced by the diet of the birds.

Generally, raw meat has little aroma and taste (Mottram, 1998; Maughan & Martini, 2012). Poultry meat has a low natural taste and aroma and it is considered as having neutral flavour and aroma compared to meat sources such as from goats and boars (Petracci *et al*. 2013). The taste and aroma of cooked chicken meat is highly dependent on the cooking method (Sanudo *et al*., 2000). The meat was prepared using the traditional method of cooking chicken in Mkambathini community which the people are familiar with and prefer. This could have affected their ratings on the aroma and taste of the panellists.

The two major contributors to poultry meat texture are the maturity of the connective tissues and contractile state of the myofibrillar proteins (Fletcher, 2002).There have been no relevant findings on any influence of β-carotene diet inclusion on the connective tissues and myofibrillar proteins of protein meat. The findings that the PABM diet had no effect on the hedonic rating of texture of the meat regardless of the sex and age of the consumers is similar to the previous report of Bartlett and Beckford (2015). The significant effect of age of the consumers on the ratings of the WM fed meat is unclear. It, however, could be because older people may have difficulty in chewing due to dentition problem and weak muscles, which is common among older people. Other older people have reduced saliva production which may affect their ability to chew and swallow (Szczesniak, 2002).

The acceptability of the meat in the current study is similar to King *et al*. (1995), who reported no differences in the β- carotene supplementation diet on the consumer's acceptability on the colour, taste, texture, and aroma of the chicken meat. Bartlett and Beckford (2015) reported that sensory characteristics of broilers fed potato root meal, a meal rich in β carotene was not affected by gender and age of the consumers. Gender of consumers had no effect on the acceptability of chicken meat. The lack of gender effect in the current study is similar to earlier reports of Dyubele *et al.* (2010) and Chulayo *et al.* (2011), except that gender of the consumers had an influence on the rating of the likeness of meat based on colour in the current study. The meat from birds fed on PABM diet had a redder colour, which is an indication of fresh, healthy meat. The thigh and drumsticks of the chickens which are dark meat were used for the sensory evaluation, the PABM fed chicken meat had a slight darker colour than the WM which made the female panellists give the PABM fed meat a lower ratings. The lower ratings of the female panellist could be attributed to their preference for light red meat while male prefer dark red meat (Fortomaris *et al*., 2006). The lower rating of colour by females could be due to their association of reddish colour of meat with unhealthy nutritive value similar to red meat such as beef (Kubberød *et al*., 2002). The higher ratings of consumers younger than 30 years olds than the older than 50 years old on the meat colour indicate that younger consumers prefer light red meat probably due to their relating redness/darkness of meat with unhealthy fat (Kennedy *et al*., 2004). Another reason for difference in ratings of colour based on age could be because as human beings grow older they tend to have chemosensory losses which diminish their ability to perceive fully sensory properties of foods (Laureati *et al*., 2006).

Sensory characteristics are affected by various factors that include locality, diet of the animal and method of cooking (Muchenje *et al.,* 2010). The cultural background and attitude of consumers as well as food preference has an impact on the acceptability of meat (Nicklaus *et al.,* 2004; Dyubele *et al.* 2010). Indigenous chicken are preferred by African consumers due to their distinct taste, colour, texture and aroma. All these sensory characteristics were not affected by the PABM diet inclusion in the feed of the chickens.

8.5 Conclusions

The type of maize fed to Ovambo chickens has no overall influence on the consumer preference of the meat produced. The influence of age and sex of the consumer on the rating of the chicken meat also had no effect on their preferences except on the colour of the meat; however this was only significant in the female consumers. It can be concluded that PABM will not influence the acceptability of the chicken meat. Chickens fed on provitamin A biofortified maize can be a possible tool for curbing VAD in southern Africa regions where there is low acceptability of the human consumption of provitamin A biofortified maize.

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CHAPTER 9

General Discussion, Conclusions and Recommendations

9.1 General discussion

The slow rate of curbing vitamin A deficiency in SSA has led to more detailed reviews of the intervention strategies that have been put in place over the years. Accessibility, affordability and availability which were lacking in these intervention strategies (supplementation, diversification and fortification) to the resource-poor households are required for optimal results. Biofortification of maize, a common staple crop in SSA, with provitamin A carotenoids was developed to combat the shortfalls of these intervention programmes. It was however discovered that although PABM has many potential benefits as a means of curbing VAD, it has low acceptability in southern Africa due to its organoleptic properties and perceptions of the majority of the people in the region. Thus, two of the pillars of food security, acceptability and utilization, were not met. To solve the issue of acceptability and utilization, it has been suggested that indigenous chickens, which are numerous in rural communities, and has been identified as an intervention to curbing malnutrition in Africa, especially among the resource-poor household Africa could be fed the PABM to improve the growth performance, meat quality as well as the level of vitamin A in chickens to enhance food and nutrition security of the resource- poor households as well as maximise the livelihood options.

Chapter 3 of the study hypothesized that the utilization of the PABM on growth performance of the Ovambo would not be affected by the age and sex of the bird. The inclusion of PABM in the diet of Ovambo chickens did not have any negative effect on ADFI, ADG and FCR of the birds. The hypothesis was, therefore, rejected. The effect of the PABM diet depended on age and sex of the birds to improve growth performance. Male birds were superior in their growth performance than their female counterparts. The PABM diet improved the FCR of the females between 19-20 weeks of age. The ADG and FCR improved between 15 and 17 weeks in male birds, 19-20 weeks in female birds.

It was demonstrated in Chapter 4 that PABM fed chickens had better dressed carcass and leg weight than the WM fed chickens. It was hypothesized that PABM, age and sex of the birds or their interactions do not influence the weight of the carcass cuts and offals of the Ovambo chickens. The interaction of the PABM diet, sex and age of the birds were considered depending on the preferred carcass cuts and edible offals. The study rejected the hypothesis and confirmed that PABM improved the body weight and dressed carcass weight of Ovambo chickens. For sale of carcass cuts, female birds have better breast and back relative weight compared to the male chickens, while the males had better leg weight than the females. At 21 weeks of age, the Ovambo birds had better growth rate and carcass characteristics than at 18 weeks old. These findings are important for the commercialisation of Ovambo chickens to optimise livelihoods of the resource- poor households.

It was important to assess that feeding PABM to chickens will not be detrimental to the health of the chickens. In Chapter 5, the haematological and serum biochemistry of the chickens were assessed. The hypothesis tested was that there was no difference in haematological and biochemical responses to PABM between the male and female Ovambo chicken. The study showed that all mean values for the haematological parameters regardless of the age, sex and diet were within the normal range. The PABM diet increased the WBC (leucocytes) of the chickens. The PABM also increased the RBC (erythrocytes) of females fed on the PABM diet than the WM fed females. The WBC of the female birds was higher than the males. The PABM reduced the cholesterol level in male birds. It was demonstrated that use of PABM, as feed and source of vitamin A did not compromise the health status of Ovambo chickens. The hypothesis was, therefore, not rejected.

In chapter 6, the meat quality of the birds was improved by the PABM inclusion as the meat and skin colour had lower lightness, higher redness and yellowness of meat which are indicators of healthy meat. The improved meat qualities which are the meat and skin colour were more apparent in male birds than the female birds. Age improved the meat quality in the male birds while it reduced it in female birds. The findings on the skin and meat colour of the Ovambo chicken in chapter 6 signified that the meat quality of meat is improved by the intake of natural carotenoids present in the PABM.

The hypothesis tested in chapter 7 was that PABM diet and sex did not influence the vitamin A concentration, proximate and mineral composition of the Ovambo meat. In the current study, however, PABM diet improved the vitamin A concentration in the meat but did not influence the proximate and mineral composition of the breast muscle. The effect of sex was only evidenced in the ash content which was higher in the female birds.

In Chapter 8, the hypothesis tested was that there was no difference between the meats from birds fed on PABM and those on the WMWM diet. It was demonstrated that PABM inclusion did not affect the consumer acceptability of the meat. The panellists did not perceive a difference in the aroma, texture and taste of the chicken meat.

9.2 Conclusions

Indigenous chickens fed PABM are a potential tool to curbing VAD in southern Africa as it increased the vitamin A concentration in the meat of the Ovambo chicken. The positive effect of the PABM diet was also evident in some carcass characteristics and meat quality of the male birds. The growth performance parameters were influenced majorly by the age and sex on the birds. The health of the chickens were not compromised by the PABM but rather increased the immunity of the chicken. Ovambo chicken fed PABM diet will be accepted easily for consumption by the resource- poor households in southern Africa as the distinct organoleptic properties of the PABM was not evidenced in the meat. Feeding PABM to indigenous chickens is an easy alternative intervention of promoting the utilisation and acceptability of PABM maize to curb VAD. This is because there is no limit to the amount of PABM that can be given to the birds as provitamin A carotenoids do not cause toxicity of vitamin A and the PABM can be planted by the resource-poor. Thus, indigenous chicken and PABM can be a cost effective combined interventions for curbing VAD. This is particularly important because in most southern African countries, chickens are owned, managed and controlled by women and children who are the vulnerable group and often at most risk of suffering or being exposed to VAD.

The indigenous chicken satisfies all the criteria mentioned plus the added benefit that the targeted group (women and children) has access and control over the chickens.

9.3 Recommendations

Vitamin A is important to the development of chickens and humans. Provitamin A biofortified maize can be grown by farmers and fed to indigenous chickens as a natural source of vitamin A by the rural households to improve the growth performance, carcass characteristics, health and meat quality of the chicken. It is suggested that provitamin A biofortified maize should be integrated into the farming system of the rural farmers in southern Africa by building the capacity of extension workers to giving trainings on the benefits of the maize based on its nutrients and growth capacity as well as monitor the sustainability of the planting of PABM.

To ensure that food and nutrition security is improved, the interventions developed should be available and accessible to all the people, all the time at reasonable/ minimal cost. It should be socially acceptable and satisfy the consumer preferences. It should also be safe, offer beneficial nutritional value and be based on what is locally available and accessible.

South Africa authorised the Food and Nutrition Security policy in 2013, and its implementation plan was launched in 2015. Although both the policy and the plan acknowledged that hidden hunger and food access are still challenges in South Africa, the interventions to combat these challenges are still the same as the previous years. More so, the policy and the plan do not really focus on using livestock as the vehicle to combat these challenges. Therefore, this study will inform the policy makers and plan implementers on innovative and cost effective interventions of eradicating food and nutrition insecurity especially amongst resource-poor households and vulnerable groups using livestock

Further research recommendations are:

1. It is suggested that the effect of PABM inclusion on day old chicks be researched till they reach maturity age to have a comprehensive understanding of how PABM is utilised.
2. Research on the vitamin A concentration in the skin and liver of the PABM fed chickens should be conducted as well as the bioavailability of the vitamin A towards the continuation of finding an alternative means to curbing VAD among the resource-poor effectively.
3. The vitamin A concentration in different chicken cuts such as the breast, thigh and drumsticks of PABM fed chickens fed should also be assessed as different parts of chickens may have varying nutritional concentrations.
4. Effects of other natural sources of carotenoids available in rural areas such as leafy green vegetables on indigenous chicken growth performances and nutrition value should be investigated for optimized use of cost effective natural sources of diet to curb VAD and improve the livelihood of the resource-poor.
5. There is also the need to investigate the retention of vitamin A concentration in the meat of chickens fed PABM after undergoing different processing and cooking methods.

6. The effect of PABM on different strains of chickens and poultry should also be investigated.
7. The effect of PABM on the cholesterol level of different strains of chickens and poultry should also be investigated.

Appendices

Appendix 1: Animal Research Ethics Approval

UNIVERSITY OF KWAZULU-NATAL™
INYUVESI YAKWAZULU-NATALI

3 June 2014

Reference: 091/14/Animal

Ms F Odunitan-wayas
Animal Science
School of Agricultural, Earth &
Environmental Sciences
University of KwaZulu-Natal
PIETERMARITZBURG Campus

Dear Ms Odunitan-wayas

Ethical Approval of Research Projects on Animals

I have pleasure in informing you that the Animal Research Ethics Committee has granted ethical approval for **2014** on the following project:

"Influence of chicken breed and age on provitamin A utilisation to improve food and nutrition security."

Yours sincerely

Professor Theresa HT Coetzer
Chairperson: Animal Ethics Sub-committee

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

Appendix 2: Humanities & Social Sciences Research Ethics Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

5 June 2015

Mrs Feyisayo Odunitan-Wayas 214583665
School of Agriculture, Earth and Environmental Sciences
Pietermaritzburg Campus

Dear Mrs Odunitan-Wayas

Protocol reference number: HSS/0482/015D
Project title: Influence of biofortified provitamin A maize on chicken age and sex to improve food security nutrition.

Full Approval – Expedited Application

In response to your application received on 12 May 2015, the Humanities & Social Sciences Research Ethics Committee has considered the abovementioned application and the protocol have been granted **FULL APPROVAL.**

Any alteration/s to the approved research protocol i.e. Questionnaire/Interview Schedule, Informed Consent Form, Title of the Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

PLEASE NOTE: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of 3 years from the date of issue. Thereafter Recertification must be applied for on an annual basis.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

………………………………………………………………

Dr Shenuka Singh (Chair)
Humanities & Social Sciences Research Ethics Committee

/pm

Cc Supervisor: Dr Unathi Kolanisi
Cc Academic Leader Research: Professor Onisimo Mutanga
Cc School Administrator: Ms Marsha Manjoo

Humanities & Social Sciences Research Ethics Committee
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Appendix 3: Approval from Mkhambathini Municipality

Private Bag X04
Camperdown
3720

Mkhambathini Municipality

Tel no: 031) 785 9300
Fax no: (031) 785 2121

FROM	:	COMMUNITY SERVICES MANAGER
TO	:	WARD COUNCILLORS.
CC	:	TRADITIONAL LEADERS
	:	OSS WAR ROOM CHAIRPERSONS
	:	WARD COMMITTEE MEMBERS
	:	CCG's, YOUTH AMBASSADORS AND CDW's
DATE	:	18 JUNE 2014
SUBJECT	:	PARTICIPATION IN FOOD SECURITY RESEARCH– UNIVERSITY OF KWAZULU NATAL

The University of KwaZulu-Natal, with the support of Mkhambathini Local Municipality, is conducting a research study on "***The consumption and utilization of indigenous chicken meat and eggs by rural communities.*** *Provitamin A biofortified yellow maize to eradicate hidden hunger*"

The target group for the said research is mainly households and cooperatives that produce and consume eggs. The anticipated duration of the research is 2 months, starting from the last week of June until end of August 2014.

Dr Unathi Kolanisi and Dr Mthulisi Siwela with 4 post graduate students (University of KwaZulu Natal), will be in contact with each ward/wardroom to explain further on the research and to set up the interviews.

Your anticipated cooperation and support of this initiative is appreciated. Please contact the Community Services Unit, details indicated above for any enquiries relating to the research study. Dr Kolanisi and Dr Siwela, of the University of KwaZulu-Natal, can also be contacted on 0730548481 and 0724 59652 respectively.

Yours sincerely,

MS G.H. BHENGU
COMMUNITY SERVICES MANAGER

Appendix 4: Consent Form for Consumer's Acceptability in English

My name is Feyisayo Odunitan-wayas and I am a full-time student at the University of KwaZulu-Natal registered for PhD in agriculture (food security). I would like you to participate in a study evaluating the acceptance of chicken meat based on diets. Therefore you will be required to taste chicken meat samples and further rate each sample using a preference test and simple picture scale indicating your views on the taste, texture, smell, colour and overall acceptability.

- Participation in this study is voluntary; participants are free to leave the study any time they wish.
- There will be no form of payment for participating in the study
- All information will be kept confidentially and will only be used for the purpose of this study.
- All information will be destroyed when it is no longer needed.
- For any further information with the study, you may contact Dr Kolanisi who is the supervisor of the study at 033 260 6342 or kolanisi@ukzn.ac.za

Declaration:

I ________________________________ (full name and surname) hereby confirm my understanding of the questionnaire and I understand that there will be no risks from the study and I may withdraw if I desire since the study is voluntary.

_________________________ _______________________

Signature Date

Appendix 5: Consent Form for Consumer's Acceptability in Isizulu

Igama lami ngingu Feyisayo Odunitan-wayas, ngingumfundi eNyuvesi yaKwazulu-Natal, ngenza i-PHD kwi Agriculture/kwezolimo (food security). Ngingathanda ukuthi ube yingxenye yalolu cwaningo mayelana nokwamkeleka kokudla okwenziwe ngamaqanda. Lokhu kusho ukuthi uzodingeka ukuthi unambithe ukudla okwenziwe ngamaqanda bese ukhombisa imibono yakho mayelane noku nambitheka, ukuzwakala emlomeni, iphunga, umbala kanye nesimo sonke jikelele.

Kubalulekile ukuthi wazi okulandelayo:

- Abantu abayingxenye yalolucwaningo ngokuvolontiya, abantu abayingxenye yalolucwaningo bavumelekile ukuthi bashiye phakathi kwalo uma bafisa akukho lutho olubi oluyokwenziwa kubona.
- Ayikho imali eyotholwa abantu abayingxenye yalolucwaningo.
- Imininingwane ezotholakala ngeke isetshenziselwe okunye okuseceleni, izosebenziswa kulolucwaningo kuphela. Imininingwane yabantu abazobe beyingxenye yalolucwaningo izogodlwa.
- Yonke imininingwane yalolucwaningo izolahlwa uma ingasadingeki.
- Uma udinga eminye imininingwane ngalolucwaningo ungathintana no Dkt. Kolanisi ongumphathi walolucwaningo.Utholakala kule nombolo-033 260 6342 noma kolanisi@ukzn.ac.za.

Izwi lobufakazi:

Mina_________________________________ (Amagama aphelele nesibongo) ngiyaqiniseka ukuthi ngichazelekile kahle ngalembuzo engizobuzwa yona futhi ngiyasiqonda isizathu salolucwaningo nokuthi yonke imininingwane etholakele izohlolwa. Ngiyavuma ukuba ingxenye yalolucwaningo, ngiyaqonda ukuthi kuyavolontiywa ukuba ingxenye yalolucwaningo nanokuthi ngingashiya phakathi uma ngifisa.

________________ ________________________

Sayina Usuku

Appendix 6: Paired Preference Test in English

Participant number: ---------------------------------

Sample number: --------------------------------------

Gender: ---

Age:|__||__|

Instructions

Please rinse your mouth with water before starting.

Please taste the two food samples in the order given, from left to right.

Please circle the number of the sample that you prefer.

_________ ________

Thank you for taking part in this study

Appendix 7: Paired Preference Test in Isizulu

Inombolo onikezwe yona: --------------------------------

Inombolo yesampulo: -------------------------------------

Ubulili: ---

Iminyaka: |__||__|

IMIYALELO

Sicela uxubhe umlomo wakho ngamanzi ngaphambi kukoqala.

Sicela uzwe lezinhlobo ezimbili zokudla ngendlela ezihlelwe ngayo, kusukela kwesokunxele kuyakwesokudla.

Sicela ubeke uphawu (X) enambeni yesampula oyikhethayo.

________ _______

Siyabonga ngokubamba iqhaza kuloluncwaningo

Appendix 8: Sensory Evaluation Questionnaire in English

5 POINT PICTORIAL HEDONIC SCALE

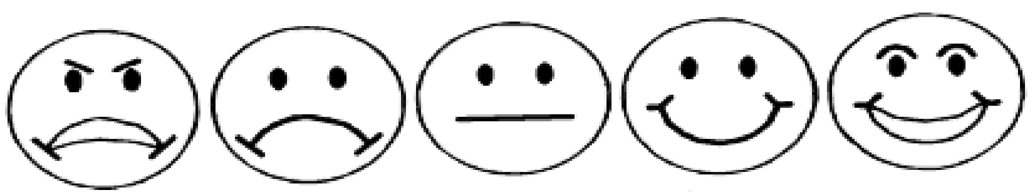
<u>Gender:</u> **Male** **Female**

<u>Age</u>: ____

<u>Number</u>: _____

<u>Sample number</u>: ______

TASTE



Very bad **Bad** **Average** **Good** **Very good**

TEXTURE

Very bad **Bad** **Average** **Good** **Very good**

AROMA

Very bad **Bad** **Average** **Good** **Very good**

COLOUR

Very bad **Bad** **Average** **Good** **Very good**

OVERALL ACCEPTABILITY

Very bad **Bad** **Average** **Good** **Very good**

Appendix 9: Sensory Evaluation Questionnaire in Isizulu

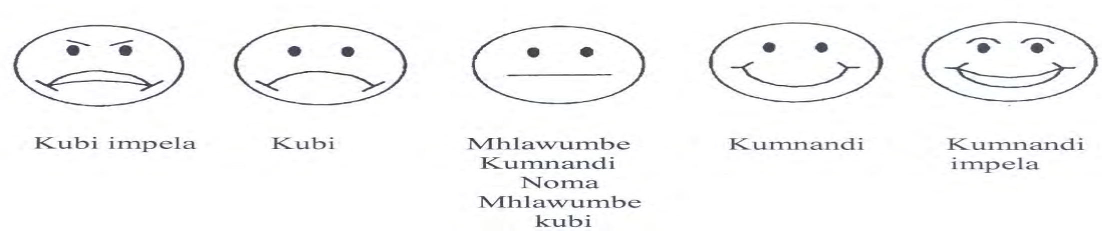
Inombolo onikezwe yona: --------------------------------

Inombolo yesampulo: -------------------------------------

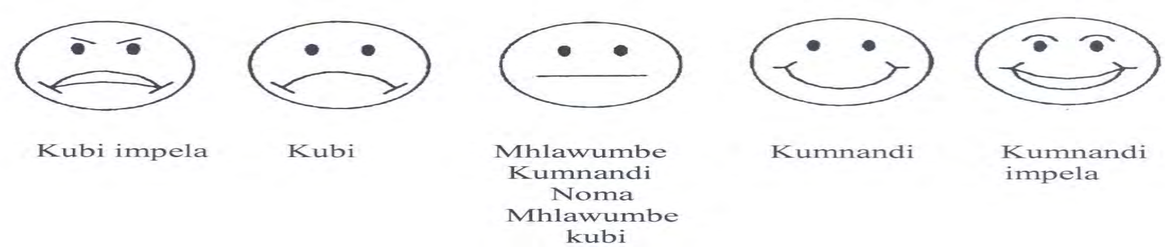
Ubulili: ---

Iminyaka: |__||__|

UKUNAMBITHEKA

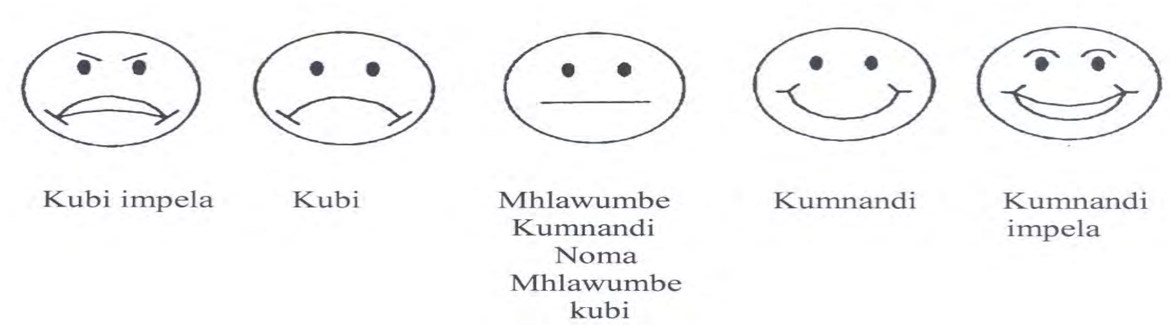


UKUZWA NGEMLOMO

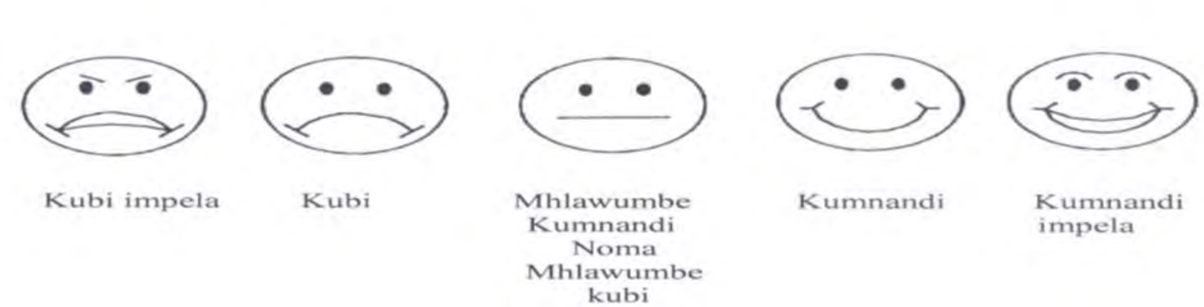


IPHUNGA

UMBALA



ISINQUMO JIKELELE (UKWAMUKELEKA)



Appendix 10: Publications

1. Odunitan-Wayas, F.A., Kolanisi, U., Chimonyo, M. and Siwela, M., 2015. The potential of crossbreeding indigenous chickens to improve rural food security and nutrition in southern Africa-a review. Indilinga African Journal of Indigenous Knowledge Systems, 14(2), 195-209.

2. Provitamin A biofortified maize to enhance chicken nutritional value for the benefit of rural households – a review. The African Journal for Physical, Health Education, Recreation and Dance (Accepted).