Physiological responses and meat quality of Potchefstroom koekoek cockerels offered canola meal as an alternative to soybean meal

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DECLARATION

I, Freddy Manyeula, declare that the thesis hereby submitted by me for the degree of Doctor of Philosophy in Agriculture in Animal Science at the North-West University is my own original and independent research work. The thesis was carried out under the supervision of Profs. V. Mlambo and U. Marume and Dr. N. A. Sebola. 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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GENERAL ABSTRACT

The current study was conducted to assess growth performance, protein utilisation efficiency, blood parameters, meat quality, bone breaking strength, density and mineral composition of Potchefstroom Koekoek (PK) cockerels offered canola meal as an alternative to soybean meal. The aim of the study was to evaluate the potential use of canola meal (CM) to replace soybean meal (SBM) in broiler grower diet as protein source. This was achieved through feeding indigenous chickens diets with incremental levels of CM with the expectation that the chicken's performance would have negatively affected. A hundred and seventy-five, 36-day old PK cockerels were randomly allocated to the dietary treatments: Control = diet with no canola meal inclusion, CM37.5 = 37.5 g canola meal/kg soybean meal, CM62.5 = 62.5 g canola meal/kg soybean meal, CM87.5 = 87.5 g canola meal /kg soybean meal, CM175 = 175 g canola meal/kg soybean meal. Canola meal had numerically higher average concentration of ash, crude fibre while dry matter, organic matter and crude protein were higher in SBM. The concentration of calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na) and sulphur (S) was numerically higher in SBM while copper (Cu), manganese (Mn) and iron (Fe) were higher in CM. Formulated diets had similar ($P > 0.05$) apparent digestibility values for minerals, dry matter and fibre but higher inclusion of CM reduced ($P < 0.05$) crude protein (CP) digestibility. Diet significantly affected growth performance parameters, protein utilisation efficiency and serum biochemistry of the PK cockerels. All the mean values of the haematological parameters were within the normal range regardless of the inclusion levels of CM. The PK cockerels fed control diet had the lowest ($P<0.05$) breast muscle, wing, drumstick and vertebrae weight. The PK cockerel fed control diet had the lowest ($P<0.05$) heart (22.20g) and liver weights (7.9 g). Higher ($P<0.05$) small intestine weights were observed on the PK cockerels fed diet CM175. The L* value and b* value of the breast muscle from the PK cockerels fed diet CM37.5 were

significantly higher than those fed the control diet. The a* value of the breast muscle increased significantly with the inclusion levels of CM

The pH_u (Ultimate pH) values of the meat from PK cockerels fed diet CM37.5 (5.97) were significantly lower ($P<0.05$) than those fed control diet (6.18). However, the PK cockerels fed the control and CM37.5 diets had the lowest shear force values. Breast muscle from cockerels fed the control diet had the highest ($P<0.05$) concentration of Ca, Mg, P, Na and K. There were no significant dietary effects on tibia length, weight, width, density, diameter proximal end, diameter distal end, breaking strength and ash percentage. Diets, however, had a significant effect on macro and trace mineral concentrations of the tibia. Tibia from cockerels fed diet CM175 had the lowest ($P<0.05$) Ca and P content. Lower ($P<0.05$) tibia Mg and Na concentration were observed in the cockerels fed diets CM87.5 and CM175 compared to tibia of those cockerels fed on the other diets. The findings of this study concluded that CM shown to have potential as an alternative to SBM in grower broiler diets and the indigenous chickens fed CM can be a tool for curbing sodium, potassium and iron and can be used to improve the meat quality of indigenous chickens in Africa.

Keywords: apparent digestibility, growth, performance, meat quality, breast, concentration

DEDICATION

This work is dedicated to my family, who have been tremendously supportive financially, morally and socially throughout my study. My Mother, Motlabaseo K. Manyeula, my cousin Jacob Seleka and not forgetting my uncle Madome Manyeula, thank you for inspiring me throughout my education. All this is for you.

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LIST OF ABBREVIATIONS/ACRONYMS

AIA	Acid Insoluble Ash
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
AWFI	Average Weekly Feed Intake
BWG	Body Weight Gain
CF	Crude Fibre
CM	Canola Meal
CP	Crude Protein
DM	Dry Matter
EAA	Essential Amino Acids
EE	Ether Extract
FAMESs	Fatty Acid Methyl Esters
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
Hb	Haemoglobin
Hct	Haemotocrit
LPO	Lipid Peroxidation
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
NIR	Near infrared reflectance
PC	Protein Consumed

PER	Protein Efficiency Ratio
PSE	Pale Soft Exudates
RBC	Red Blood Cells
RP	Reducing Power
SBM	Soybean meal
SOD	Superoxide dismutase
TP	Total Protein
TPC	Total Phenol Content
TPC	Total Phenol Content
WBC	White Blood Cells
WBSF	Warner Blatzer Shear Force
WHO	World Health Organization.

CHAPTER 1 – GENERAL INTRODUCTION

1.1 Background

In rural villages of South Africa, indigenous chickens provide meat and eggs, which are sources of readily accessible high-quality protein (Aganga *et al*., 2000), vitamins and micro minerals. However, the productivity of indigenous chickens under extensive production systems is low due to poor feeding and the slow growing nature of the birds. In order to meet the food and nutrition security requirements of people living in rural areas, there is a need to improve the productivity of indigenous chickens. A major challenge to improving the productivity of these chickens is the inadequate supply of nutrients, particularly protein, required by indigenous chickens for optimum production.

The nutrition of indigenous chickens can be improved by offering feed resources of higher quality and quantity as they contribute to the total cost of production. Indeed, for the scavenging indigenous chicken, protein and energy supplies are often limiting (Okitoi *et al*., 2009). Energy and protein are the two most expensive nutrients used in poultry rations, hence they are of greatest concern to poultry nutritionists (Rose, 1997). Mengesha (2012) stated that these two nutrients contribute 90% of the total cost for production. As a result, there is a need to identify and evaluate some non-conventional feed resources as alternatives that may be used to improve the efficiency of poultry production in rural areas. Some of the non-convectional feed resources are sunflower (*Helianthus annuus)* cake and canola (*Brassica napus*) meal. These cakes are available in South Africa, are rich in protein and energy and are usually used in livestock feeds (Babiker, 2012)

1.2 Problem statement

The major source of feed for indigenous chickens in rural households is from scavenging, with the diet consisting of anything edible found within the environment, including household waste, crop by- products and a range of food products from gardens, fields and wasteland, which are low in protein and energy (Alders *et al*., 2009). In South Africa, the productivity of indigenous chickens is generally low because of poor nutrition, poor housing and poor healthcare under traditional rearing systems (Alders *et al*., 2001). Alders *et al*. (2009) also reported that the quantity and quality of the feed base for indigenous chickens are usually the main factors limiting chicken production. The strategy for ameliorating poor nutrition for the scavenging chickens could be by improving the quantity and quality of feed resources. A sustainable improvement in this nutrition requires supplementary feeding using some non-conventional, by-products and low-cost, readily available indigenous feed resources. In South Africa, examples of such feed resources include sunflower cake and canola meal, which are the most common by-products of oilseed extraction. However, few attempts have been made to explore the efficacy of the use of these feed sources in formulating diets for indigenous chickens.

1.3 Justification

Chicken meat and eggs are sources of essential amino acid and fatty acids required for human brain development. However, the productivity of indigenous chickens under extensive production systems is generally low due to factors such as poor nutrition, poor housing and poor healthcare. Conventional poultry production has always been dependent on expensive protein and energy sources such as soybean meal (*Glycine max* L. Merr), fishmeal and animal protein concentrates. These feedstuffs account for nearly 80% of the total costs of production (Nworgu

& Fasogbon, 2007), which in turn places chicken meat and eggs out of reach for the ordinary consumer. Soybean meal in particular has become expensive due to competition between humans and animals, resulting in rising prices on the world market. The current acute shortage of animal protein in developing countries (Abou-Eless *et al*., 2011) justifies research on the potential use of low cost and locally available feed resources and by-products. This is an important step towards reducing feed costs and improving the profitability of indigenous chickens.

1.4 Objectives

The broad objective of this study was to investigate the effects of feeding canola meal (CM) as an alternative to soybean meal (SBM) on growth performance, blood biochemistry and meat quality and bone development in the Potchefstroom Koekoek (PK) cockerels reared under an intensive production system.

The specific objectives of the study were to:

1. determine the chemical composition of SBM and CM;
2. investigate the effects of partial replacement of the SBM with graded levels of the CM on nutrient digestibility of PK cockerels reared under intensive system;
3. investigate the effects of partial replacement of the SBM with graded levels of the CM on growth performance and blood biochemistry of the PK cockerels reared under an intensive feeding system,
4. assess the effects of partial replacement of the SBM with graded levels of the CM on carcass characteristics, viscera macromorphometry and meat quality of the PK cockerels reared under an intensive feeding system; and

5. determine the effects of partial replacement of the SBM with graded levels of the CM on bone breaking strength, density and mineral composition of the PK cockerels reared under an intensive feeding system.

1.5 Hypotheses

This study tested the hypotheses that:

1 there are no significant differences in the chemical composition of the SBM and CM;

2 partial replacement of the SBM with CM in poultry diets has no significant effect on nutrient digestibility of the PK cockerels reared under an intensive system;

3 partial replacement of the SBM with CM in poultry diets has no significant effect on growth performance and blood biochemistry of the PK cockerels reared under an intensive production system;

4 there are significant differences in carcass characteristics and meat quality of PK cockerels fed diets in which the SBM has been partially replaced with CM as a protein source; and

5 partial replacement of SBM with CM in poultry diets has no significant effect on bone development and mineralisation of PK cockerels reared under an intensive production system.

CHAPTER 2 - LITERATURE REVIEW

2.1 The role of local chickens

There are several local chicken breeds in South Africa such as the Potchefstroom Koekoek, the Venda, the Naked Neck, the Ovambo, the Natal Game, the Zulu and Nguni, to mention but a few. Local chickens are raised extensively in relatively small flocks numbering between 1 and 50 and they are generally less productive in terms of egg and meat production (Alabi *et al*., 2012) due to poor genes, various threats (ranging from predation, poor health, cold or heat stress) as well as poor nutrition (Muchadeyi *et al*., 2004; Mapiye *et al*., 2008). These authors further state that, due to harsh conditions, indigenous chickens have gone through overtime several genetic changes which resulted in them having reduced body sizes in areas experiencing feed scarcity and larger body sizes in areas with plenty of feed.

Indigenous chickens play vital roles in the socio-economic lives of resource-poor communities of South Africa, particularly those who live in rural areas. The most obvious role of indigenous chickens is provision of eggs and meat as a source of income and good nutrition. Indigenous chickens are often sold to meet emergency cash needs (Dinka *et al*., 2010). Consumers prefer attributes of the indigenous chicken meat (leanness and flavour) and consider the meat as an organic product. Chickens are also important in traditional ceremonies such as marriages and burials. Aganga *et al*. (2000) reported that meat and eggs from chickens are a good sources of essential amino acids and fatty acids for general well-being and optimal development of the human brain during the perinatal period. Moreki (2010) also reported that meat and eggs from chickens are good sources of vitamins and minerals, all of which are essential for good health, growth and well-being. Chickens can also be exchanged to obtain other animals like goats and cattle.

2.2 The Potchefstroom Koekoek chicken

The Potchefstroom Koekoek is a locally developed breed, which was bred during the 1950s by Mr C.L. Marais at the Potchefstroom Agricultural College through a crossing of the Black Australorp cockerels with the White Leghorn hens and a subsequent mating of the F1 hens and the cockerels (Fourie & Grobbelaar, 2003). This breed is one of the most promising breeds, second to the White Leghorn in terms of the hen-house egg production per hen and hatchability (Wondmeneh *et al*., 2011). The Potchefstroom Koekoek was developed specifically for its brown coloured shelled egg production trait - to meet consumer preferences as consumers preferred brown shelled eggs to white shelled eggs (Grobbelaar *et al*., 2010). It is characterised as a heavy breed, with a high egg production and adaptability. Its average adult body weight ranges from 3 to 4 kg for cocks and 2.5 to 3.5 kg for hens and can reach sexual maturity in 130 days. The average egg weight is 55.7 g (Grobbelaar *et al*., 2010). Phenotypically, the chicken is characterised by a black and white speckled colour pattern on feathers, also described as barred, which is present in as many as nine different poultry breeds. The colouring is a sex-linked trait making gender identification easier and because of this, they are popular in breeding programs. This sex-linked trait gives the males distinguishable light grey bars on the feathers (Van Marle-Köster & Casey, 2001). However, little is known about feeding the Potchefstroom Koekoek chicken a canola meal diet.

2.3 Available feeds for indigenous chickens

Indigenous chickens are scavengers by nature. They scavenge insects, earthworms, green grass, kitchen leftovers (Salafaoh, 1997) and obtain minerals from the soil (Aganga *et al*., 2000). Kusina *et al*. (2001) stated that the scavenging of a feed resource base changes with season and

household farming activities. A few farmers supplement their indigenous chickens with maize and bran (Badubi *et al*., 2006).

The requirements of dietary protein depend on species, age and breed (Alam *et al*., 2004). Laudadio *et al*. (2012) stated that dietary protein is an important regulator of poultry performance, but also of the development and ecology of the gastrointestinal tract. The protein requirement of indigenous laying hens ranges from 16 to 18% of the provided diets and is needed for egg production, maintenance and growth of tissue and feathers (Tadelle & Ogle, 2001). The crude protein requirements for heavy (1.66 - 2.14 kg) growing indigenous chickens vary from 20, 16 and 14 % while those of the light (1 - 1.65 kg) growing indigenous chickens vary from 17, 14 and 12 % during the 5 – 8-, 8 – 14- and 14 - 21-week growth periods, respectively (Chemjor, 1998). Adjetery *et al*. (2014) reported an 18% crude protein level as a requirement for laying indigenous guinea fowls during the 28 – 36-week of age for achievement of the optimum egg production.

Poultry diets are formulated based on the bird's digestible amino acid requirement (Table 2.1 and Table 2.2). Chickens require essential amino acids and other non-essential amino acids in order to synthesise proteins at acceptable rates (Pesti, 2007).

Table 2.1 Amino acid profile expressed as % of lysine in the National Research Council (1984), NRC (1994) and Illinois ideal chick's protein (IICP) systems

Amino acids	**NRC (1984)**	**NRC (1994)**	**IICP**
Lysine	100	100	100
Arginine	120	114	105
Histidine	29	32	37
Methionine	42	46	36
Cysteine	36	36	36
Phenylanine	60	66	55
Tyrosine	52	56	50
Threonine	67	73	67
Leucine	113	109	111
Isoleucine	67	73	67
Valine	68	82	77
Tryptophan	19	18	16
Glycine + Serine	125	114	65
Proline	44	55	44

Source: Baker & Han (1994): NRC= National Research Council

Table 2.2 NRC (1994) requirement for crude protein and the most rate limiting amino acids for broilers (%) and laying hens (mg/100 g feed/day)

	Age of broilers (weeks)			
Nutrient	**0-3**	**3-6**	**6-8**	**Layers**
Crude protein	23.00	20.00	18.00	15.00
Methionine	0.50	0.38	0.32	300
Tsulf AA	0.90	0.72	0.60	580
Lysine	1.10	1.00	0.85	690
Threonine	0.80	0.74	0.68	470
Tryptophan	0.20	0.18	0.16	160
Isoleucine	0.80	0.73	0.62	650
Arginine	1.25	1.10	1.00	700
Valine	0.90	0.82	0.70	700

Source: Applegate & Angel (2008); Tsulf AA= Total sulphur Amino acid.

The sum of the essential and non-essential amino acids may also be called crude protein. In South Africa, indigenous chickens are fed with commercial (layer or broiler) feeds using expensive soybeans meal as the protein source. However, rural farmers do not have enough cash to buy this expensive source of protein for their chickens. Animal nutritionists have resorted to searching for agro-industrial by-products such canola meal, sunflower cake, Bambara waste meal, palm kernel cake and groundnuts cake to use as alternative protein sources to the expensive soybeans meal.

2.4 Canola and canola meal

Canola is a highly productive crop produced primarily for its oil. Canola is the name given to varieties of rapeseed that are low in glucosinolates (<30 μmol/g) and erutic acid (<2%) (Bell, 1993). Its oil is used to produce margarine and as a source of renewable energy (bio-diesel). Canola meal is the by-product of oil extraction, which may be used as a valuable protein source in animal feeds. The process of oil extraction from canola seeds includes seed cleaning, pre-conditioning and flaking, seed cooking, seed pressing, solventising of the press cake, desolventising and toasting of the meal. The cleaned seed is firstly flaked by roller millers to rapture as much cell wall as possible without damaging the overall quality of oils. The seeds are then cooked at temperatures between 80 and 90^{o}C and then pressed by expellers to remove 50% to 60% oils. The remaining oil is extracted by a solvent, which is usually hexane (Newkirk, 2009). After extraction, the solvent is removed from the meal in a desolventiser-toaster at a temperature of 80 - 115^{o}C with moisture being added during the process (Canola Council, 2009).

The effect of processing on canola meal quality has been reviewed (Newkirk, 2009; Kasprzak *et al*., 2016). During seed cooking, the temperature ranges from 80 to 90 ^{o}C and the moisture ranges between 6 and 10%. This step is needed to deactivate the myrosinase and to prevent hydrolysis of glucosinolates into toxic metabolites (aglucones). It is well-documented that excessive heating may result in Maillard reactions that can cause protein damage and a reduced digestibility of amino acids in animals (Bell, 1993; Newkirk *et al*., 2003). In addition, additives such as gum and soap stocks may be included in the process to reduce the dustiness of the meal. This increases the total oil content in canola meal by 1 to 2% (Spragg & Mailer, 2007; Newkirk, 2009; Barthet & Daun, 2011). Canola meal, the by-product obtained from the processing of canola seed is canola meal, which is used in the poultry industry (Naseem *et al*., 2006) and animal feeds (Babiker, 2012). Vast literature shows the effects of canola meal on performance of broilers (Leeson *et al.*, 1987; Naseem *et al*., 2006), layers (Gheisari *et al*., 2008) and quails (Karayagız, 2015) performance. Nonetheless, the effect of canola meal on the Potchefstroom Koekoek chicken has never been focused on nor evaluated.

2.5 Chemical composition of canola meal

The chemical composition of canola meal varies due to cultivar, environmental conditions during growth, harvesting periods and crushing conditions (Bell & Keith, 1991). Additionally, the meal quality is influenced by the type of oil extraction process (i.e. expeller and solvent-extraction) (Spragg, 2013). The main components of canola meal include protein, carbohydrates (simple sugars, sucrose, oligosaccharides, starch), dietary fibre (non-starch polysaccharides, lignin with associated polyphenols, glycoproteins), fat and ash.

2.5.1 Amino acid composition and digestibility

Canola meal is a good source of vegetable protein for livestock feeding (Table 2.3). It has a high crude protein content (34.8 %) which can be utilised by poultry. Higher crude protein content results in higher proportion of amino acid (methionine, 0.73%, lysine, 1.74% and threonine, 1.50%) in the meal (Pottguter *et al*., 2006). According to Bell (1984), the concentration of protein and amino acids (Table 2.4) in canola products varies depending on variety, environmental factors, canola seed composition and the amount of residual oil and carbohydrates in the meal.

Amino acid content varies with protein content and can be calculated by multiplying the crude protein content of the meal by the proportion of amino acid as a percentage of the protein. In canola protein, the concentration of methionine, cysteine and threonine are higher, while that of lysine and tryptophan is less than in soybean meal (Khajali & Slominski, 2012). It is documented that the canola meal (CM) has a well-balanced amino acids profile compared to soybean meal. Since sulphur containing amino acids (methionine and cysteine) is one of the first limiting amino acids in poultry feeds (Farkhoy *et al*., 2012), any reduction in methionine availability seriously affects the competitive position of CM for use in poultry feeds (Pearson *et al*., 1992). The protein quality and quantity of CM for the PK cockerels is scantly documented and the previous findings are based on other poultry species (broilers, layers, breeding hens and turkeys).

Table 2.3 Crude protein content in percentage of different types and forms of canola meal (% as fed basis)

Canola Form	Canola Type	CP content	References
Expeller		36.3	Wickramasuriya *et al.,2015:* Landero *et al.*, 2012
Expeller		34.5	Kong & Adeola. 2013
Expeller		32.0	Mailer. 2004
Expeller		35.1	NRC. 2012
High fibre		35.2	Mustafa *et al*., 1996
Low fibre		40.2	Mustafa *et al*., 1996
Meal		35.5	Khajali &Slominski. 2012
Meal		40.0	Chen *et al*., 2015
Meal	Black	36.9	Mejicanos *et al.*, 2015
Meal	Yellow	41.0	Mejicanos *et al.*, 2015
Meal		37.5	NRC. 2012
Meal		41.1	Maison. 2013
Solvent		37.3	Wickramasuriya *et al.*, 2015
Solvent		34.0	Mailer. 2004

Table 2.4 Amino acid composition of canola meal (% as fed basis)

	References			
Amino acid	**Mejicos *et al*. (2016)**	**CCC (2015)**	**Maison (2013)**	**Gorski. (2015)**
Alanine	1.49	1.57	_	2.49
Arginine	2.28	2.38	2.28	_
Aspartate	2.62	2.61	_	0.82
Cysteine	0.80	0.82	0.86	_
Glutamine	6.60	6.53	_	_
Glycine	1.85	1.77	_	0.91
Histidine	1.18	1.22	0.07	1.35
Isoleucine	1.21	1.25	1.42	2.53
Leucine	2.43	2.22	2.07	2.02
Lysine	2.02	2.13	2.45	0.68
Methionine	0.68	0.70	0.71	_
Proline	2.54	2.15	_	1.38
Phenylalanine	1.48	1.46	1.48	_
Serine	1.69	1.44	1.55	1.49
Threonine	1.62	1.54	_	0.49
Tyrosine	0.46	0.48	0.43	0.46
Tryptophan	0.93	0.90	1.06	_
Valine	1.66	1.78	1.78	1.72

- = no data were available, CCC= Canola Council of Canada.

2.5.2 Carbohydrates and fibre

Carbohydrates in canola oilseeds are categorised into soluble sugars, insoluble carbohydrates and fibre (Barthet & Daun, 2011). The concentration of soluble carbohydrates (sucrose, raffinose, stachyose, fructose and glucose) in mature seeds is approximately 10% of the oil-free weight (Barthet & Daun, 2011). Different studies have shown variation in concentration of carbohydrate and fibre components of canola meal (Table. 2.5). However, there is limited information on the impact of carbohydrates and fibre in canola meal on PK cockerel performance.

2.5.3 Minerals and vitamins

The concentration of minerals in canola products is often a result of differences in soil mineral contents and growth environment (Bell & Keith, 1990; Mahan *et al*., 2005) (Table 2.6). When compared to the other vegetable-origin oilseeds, canola products are a relatively good source of essential minerals (Canola Council of Canada, 2009). Research findings suggest that minerals in CM are well-utilised since supplemental minerals in many studies have never increased performance (Summers & Lesson, 1985). Summers (1994) showed that high sulphur levels in canola meal may partially cause depression in performance because the element changes the cationic and anionic balance. This problem can be partially overcome by increasing the dietary calcium levels (Summers, 1994) and endogenous amount of phytase that are required to hydrolyse the phytate-phosphorus complex (Selle & Ravindra, 2007; Akinmusire & Adeola, 2009; Hanna, 2015).

Table 2.5 Carbohydrate and dietary fibre components (%) of canola meal

Components	References					
	Bell (1993)[a]	**CCC (2009)[b]**	**Khajali & Slominski (2015)[c]**	**Mailer (2007)[c]**	**Liang (2000)[b]**	**Wickramasuriye *et al*. (2015)[b]**
Starch	2.5	5.1	2.4	5.2	_	5.1
Sugar	_	6.7	_	8.0	_	_
Sucrose	7.7	6.2	6.0	_	_	5.2
Fructose +glucose	_	0.3	_	_	_	0.6
Oligosaccharides	2.5	2.2	2.0	2.3	2.3	2.3
NSP	17.9	15.7	18.0	16.1	16.1	18.9
Soluble NSP	1.3	1.4	_	1.4	1.4	_
Insoluble NSP	16.4	11.7	11.6	12.0	12.0	11.2
Crude fibre	14.6	11.7	11.6	12.0	12.0	11.2
Acid detergent fibre	19.8	16.8	18.2	17.2	17.2	16.2
Acid detergent lignin	_	5.1	_	_	_	5.8
Neutral detergent lignin	_	20.7	_	_	_	_
Total dietary fibre	_	_	_	_	_	1.2

[a] Oil-free, dry matter, [b]12 % moisture basis, [c]10 % moisture basis

NSP=Non-starch polysaccharides

CCC = Canola Council of Canada

Table 2.6 Mineral composition of canola meal

Minerals	References					
	Bell & Keith (1991)[b]	**NRC (1994)[d]**	**Mailer (2004)[a]**	**Khajali & Slominski (2012)[c]**	**Slominski (2015)**	**Mejicanos *et al.* (2016)**
Calcium, %	0.70	0.68	0.56	0.67	0.65	0.70
Phosphorus, %	1.13	1.17	0.96	1.02	0.99	1.2
Phytate phosphorus, %	0.83	0.64	_	_	0.38	0.64
Sodium, %	_	_	_	0.08	0.07	0.08
Chlorine, %	_	_	0.10	0.10	0.10	_
Potassium	1.35	1.29	1.26	1.17	1.13	1.29
Sulphur, %	0.94	_	0.62	0.65	0.63	_
Magnesium, %	0.57	0.64	0.47	0.56	0.54	0.60
Copper, mg/kg	6.34	10	3.9	_	4.7	_
Iron, mg/kg	157	_	138	_	159	162
Manganese, mg/kg	54.7	159	52	_	162	_
Molybdenum, mg/kg	1.5	_	_	_	1.4	_
Zinc, mg/kg	57.8	71	45	_	47	_
Selenium, mg/kg	1.22	1.00	_	_	1.1	_

[a] As fed basis (n = 26), [b] Dry matter basis (n = 28), [c]10 % moisture basis, [d]As fed basis

Canola meal has high amounts of calcium, phosphorus, magnesium, manganese and selenium but low potassium and copper contents (Table. 2.6). However, approximately 85% of the total phosphorus in CM is present as hexaphosphoinositol (phytate) (Canola Council of Canada, 2015). The latter makes P largely unavailable to poultry because they lack sufficient endogenous amounts of the necessary enzyme to hydrolyse. Canola meals have high amounts of vitamins (Table 2.6) but there is still limited information on the vitamin composition of CM. The CM contains high amounts of biotin, folic acid, niacin, riboflavin and thiamine (Bell, 1993; NRC, 2012) (Table 2.7).

2.5.4 Energy

Metabolisable energy (ME) is an expensive component of poultry diets and represents a large portion of the total cost of broiler production (Rose, 1997). The energy contribution of poultry diets is usually described in terms of ME and/or net energy (NE). Metabolisable energy can be accurately determined from a difference between the gross energy of the feed and the gross energy of the excreta derived from such a feed (NRC, 1994). The concentration of energy in the diet greatly influences the intake of other nutrients and utilisation of the ME (Ferket & Gernat, 2006). Broilers exhibit the ability to control energy intake by adjusting their feed intake as dietary energy concentration changes (Leeson *et al*., 1996). Canola meal contains between 2.77 and 3.27 Kcal/kg digestible energy **(**Mejicanos *et al.*, 2016**)**.

Table 2.7 Vitamin content of canola meal (mg/kg as fed basis)

Vitamins	References				
	Liang (2014)	**Bell (1993)**	**CCC (2009)**	**Choo *et al.* (2014)**	**Mejicanos *et al.* (2016)**
Vitamin E (IU/Kg)	20.39	21.64	13	_	_
Pantothenic acid	9.5	9.5	9.3	9.5	9.5
Niacin	160	160	156	160	169.5
Choline	6700	6700	6500	6700	_
Riboflavin	5.8	5.8	5.7	5.8	3.7
Biotin	1.1	1.07	0.96	0.98	1.0
Folic acid	2.3	2.3	0.8	0.83	2.3
Pyridoxine	7.2	7.2	7.0	_	_
Thiamine	5.2	5.2	5.1	5.2	5.2

- = no data were available
CCC = Canola Council of Canada

The energy level and digestibility in CM vary depending on nutrient composition, especially for protein, oil and fibre (Canola Council of Canada, 2009; Wickramasuriya *et al*., 2015). These factors are influenced by variety and seed quality along with the feed processing technology (Bell, 1993). Canola meal contains about 3346 kcal/kg digestible energy while the de-hulled CM contains approximately 4063 kcal/kg digestible energy, due to a reduction of fibre component in hulls, which comprises about 12–16 % of the canola seeds (Bell, 1993).

The greater the concentration of ether extract and gross energy in canola meal, the greater the digestible and metabolisable energy when used in poultry diets (NRC, 2012). In comparison, a greater concentration of the neutral and acid detergent fibres in CM results in a decreased digestible and net energy in growing poultry diets. For example, an expelled meal, which contains an average 10.0% of ether extract (EE), had 4873, 3779, 3540 and 2351 kcal/kg for GE, DE, ME and NE, respectively. On the other hand, a pre-press solvent extracted CM, which contains lesser EE (3.2% on average), had 4332, 3273, 3013 and 1890 kcal/kg for GE, DE, ME and NE respectively (NRC, 2012).

2.6 The anti-nutritive factors of canola meal

The nutritive value of canola meal is limited by the presence of several anti-nutritive factors, which include glucosinolates, sinapine, phytic acid, polyphenolic compounds, erucic acid, protein inhibitors and the indigestible non-starch polysaccharides (Canola Council of Canada, 2015).

2.6.1 Glucosinolates

Glucosinolates are a group of over 130 nitrogen and sulphur containing natural products found almost exclusively in plants families of the Brassicaceae and other related families of the order Capparales (Fahey *et al*., 2001). In plants, they co-exist with an endogenous enzyme myrosinase, a thioglucosidase. They share a core structure containing a β-D glucopyranose residue linked via a sulphur atom to a (Z)-N-hydroximino sulphate ester and are distinguished from each other by a variable R group derived from one of the several amino acids (**Figure 2.1**).

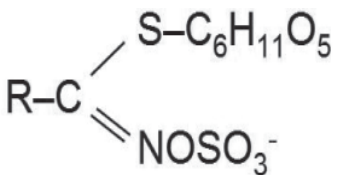

Figure 2.1 Structures and names of the most abundant glucosinolates of canola meal. r = 3-butenyl- (gluconapin); r = 4-pentenyl- (*glucobrassicanapin*); r = 2-hydroxy-3-butenyl- (progoitrin); r = 2-hydroxy-4-pentenyl- (*gluconapoleiferin*); r = 3-indolylmethyl- (*glucobrassicin*); r = 4-hydroxy-3-indolylmethyl- (*4-hydroxyglucobrassicin*)
Source: Zukalova & Yasak, 2002).

Physical tissue or cell injury normally leads to the breakdown of glucosinolates through a hydrolytic action of myrosinase, resulting in the production of compounds including isothiocynates, thiocyanates and nitriles (Hirani *et al.*, 2012). Glucosinolates do not cause any harm to animals. However, the breakdown products of glucosinolates either through enzymatic hydrolysis or by the non-enzymatic factors (heat, low pH, anatomical and physiological structure of the gastrointestinal tract, digesta transit time and microbial activity) cause harmful effects to animals (Bell, 1993; Brand *et al*., 2007).

Literature shows that glucosinolates and their transformed products have different biological effects on animals including the anti-carcinogenic property in human, insect repellent and anti-fungal depending on the specific chemical structure (Mithen *et al*., 2000; Brader *et al*., 2006; Andersen *et al*., 2010). The concentration of glucosinolates found in canola seed ranges from 3.6 to 9.2 μmol/g (Bell, 1993). Glucosinolates defend plants against insects, herbivores and microbial pathogens (Newkirk, 2009). The hydrolysed products from glucosinolates can cause goiter, hemorrhagic liver (Campbell & Slominski 1991), have a bitter taste (Mailer *et al*., 2008; Khajali & Slominski, 2012), reduce voluntary feed intake (Lee & Hill, 1983) and can also reduce animal performance (Woyengo *et al*., 2011; Newkirk, 2009).

Glucosinolate compounds cause an enlargement of the thyroid gland and inhibit synthesis and secretion of the thyroid hormones (Schöne *et al*., 1997). Additionally, the intake of glucosinolates results in liver and kidney damage through causing necrotic cell death (Tripathi & Mishra, 2007). The negative effects of glucosinolates depend on the concentration and composition of such compounds and their degradation products. The tolerance for such molecules is relatively different among different animal species (Tripathi & Mishra, 2007).

Growing pigs can tolerate a maximum of 2.0 - 2.5 μmol/g of glucosinolates in the diet. In poultry, up to 4 μmol/g for layers and 1.5 μmol/g for broilers were recommended. Therefore, limited information is available on the maximum tolerable level of these compounds in indigenous chickens. Moreover, as the CM contains lower levels of glucosinolates than the rapeseed meal, the aforementioned problem may not exist with the CM when fed to PK cockerels.

2.6.2 Sinapine

Sinapine is a choline ester of sinapic acid. It is one of the major phenolic choline esters in oil-extracted rapeseed meal (Blair & Reichert, 1984). Some literature states that sinapine accounts for up to 70% of the sinapic acid esters in CM. Kozlowski *et al*. (1990) propose that the dark colour, bitter taste and astringency in CM are due to sinapine and sinapic acid and they account for approximately 1% of the CM (Khajali & Slominski, 2012; CCC, 2015). Among the phenolics in CM, sinapine is the most abundant. It produces fishy tainted eggs in hens (Wickramasuriya *et al*., 2015; Bell, 1993; Zhou *et al.*, 2005).

Hens are unable to convert trimethylamine to the odourless N-oxide (Khajali & Slominski, 2012). Qiao & Classen (2003) reported that the bitter taste of sinapine did not affect the feed intake and growth rate in broilers. Interestingly, an improvement in the broilers supplemented with purified extract is reported when metabolisable energy content and protein digestibility of the diet. Furthermore, the concentration of sinapine in CM is between 1.0 and 1.5% (Mailer, 2004) and has no negative effects on the broiler performance. Use of up to 20% of the CM on broilers (Ahmed *et al*., 2015; Gopinger *et al*., 2014) and 40% on quails (Karayagız, 2015) did not affect carcass characteristics

2.6.3 Phytate/Oxalate

Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakis hydrogen phosphate) is regarded as the primary storage form of phosphorus in the plant tissue (Khajali & Slominski, 2012). It is an anti-nutritional factor because in the gastro-intestinal tract it forms insoluble complexes with proteins and some minerals (calcium, iron, zinc manganese, phosphorus and magnesium) to rendering them unavailable (Mansoori *et al*., 2015). Phytate also binds to the amino acids: arginine, lysine

and histidine, resulting in insoluble phytate-protein complexes that less available for absorption (Anderson, 1985; Nahashon *et al*., 1994). The digestibility of phosphorus in CM is about 25-30% of the total phosphorus (Sauvant *et al*., 2004). The concentration of phytate phosphorus in CM ranges from 0.65 - 0.87% (NRC, 2012). Phytic acid affects animal performance by reducing nutrient digestibility through binding to nutrients and the digestive enzymes or both. This in turn, result in increased endogenous loses of amino acids (Woyengo & Nyachoti, 2013).

The enzyme phytase can hydrolyse phytic acid to inositol and inorganic phosphorus, leading to improved phosphorus utilisation and overall growth performance of birds by means of improving gut morphology (Nelson, 1967; Peng *et al*., 2003; Debnath *et al*., 2005; AL-Massad *et al*., 2011). With phytase, carbohydrase and proteinase enzymes, the feeding value of CM can be enhanced with potential health benefits in the PK cockerel's diets. Furthermore, supplementation of phytase does not affect the true digestibility of amino acids and can improve growth performance as well as enhance the insulin liver receptor sensitivity in boilers (Józefiak *et al*., 2010; Kong & Adeola. 2011).

2.6.4 Polyphenolic compounds

Polyphenolic compounds are complex polyphenol compounds having molecular weights ranging from 500 to 3000 Da (Khajali & Slominski, 2012). They are divided into hydrolysable and condensed fractions. In canola, the condensed polyphenolic compounds are found in hulls with the brown hull containing more tannin than the yellow (Mejicanos *et al*., 2016). The total amount of polyphenolic compounds in canola hulls ranges from 1.9 to 6.2 g per 100 g of oil-free hulls (Naczk *et al*., 2000) of which 70 to 96% of this total are insoluble and give the meal a dark

unattractive colour (Mejicanos *et al*., 2016). Dietary polyphenolic compounds reduce the digestibility of nutrients and increase endogenous losses, resulting in reduced growth performance of broiler chickens. Mansoori & Acamovic (2002) argued that adding soluble polyphenolic compounds to broiler diets resulted in growth depression. Silverstein *et al*. (1996) concluded that dietary polyphenolic compounds alter the intestinal absorption of amino acids, minerals and simple sugars and the extent depends on the concentration of tannin in the diets (Jansman, 1993). However, most of the polyphenolic compounds are removed in the oil extraction process **(**Kozlowska *et al*., 1990). Canola meal usually contains less than 1.5% polyphenolic compounds (Mailer, 2004). The current available literature has no evidence of the effects of polyphenolic compounds in CM on the production and meat quality of PK cockerels.

2.6.5 Erucic acid

Erucic acid, also known as *cis*–13-docosenoic acid, is 22-carbon chain unbranched, monounsaturated fatty acid with a single double bond in the omega 9 position. It constitutes 30 – 60% of the total fatty acids of rapeseed, mustard seed and wall flower seed (Canola Council of Canada, 2015). Ecke *et al*. (1995) argue that consuming a high erucic acid oil can cause some health risks to humans. In rats diets with high amounts of erucic acid (more than 10%) caused the accumulation of erucic acid in tissue lipids and a reduction in growth rate (Green & Innis, 2000). These negative effects on growth persuaded plant breeders to reduce the amount of erucic acid in rapeseed varieties. Inclusion of up to 15% of low erucic acid rapeseed oils in the diets of broilers was nutritionally satisfactory with regards to growth performance (Vogtmann & Clandinin, 1974). Potchefstroom Koekoek cockerels appear less sensitive and an inclusion level of up to 20% of the rapeseed meal does not affect the weight of breeding chicks (Gheisari, 2014).

Moreover, as the CM contains a low amount of erucic acid than rapeseed, the afore-mentioned problems may not exist when CM is fed to PK cockerels.

2.6.6 Protein inhibitors

Canola seed contains large amounts of stored nutrients that are used to sustain germination and seedling growth. Proteins in the seed are enzymatically hydrolysed to support early plant growth. Amylase inhibitors, lectins and trypsin inhibitors are proteins that protect the seed from attack by insects. Protein inhibitors in canola seed have a major impact on nutritional value as they inhibit pancreatic serine proteinases. This impairs the absorption of nutrients decreased growth (Guillamon *et al*., 2008). Mansour *et al*. (1993) found no trypsin inhibitor in canola meal due to the chemical and mechanical processes it underwent when its seed was processed to a meal in the processing plant. Information on nutritional effects of protein inhibitors on PK cockerels is currently very limited.

2.7 Canola meal inclusion in poultry diets

Several studies have been conducted to evaluate the effects of different inclusion levels of the CM in poultry diets (Table 2.8). Balancing diets to ensure adequate levels of the essential amino acids is always necessary in the formulation of poultry diets. Due to low energy levels of the meal the maximum recommended levels of CM for starter and grower phase in broiler production are 20 and 30%, respectively (Canola Council of Canada, 2015). McNaughton *et al*. (2014), compared a genetically modified, a near-isogenic but not genetically modified and a commercial canola meal on the performance of broilers and reported no significant differences in body weight gain, feed intake and mortality. It was concluded that the meal derived from

genetically modified canola seeds was nutritionally equivalent to meals produced from the non-genetically modified canola seeds.

Leeson *et al*. (1987) conducted an experiment to investigate the effects of replacing a dietary SBM with a canola meal in the diets of laying hens with emphasis on the efficiency of mineral utilisation. The dietary treatments consisted of 0, 25, 50, or 100% replacements of SBM with canola meal which, equated to canola meal levels at 0, 6.32, 12.64 and 25.28% of the diet, respectively. Such dietary treatments had no effect on feed intake even though the birds that were fed diets supplemented with canola meal were numerically heavier than those that were fed the control diet.

Table 2.8 Inclusion levels of canola meal in poultry diets

Reference	Species	Parameter	Recommended level
Leeson *et al.,* 1987	Layer and broiler chicken	Production and Reproduction	Up to 100% canola meal
Naseem *et al.,* 2006.	Broiler chicks	Production parameter	Up to 25% canola meal
Gheisari *et al.,* 2008	Layer chicken	reproduction	10% rape seed
Riyazi *et a*l., 2009. Iran	Commercial layer hens	Egg quality	10% canola meal
Gheisari *et al* 2011	IBH	Chick weight	15% rape seed
Payvastagan *et al* 2012	Broiler chicks	Production parameter	10% canola meal
Hameed *et al*., 2012	Japanese quail	Production performance	Up to 15% canola meal
Gheisari 2014. Iran	IBH	Egg quality	15-20% rape seed
Gopinger *et al*., 2014	Broilers chicken	Meat and sensory	Up to 20% canola meal
Karayagız, 2015	Quail	carcass quality	Up to 40% canola meal
Ahmed *et al*., 2015	Broiler chickens	Growth and carcass traits	Up to 20% canola meal

IBH = Indigenous breeding hens

The study by Gheisari *et al*., (2008) also observed no effect of the dietary treatments on egg production, egg weight and eggshell deformation. Leeson *et al*. (1987) reported out no CM mediated differences in nutrient retention and bone mineralisation. It was therefore concluded that canola meal can totally replace a SBM without any adverse effects on performance, nutrient retention or mineral metabolism in the diets of laying hens.

Taraz *et al*. (2006) conducted an experiment to study the effects of a rapeseed meal on organ weights, blood biochemistry and performance of broiler chickens. The rapeseed meal replaced the SBM at the levels of 0 (control), 25, 50, 75 and 100 % for the periods of starter (1-21 days), grower (21-42 days) and finisher (42-49 days) feeding systems. Body weight gain was lower in the 100% treatment during the starter, grower and total feeding periods, while there were no differences between the control and 25% treatments. The size of livers and relative weight of gall bladders increased with increasing levels of dietary rapeseed meal. The lowest weight for abdominal fat pad, carcass and whole body were seen in 100% treatment and the highest ratio of carcass to whole weight was seen in 25% treatment, which was significantly different from other treatments. Taraz *et al*. (2006) therefore concluded that 25% of SBM can be replaced with rapeseed meal in the diet for broilers.

Mikulski *et al*. (2012) conducted an experiment on the effect of different dietary levels of rapeseed meal on growth performance, carcass traits and meat quality in turkeys. The diets were iso-energetic and isonitrogenous, containing 0, 60, 120 and 180 g/kg of rapeseed meal. No effects on body weight, carcass dressing percentage and meat fat content were observed. Increase in the inclusion rate of rapeseed meal was followed by a linear increase in feed conversion ratio.

In the meat, the concentrations of margaroleic acid and the saturated fatty acids decreased linearly with an increase in the rapeseed meal while oleic acid and the polyunsaturated fatty acids increased linearly with an increase in the rapeseed meal. Mikulski *et al*. (2012) concluded that the use of a good quality, low-glucosinolate rapeseed meal as a substitute for a SBM, in amounts of up to 180 g/kg, had no detrimental effect on growth performance, carcass traits and meat quality in turkey.

Jia *et al*. (2012) fed a 30% CM that was derived from either the yellow-seeded (*Brassica napus*) or black-seeded (*Brassica napus*) canola seeds to broiler chickens from 3 to 17 days of age. Although the broiler chickens that were fed with a canola meal that was derived from the yellow-seeded (*Brassica napus*) CM had a better energy utilisation than those birds fed with a black-seeded CM, there were no observed differences in their growth performances. Vast literature has so far made extensive researches to find out the inclusion levels of the CM on broilers. Nonetheless, the effect of CM inclusion levels on the performance of PK cockerels has not yet been investigated.

2.8 Evaluation of protein quality

Knowledge of the availability of amino acids in feedstuffs is an important feature of the dietary protein quality. Availability is a function of two processes: digestion and metabolism while protein quality is the measurement of availability of amino acids in the feeds and the rate at which amino acids are digested, absorbed and finally utilised by the animal body to yield energy or needed compounds. Millwards *et al*. (2008) describes protein quality as the characteristics of a protein in relation to its ability to achieve a defined metabolic action. Protein quality can be

defined as the ability of a feed protein to meet the body's metabolic demand for amino acids and nitrogen and is determined by the amino acid composition and digestibility of the protein as well as the bio-availability of the individual amino acids (Boye *et al*., 2012). Birds excrete faeces and urine plus the endogenous materials together as excreta. Earlier studies therefore suggested different methods to determine the digestibility of different feedstuffs fed to poultry in order to evaluate the bio-availability of amino acids, of a single ingredient or synergism of a mixture. Numerous methods have been developed and used to estimate the bio-available amino acids, either through *in vivo*, *in vitro* and chemical assays.

2.8.1 Indirect in vivo protein quality assays

In the opinion of Sibbald (1987), indirect methods of determining amino acid bio-availability include microbiological assays, insect assays and plasma amino acid assays. Ravindran & Bryden (1999) postulated that the indirect method of measuring blood plasma amino acids is based on the principle that the blood will transport any products of digestion and absorption (peptides and free amino acids) to tissues in the body. However, the indirect measuring of plasma amino acids is based on the relationship between free amino acids and amino acid absorption. Plasma amino acid concentration from starvation are used as a reference and compared to post-prandial plasma amino acids.

2.8.2 Growth assays

Growth assays usually involve the addition of graded levels of a specific amino acid or test feedstuff to an amino acid deficient diet. This method is called the slope-ratio method but if more than one test feedstuff is fed, one can also use the standard curve method if only one level of the

test feedstuff is fed. Bio-availability is then calculated by regression analysis and from a ratio of the slopes of the growth lines for the test feedstuff and amino acid of interest (Parsons, 2002). The measurement of the growth response to the dietary amino acid levels is favourable because this includes digestion, absorption and utilisation of the amino acid. However, these types of assays are expensive, time consuming, can only measure one amino acid at a time and require expensive purified or semi-purified diets (Ravindran & Bryden, 1999).

2.8.3 Digestibility assays

Digestibility assays for poultry can be conducted by collecting either excreta (faeces and urine) voided from the birds or by collecting digesta from the ileum. Excreta assays are based on the principle of measuring amino acids that are voided in the excreta, which are then subtracted from the dietary amino acids. Even though faeces and urine are collected together, it has been shown that the amino acid content of urine is small and will have little effect on the amino acid digestibility values (Ravindran & Bryden, 1999). Adult roosters are preferred experimental animal subjects because they do not lay eggs, which when broken, can contaminate the excreta sample. The excreta method is a simple assay to conduct and many animals may be utilised without euthanising or making surgical modifications.

The birds' nutrients absorption occurs in the jejunum and ileum. The main site for microbial fermentation of avian species is the ceca, which are located near the terminal ileum and colon. Parson *et al*. (1982) reported a microbial contribution of approximately 25% of the total excreta protein. This microbial modification of amino acids and a microbial protein contribution may change the final excreta analysis value, which influences the final digestibility value calculated

from the total excreta analysis (Ranvindra & Bryden, 1999). To overcome this obstacle, cecectomy and ileostomy techniques are used to collect excreta for digestibility assays.

The most common method to collect excreta is the precision-fed rooster assay. The surgical removal of ceca in order to accurately evaluate amino acid digestibility is known to be the precision – fed cecectomised rooster assays. Cecectomy techniques were firstly used in cockerels (Green *et al*., 1987) and then in ducks (Ragland *et al*., 1999). The cockerels were firstly deprived of solid feed for 24 hours before the removal of ceca. After being anaesthetised and removal of ceaca, solid feed was withheld for 24 hours but water was provided *ad-libitum* (Green *et al*., 1987). After 10 days, the skin sutures were removed and the cockerels were then ready for experiment use for 4 weeks after surgery. Cecectomy is considered simpler and more rapid in comparison to other surgical procedures such as ilea1 cannulation (Parsons, 2002). Since the primary site of microbial fermentation will be removed, a more accurate value of digestibility will be determined.

The other method that can be used to determine protein quality is the ileal digestibility assay which is considered more accurate (Payne *et al*., 1968). The ileal amino acid digestibility assay can be carried out either by inserting a cannula in the terminal ileum or slaughtering birds and collecting digesta from the distal small intestine. The ileostomy technique can be used with adult cockerel of 2.8 – 3 kg into which a cannula is surgical inserted in the terminal ileum to facilitate collection ileal digesta. The ileal digesta were then assayed for amino acid digestibility (Schutter *et al*., 1991). Digesta are generally collected for the entire ileal region between the Meckel's diverticulum and the ileo-cecal junction, however, it has been suggested that a collection from

only the last 15 - 20 cm of the small intestine may be preferred (Kadim & Moughan, 1997). Birds are fed an experimental diet with an appropriate marker over a period of several days or weeks, after which contents of the ileum are collected.

2.8.4 Digestibility markers

The use of the ileal digesta technique requires chromic oxide (Cr_2O_3), indigestible marker acid insoluble ash (AIA) and titanium dioxide (TiO_2) to relate the amino acid contents in the ileum in relation to those in the diet. The most effective markers will be inert materials that are not digested or absorbed within the gastrointestinal tract and have no effect on the digestive system. Amino acid digestibility is determined by the ratio of the concentration of the marker in the diet to the concentration in the ileal digesta or faeces.

2.8.5 In vitro protein quality assays

In vitro methods, like chemical, microbiological and near infrared reflectance (NIR) spectroscopy assays are preferred for their simplicity. These methods are generally reproducible and require no animal use. *In vitro* methods can also give insight into the degree of heat damage of proteins, which can adversely affect the digestibility of amino acids, especially lysine. The results of a study by Kim (2010) showed that chemical assays are routinely carried out in the laboratory using an ion-exchange chromatography technique.

2.9 Factors affecting protein digestibility and utilisation

Several factors affect protein utilisation including site of gastro intestinal tract, anti-nutritional factors, age, dietary level of the protein, carbohydrates, fibre and fats. These factors are briefly described in the sections that follow.

2.9.1 Site of digestion

In birds, digestion of dietary protein begins in the proventriculus (stomach) but occurs mainly in the small intestine. The undigested proteins and unabsorbed peptides of either dietary or endogenous origin, enter the large intestine. In the large intestine, these components are subjected to fermentation by the intestinal microflora. Proteins not digested in the small intestine enter the caecum where they undergo fermentation (Nesheim & Carpenter, 1967). Microflora of large intestine affect protein digestibility. The amino acids that are not absorbed and undigested proteins flow into the colon and end up being excreted through faeces, leading to environmental pollution (Juquiera *et al*., 2006).

2.9.2 Anti-nutritional factors in feeds

It has been known for some time that the major influence of anti-nutritive factors on protein nutrition is a reduction in digestibility. The presence of anti–nutritional factors in diets constitutes a great danger to chicken health and to their performance. The existence of trypsin inhibitors, polyphenolic compounds, lectins, saponins and chromogenic compounds affect the nutritional value, digestibility and utilisation of proteins, causing digestive and metabolic diseases of chickens as well as reduced performance. For example, the study by Gu *et al*. (2010)

found that soybean varieties containing high amounts of trypsin inhibitor and lectin resulted in low body weight gain, protein and fat digestibility in rats.

Anti-nutritional factors are known to bind protein within the ileal epithelium, thus disrupting the digestive and absorption process of protein and consequently, lowering body weight. They also inhibit the activity of other proteolytic digestive enzymes which result in a slow release of essential amino acid leading to growth depression. Some stimulate digestive enzyme secretion into the gut and increase mucin production and the sloughing of epithelial cells, resulting in a reduced apparent amino acid digestibility.

2.9.3 Age and physiological state of the chicken

The ability of poultry to digest and absorb dietary protein is known to be influenced by age and physiological state. The chick's digestive system and enzyme concentration improves with age. Nitsan *et al*. (1991) reported an increase in enzyme concentration in the first 14 days of age. Between 1 and 14 days ages of development, dietary nutrients may be poorly utilised. In a study involving the effects of age on nutrient digestibility in chicks fed with different diets, Bata & Pearson (2002) concluded that the digestibility of amino acid increased and improved with age.

2.9.4 Dietary level of protein and amino acids

The level of dietary protein influences protein absorption and utilisation. In a balanced diet, approximately 90% of the dietary amino acids are absorbed by the gut and 30 - 50% will be used by the intestine itself, while the rest will be absorbed by the portal system (Have *et al.,* 2007). A high dietary protein level depresses the efficiency of absorption. Protein levels less than 16% in

the diets lead to higher percentages of protein being extracted by the gut (Junqueira *et al*., 2006). Joseph *et al*. (2000) found similar results on body weight when feeding a different level of protein (18 versus 16% crude protein) on breeder chickens. The authors concluded that utilisation of the dietary protein, as indicated by protein efficiency ratio, was higher in birds fed with low protein diets.

Previous studies (Cheng *et al*., 1997; Aletor *et al*., 2000) reported a significant increase in protein efficiency ratio with a reduction in the dietary crude protein content in broiler chicken diets. Patras *et al*. (2009) investigated the effects of dietary fibre and protein level on the nitrogen excretion pattern of pigs and found that total nitrogen excretion was higher in high protein diets (18.8 g/day) compared to low protein diets (12 g/day). The authors concluded that a reduction of protein content in the diet was an effective way of reducing nitrogen excretion, thereby lowering ammonia emission.

Thus, the metabolic utilisation of amino acids in the gut depends on the composition of the meal with respect to the presence or absence of indispensable and dispensable essential amino acids. A high concentration of threonine in the ileal digesta has been attributed to a high production of mucin, leading to a low ileal apparent digestibility coefficient for threonine in the diets (Lien *et al*., 1997).

2.9.5 Fibre level

Fibre is a well-known dietary ingredient that stimulates long-term mucosal growth, especially in the colon. It is expected that if the structural changes only take place in the colon, they will then

not influence amino acid absorption in the small intestine. Kluth & Rodehutscord (2009) concluded that a high fibre (cellulose) concentration in the diet of broiler chickens increases essential amino acid loss. High dietary fibre increases mucin production in the gastrointestinal tract, hence, low ileal apparent amino acid digestibility (Adedokun *et al*., 2011). Mucin is a polymeric glycoprotein, which comprises of a mucus layer that covers the entire epithelium of the gastrointestinal tract. According to Hoskins (1984), mucin is secreted and renewed and its high proportions remain undigested in the distal portions of the small intestine.

Cao *et al*. (2003) investigated the effects of dietary cellulose levels (0, 3.5 and 10.0%) on growth, retention time of diets in the digestive tract, nitrogen utilisation and caeca microflora of chickens. Cao *et al*., (2003) found that there was no difference in nitrogen utilisation by chicks fed with 0 and 3.5% levels but nitrogen retention was lower in the group fed with the high levels (10.0%). The authors concluded that the high cellulose diets negatively influenced nitrogen retention. In general, as the level of fibre incorporation in the diet increased, protein efficiency, nitrogen balance and the apparent protein digestibility decreased while the faecal and urinary nitrogen excretion increased (Bach Knudsen, 2001). On the other hand, if insoluble fibre is included in the poultry diets at recommended concentrations, the performance of birds will not be affected despite a reduction in the nutrient concentration (Hetland *et al*., 2002). In view of these findings, it remains unclear what inclusion level of the CM would be optimum for production in PK chickens.

2.10 Effects of dietary protein on growth, health and meat quality in chickens

2.10.1 Growth

Kingori *et al*. (2007) conducted an experiment to investigate the effects of protein intake on growing indigenous chickens on free-range and concluded that a protein supplementation of 3.2 g/bird/day is mandatory for optimum growth. Similarly, Nieto *et al*. (1995) observed that an improved quality of dietary protein improves body weight gain (growth). Salahuddin *et al*. (2012) conducted an experiment in which the effects of protein were tested on growth performance and haemo-biochemical profile in broilers and they found that 20% crude protein in broiler diets increased body weight gain. Temim *et al*. (1999) found that feeding broilers with high CP diets (25 vs. 20%) at a high ambient temperature (32°C) during the growing period, improved weight gain. In contrast, Nemavhola & Ndlovu (2000) reported that a high level of protein and energy did not have significant effect on the growth rate and feed intake of indigenous chickens. This was corroborated by Junqueira *et al*. (2006) who observed that a higher protein level did not have any effects on the birds' performance. The authors further stated that, supplying protein in excess (more than 16%), did not improve performance but rather caused higher faecal nitrogen losses through faeces and contributed to environmental pollution. Increasing dietary protein beyond 18% CP level led to a decrease in weight gain in the Ugandan local chickens (Megala *et al*., 2012). This is supported by the study of Laudadio *et al*. (2012), who found that body weight and feed conversion ratio were not affected by feeding a relatively low protein (18.5%) diet compared to feeding dietary protein at higher levels (20.5 and 22.5% crude protein).

In another study by Widyaratne & Drew (2011), it was documented that a high protein level of 20% with high digestibility, have a high final body weight (growth) in the broiler chickens. Kermanshashi *et al*. (2011) studied the effects of dietary crude protein fluctuation on performance, blood parameters and nutrients retention in broiler chicken during a starter period and concluded that reducing crude protein, reduced body weight gain. Jones *et al*. (1967) also concluded that the growth rate of cockerels was reduced by feeding a low dietary protein during the growing period. The author further stated that body weight increased rapidly when broilers were fed on a 17% protein diet. It was found (Tarasewicz *et al*., 2006) that in quails and broilers; the protein content of feed affected the bird's growth, mostly in the first stages of life.

2.10.2 Immunity

Proteins have serious implications on the defensive systems of chickens. An appropriate amount of protein in diets can modulate the quantitative and qualitative aspects of the immune response to pathogens. In poultry, it has been shown that a deficiency or an excess of dietary protein or amino acids, changes the immune responses. A deficiency of a dietary protein or amino acids has long been known to impair the immune function and increase the susceptibility of animals to infectious diseases (Li *et al*., 2007).

2.10.3 Carcass characteristics

In their study, Corzo *et al*. (2011) observed lower carcass and meat yields for broiler chickens fed with a reduced crude protein diet (18 versus 20% crude protein). Malik *et al*. (2013) investigated the effects of dietary protein levels (21 versus 23% crude protein for starter diets and 19 versus 21% crude protein for finisher diets) on carcass characteristics of heat-stressed

broiler chicks and found that a high dietary protein level (23% crude protein) resulted in higher carcass traits (drumstick, thigh, chest, back and wing weight) as compared to broilers fed with a low dietary protein level (21% crude protein). Breast muscles synthesised from amino acids accounted for up to 30% of the edible meat and as much as 50% of the edible protein in the carcass (Summer *et al*., 1998), hence, protein quality and quantity have effects on carcass characteristics. Hickling *et al*. (1990) found that an extra l5 - 20 g of breast meat per bird can be obtained by increasing the dietary methionine or lysine by 12% over the national research council requirement.

2.10.4 Meat quality

Meat quality is a set of characteristics that gives meat the ability to satisfy the expressed and implicit needs of its consumer. It refers to the overall meat characteristics including its physical, chemical, morphology, biochemical, microbial, sensory, technological, hygiene, nutritional and culinary properties (Toughan *et al*., 2013).

2.10.4.1 Meat colour

Many studies show that meat colour is one of the most important quality attributes and plays an important role on the acceptance of the meat and consumer perception of the freshness of the product (Fletcher, 1999; Qiao *et al*., 2002; Muchenje *et al*., 2009). The colour of the lean and external fat of meat has been shown to be influential on the acquiring capacity and visual acceptability by the consumer. Literature show that colour is affected by many factors such as sex, age, strain, processing procedure, chemical exposure, dietary and environmental (feed, housing condition etc.) variables (Fletcher, 1999; Toyomizu *et al*., 2001; Milan & Klaus, 2010;

Lyon *et al*., 2004.). Chicken skin colour varies from cream to yellow and this is due to the type of feed consumed (Fletcher, 2002). Muscle pH strongly affect meat colour, with low pH producing a pale colour and high pH resulting in a much darker colouration. The oxymyoglobin will produce a bright red colour on the muscle whereas the metmyoglobin will impart a browner colour (United States Department Agriculture, 2011).

Toyomizu *et al*. (2001) demonstrated that feeding a diet with spirulina could induce redness, saturated at 40 g/ kg, whereas yellowness increased with an increased dietary in spirulina. This means the diet with spirulina which is rich in protein sources affects colour. The study by Mikulski *et al*. (2012) revealed that an inclusion of 180 g/kg of rapeseed as a source of protein, increased the meat yellowness of turkeys. However, these results are in contrast with those of Gopienger *et al*. (2014) who found that an inclusion of the CM had no effects on the colour of the broiler meat. The discrepancy between the two studies might have been due to the use of different types of birds (Turkey versus Broiler chicken). This would agree with other studies that showed that carcass colour is affected by genetic strains (Fanatico *et al*., 2007; Gordon & Charles, 2002).

The colour of the meat is less directly influenced by the component diet or feeding system. Adeola & Ball, (1992) reported a reduced pale soft exudates (PSE) when chickens are supplemented with high tryptophan level while a high dietary lysine supplementation in broilers lead to a high lightness and yellowness value but a reduced redness value (Bouliuane & King 1998). Tang *et al*. (2007) reported a decreased muscle yellowness when a high lysine content

was supplemented to broilers. A low lysine density produced a redder and more yellow pork than a high density of dietary lysine (Cameron *et al*., 1999).

2.10.4.2 Meat pH

Meat pH ranges from pH 5.2 to 7.0 with the highest quality meat product falling between a pH range of 5.2 and 6.0. It has been reported that the meat pH of broilers chicken ranges from 5.5 to 5.6 (Abdullah *et al*., 2010) while that of Thai chickens is 5.80 to 5.93 for the *pectoralis* muscle and 5.85 to 6.06 for the *biceps femoris* muscle (Wattanachant *et al*., 2004). Adeyanju *et al*. (2013) found the meat pH ranging from 6.26 to 6.29 of the indigenous cockerels. The pH of meat may be influenced by other internal factors such as muscle type, chicken strain (Santos *et al*., 2005) and external factors including feed, fasting, electrical stimulation and chilling. The changes that occur in the muscle post-mortem can be measured by the level of pH and temperature (Deiss *et al*., 2009). However, an increase or decrease in pH in chicken meat depends on glycogen and lactic acid in the muscle (Zhang *et al*., 2010). Dyubela *et al*., 2010 postulated that an accumulation of lactate in the muscle lowers the intracellular pH and it occurs until it reaches the ultimate pH (pH_u) of about 5.4 to 5.70. The highest muscle pH values at 20 min and 24 hours post-mortem were associated with the highest body weights and breast meat yields (Le Bihan-Duval *et al*., 2003) which might be contributed to the quality and quantity of the dietary protein. The dietary-induced changes of muscle growth rate increased growth rate as a result of increases protein turnover. Literature shows no effects of dietary lysine supplementation on meat pH, drip loss, firmness and yellowness on pig muscle **(**Apple *et al*., 2004) whereas, a study by Tang *et al*. (2007) reported no influence on pH, shear force, value and meat colour. Mushi *et al*. (2009) stated that a high pH (pHu) in meat can be associated with a

lower glycogen reserve due to insufficient nutrition. However, we are not aware of any reports on the effects of using CM on the pH of PK cockerel meat.

2.10.4.3 Drip losses

From the point at which a bird is slaughtered during the meat production process, it is inevitable that water will be lost (drip loss) from the carcass. Water represents between 70% and 80% of the weight of raw poultry meat. The loss of water (drip loss) is a key concern for meat producers as this water content is said to contribute to the sensorial, organoleptic (juiciness, tenderness texture, smell and colour) and technological quality traits (Arango & Restrepo, 2003) of the meat products. However, this impacts on consumer opinion, thus affecting demand and the saleable value (Prevolnik *et al*., 2010; Mason *et al*., 2016). According to Gil *et al*. (2008), drip loss is related to sensory qualities, such as hardness and juiciness. Muscles with a high drip loss have a high Warner Bratzler shear force. The results of a study by Mukulski *et al*. (2012) showed that a substitution of soybeans with a rapeseed meal at 120 g/kg increased the drip loss as compared to the control diet.

2.10.4.4 Cooking loss

Cooking loss depends on shape and size of the sample, temperature profile during cooking, final cooking temperature and environment during cooking (Honike & Hamm, 1994). Honike & Hamm (1994) further stated that the higher the final temperature and the slower the speed of heating, the higher was the cooking loss. Mikulski *et al*. (2012) studied the effects of a rapeseed meal at levels of 0, 60, 120 and 180 g/kg in a diet of a growing turkey and found that an inclusion level of 180g/kg increased cooking loss. On the contrary, Gopienger *et al*. (2014) found no significant differences in cooking loss of the meat of broilers fed with incremental

levels of the CM in place of SBM. A cooking loss percentage of a commercial cross of broiler strains of chickens was reported to be 29.56% in male and 27.95% in female birds but there was no difference ($P<0.05$) between the four different strains (Lohman, Hubbard JV, Hubbard classic and Ross) (Abdullah *et al*., 2010).

2.10.4.5 Meat tenderness

Meat tenderness is generally considered one of the most important determinants of meat quality (Rawdkhuen *et al*., 2013). A Warner Blatzer shear force is the most widespread method used as an indicator of tenderness. Kamp & Parr (2012) revealed that tenderness depends on the amounts of intramuscular, connective tissue, the length of the sarcomere and the proteolytic potential of the muscle. Meat tenderness originates in the structural and biochemical properties of skeletal muscle fibres, especially the myofibrils and intermediate filaments and of the intramuscular connective tissue, the endomysium and perimysium, which are composed of the collagen fibrils and fibres. Tenderness may be influenced by species, breed, age, sex, diet and individual skeletal muscle tissue of fowls (Wattanachant, 2008). In a study by Gopienger *et al*. (2014), the effects of substituting SBM with CM were studied on meat and carcass yields of broiler chickens. They reported that CM had no effects on the shear force of the breast meat.

2.10.5 Carcass and meat characteristics

Gopinger *et al*. (2014) conducted an experiment to compare the effect of the different dietary levels of canola meal on growth performance, nutrient digestibility and meat characteristics in broiler chickens from 8 to 35 days of age. Dietary treatments had no effect on the sensory evaluation parameters and chemical composition of the leg meat but some linearly increasing

responses were found on the dry matter and ether extract content of the breast meat. No significant effects on leg and wing yield were observed but a linear decrease in the breast yield was observed. The authors concluded that SBM can be replaced by canola meal at concentration of up to 20% of the total diet without affecting the carcass yield, composition of the meat or the sensory characteristics of meat. Nemavhlola & Ndlovu (2000) investigated the effects of different diets on growth rate and meat quality of indigenous chickens and found that proteins have no effects on juiciness and tenderness of the meat of indigenous chickens and only the breed had significant effects. Manyeula *et al*. (2011) found no effects on odour, odour intensity, juiciness, tenderness and flavour of the meat of indigenous chicken fed with the *Imbrasia belina, Tylosema esculentum* and *Vigna subterranea* as protein sources.

Naseem *et al*. (2006) concluded that a canola meal (with 85% KOH solubility) of up to 25% can be incorporated in the broiler starter and finisher diets without any adverse effects on production parameters. On the other hand, Hameed *et al*. (2002) reported that high amounts of canola meal in Japanese quail diets (20, 25 and 30%) did not show good results. The results showed that dressing percentage of quail fed a diet with 15% canola meal was the highest while that of the quail fed a diet with 30% canola meal had the lowest dressing. The authors suggested that the presence of intrinsic anti-nutrient factors like glucosinolate (found in canola meal in minute) amounts may have impaired performance, while a high percentage of fibre may also have had a confounding effect when high levels of canola meal are included in the diet. The differences in results on the inclusion levels of CM may be species specific. Research by Karayagız & Bulbul (2015) also supported the findings of Naseem *et al*. (2006) who concluded that hot, cold carcass weights and yields as well as relative weight of liver, heart, spleen, gizzard, proventriculus and

abdominal fat were not affected by 10, 20, 30 and 40% combinations of CM and sunflower meal added at the expense of soybeans.

Woyengo *et al*. (2011) also observed that an increase in the dietary levels of an expeller-extracted CM on broiler diet from 0 to 40% resulted in a linear increase in the liver weight relative to the body weight. However, the related kidney weight showed a quadratic response, decreasing with an increase in the dietary expeller-extracted CM from 0 to 30% and a similar kidney weight increase when the dietary levels of an expeller-extracted CM was increased from 30 to 40%. No statistically significant differences were observed for the carcass yield or any other individual part yield between control and the 73496 or 73496(S) CM groups when fed to broilers (McNaughton *et al*., 2014). Results of yet another study by Payvastegan *et al*. (2013) showed that dietary CM also numerically increased ($P< 0.05$) weights of the gizzard, liver and pancreas relative to live body weight as the dietary inclusion of CM increased from 0 to 10% and these same weights also significantly increased when the dietary level of CM was increased from 0 to 20%. The workers also found a decrease in breast weight, as a percentage of the live body weight due to the addition of 20% CM to broiler diets. Decrease in breast yield by the CM substitution may be due to the fact that the dietary lysine required by broilers to achieve the maximum breast meat yield was higher than that required for the maximum body weight gain (Mushtaq *et al*., 2007).

Mikulski *et al*. (2014) concluded that the use of good quality, low-glucosinolate rapeseed meal as a substitute for soybean up to 180 g/kg, had no effect on the carcass trait of turkeys, including muscle yield and carcass fat content. The high rapeseed meal content in the diets contributed to

an increase in the concentrations of polyunsaturated fatty acids in meat. The changes in the fatty acid profile may be considered desirable because they cause a deterioration in the oxidative stability of meat stored for a long period. Changes in the fatty acid composition of meat were accompanied by changes in some of its functional properties, leading to a significant increase in the drip loss in groups fed with 120 and 180 g/kg of the rapeseed meal and a decrease in the tenderness values in the groups fed with 180 g/kg of the rapeseed meal. Stanacev *et al*. (2011) also observed that canola could change the fatty acids composition of lipids in the meat and with an increase of the extruded canola seed meal in chicken feed, the amount of stearic, palmitic and linoleic acids decreased while that of oleic and linoleic acids increased.

2.10.6 Dietary effects on blood parameters of chickens

Blood parameters are vital tools that help to detect any deviations from normality in the animal's body (Ogunbajo *et al*., 2009; Etim *et al*., 2014). They provide information on the health and nutritional status of the animal (Orawan & Aengwanich, 2007; Khawaja *et al*., 2012). Nutritional status of an animal depends on its dietary intake and the effectiveness of its metabolic processes (Etim *et al*., 2014. A lower than normal white blood cell count is an indication of a greater challenge to the immune system of birds. An increase in the neutrophils-lymphocytes ratio is a good indicator of nutritional stress (Etim *et al*., 2014). Nwanbe & Elechi (2009) reported that values below the standard range of the packed cell volume and haemoglobin imply a high level of blood dilution and a low efficiency of cellular oxygen transportation.

There is a direct relationship between quality of feed and the composition of broilers chickens' blood (Mirtuka & Rawnsley, 1997). Significantly higher values of red blood cells (RBC), Hb and PCV were recorded in broilers fed with high protein diets than those fed with low protein. Brown

et al. (2000) postulated that increased red blood cell values are related to a high-quality dietary protein and with disease-free birds. Ahmed *et al*. (1994) observed that mean corpuscular haemoglobin concentration (MCHC) values decrease with an increase in the level of protein. Haemoglobin (Hb) concentration, PCV and MCHC are very sensitive to the level of protein intake by poultry (Egubunike *et al*., 2009). However, limited information is available on the haematological indices and serum metabolites of PK cockerels fed with alternative protein sources.

2.11 Summary

Indigenous chicken production is one of the most important enterprises in low-income communities. The chickens serve as a source of protein, income and play social-cultural roles. Despite their importance, their production remains low in terms of quality and quantity primarily due to the poor feeding standard. An improved nutrition management is the only solution to achieve optimum production. Alternatives but cheaper protein sources, which can reasonably replace the inadequate supply of the more expensive SBM, need to be researched and developed. Canola meal is a possible alternative protein source, which can replace the SBM in poultry diets. Canola meal has a well-balanced amino acid profile, rich in sulphur amino acids that are considered to be the first limiting amino acids for chickens. It is also richer in vitamins and minerals as compared to other oilseed protein sources. However, anti-nutritive factors like glucosinolates, sinapine, phytic acid, polyphenolic compounds, erucic acid, protein inhibitors and the indigestible non-starch polysaccharides are present. Information on the feeding levels of CM as well as its potential physiological and meat quality effects on indigenous chickens is inadequate. It is therefore essential to assess the effects of the inclusion levels of CM as a

possible replacement for the SBM in indigenous chickens as well as to determine optimum inclusion levels.

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CHAPTER 3 - CHEMICAL CHARACTERISATION OF CANOLA AND SOYBEAN MEAL AND THE APPARENT NUTRIENT DIGESTIBILITY OF CANOLA-BASED DIETS OFFERED TO POTCHEFSTROOM KOEKOEK COCKERELS

Abstract

The current study was conducted to assess the chemical composition of canola and soybean meal and the apparent nutrient digestibility of canola-based diets offered to Potchefstroom Koekoek cockerels. Soybean meal (SBM) was obtained from Nutrifeeds in Mafikeng, South Africa while canola meal (CM) was purchased from Southern Oil (Pty) Ltd Company in Western Cape, South Africa. Three samples were taken from three different bags and ground to pass through a 1 mm sieve. After proximate and mineral analysis, the protein sources (CM and SBM) were used to formulate chicken grower diets by replacing the SBM component in a commercial grower diet on a crude protein (CP) basis with canola meal as follows: control (diet with no canola meal inclusion), CM37.5 (37.5 g canola meal/kg soybean meal), CM62.5 (62.5 g canola meal/kg soybean meal) CM87.5 (87.5 g canola meal /kg soybean meal) and CM175 (175 g canola meal/kg soybean meal). Chemical characterisation data were not statistically analysed but was used to describe the two dietary ingredients of interest in this study, canola meal and soybean meal. The information was also used in ration formulation. Twenty-five 75-day old Potchefstroom Koekoek (PK) cockerels were used in the digestibility trial. Results showed that canola meal had numerically higher average concentration of ash, crude fibre while dry matter, organic matter and crude protein were higher in SBM. The concentration of Ca, P, Mg, Na and S was numerically higher in SBM while Cu, Mn and Fe were higher in CM. Formulated diets had similar ($P > 0.05$) apparent digestibility values for minerals, dry matter and fibre but higher inclusion of CM reduced ($P < 0.05$) CP digestibility such that CM175 diet had the lowest

(72.42%) digestibility. In conclusion, substituting 175 g/kg of SBM with CM in chicken grower diets for Potchefstroom Koekoek cockerels did not affect the nutrient digestibility with the exception of negative effects on crude protein digestibility at this level. The findings indicate that CM can be added up to 8.75% of the ration without affecting digestibility.

Key words: nutrient digestibility, proximate analysis, mineral analysis, canola meal, soybean meal.

3.1 Introduction

Poultry production in South Africa has always been dependent on expensive protein and energy sources such as soybean meal (*Glycine max* L. Merr), fishmeal and animal proteins concentrates. These feedstuffs account for 80% of the total costs of production (Nworgu & Fasogbon, 2007), which in turn makes chicken meat production less profitable. Soybean meal, in particular, has become very expensive due to competition between humans and animals resulting in rising prices on the world market. Consequently, the search for alternative plant protein sources as partial or complete substitute to SBM remains a major challenge for animal nutritionists.

The use of agro-industrial by-products (Eshiet & Ademosum, 1981) and locally available feeds resources (Mwale *et al*., 2008; Sayda *et al*., 2011) such as canola oil cake is, therefore, fundamental. Canola meal is a potential alternative plant derived dietary protein source, which may be used to replace SBM in poultry diets. It contains a well-balanced amino acid profile and is rich in sulphur amino acids, which are considered to be the first limiting amino acids for chickens. It is also rich in vitamins and minerals as compared to other oilseed meals. The major constraint on the use of canola meal in poultry diets is the possible presence of high amounts of anti-nutritional factors (ANF) like glucosinolates, sinapine, phytic acid, polyphenolic compounds, erucic acid, protein inhibitors, fibre and indigestible non-starch polysaccharides that depress poultry performance (Wiryawan & Dingle, 1998). Polyphenolic compounds and phytates interfere with protein and carbohydrate digestion (Bell, 1993; Bower *et al*., 1998). This is because they form complexes with proteins, carbohydrates and minerals (Gu *et al*., 2010; Kumar *et al*., 2012). Inclusion of canola meal in poultry diets is, therefore, likely to affect the ability of the chicken to extract nutrients from the diet. Higher inclusion levels of canola meal are likely to

result in diets with higher levels of fibre and anti-nutritional compounds. While the tolerance levels of Potchefstroom Koekoek cockerels to fibre and anti-nutritional compounds is largely unknown, a strong possibility exists that digestibility of their diets is likely to be negatively affected by higher inclusion levels of canola. For this reason, the determination of the coefficient of nutrients digestibility of canola meal-based diets aids in the optimisation of the use of this protein source in the diet of indigenous chickens.

To formulate good quality rations using canola meal, accurate information on its nutrient content and bioavailability is required. In addition, oil extraction parameters such as processing temperature and moisture affect the chemical composition and digestibility of feedstuffs such as canola meal. Thus, it is critical to determine the chemical composition of canola meal with a view to use it as an alternative protein source for indigenous chickens of South Africa. It is in this context that the present study was designed to chemically characterise canola and soybean meal and to determine the nutrient digestibility of canola meal-based experimental diets. It was hypothesised that partial replacement of the SBM with the CM in poultry diets would not affect apparent digestibility of nutrients in Potchefstroom Koekoek cockerels.

3.2 Material and methods

3.2.1 Study site

This study was conducted at the North-West University experimental farm (Molelwane), in the North-West province of South Africa. The study area is located 25.80°S and 25.50°E and experiences summer climate from August to March with temperatures ranging from 22 to 35°C and average annual rainfall ranging from 200 to 450 mm per annum. The study site experiences

winter from May to July, with sunny dry days and chilly nights with average minimum and maximum temperatures of 2 and 20°C, respectively.

3.2.2 Source and sample preparation

Soybean meal was obtained from Nutrifeeds in Mafikeng, South Africa while CM was purchased from Southern Oil (Pty) Ltd Company in Western Cape, South Africa. To produce CM, oil was extracted from canola seeds following four steps (expelling, solvent washing, desolvetising and cooling and drying). The cooked canola was pressed in series of expellers to remove 58% of oil at 7% moisture and a temperature of 90-110^{o}C. The press cake from expeller containing 18% oil, was solvent (hexane) extracted at 55^{o}C and 4% moisture content. The meal was then heated to 110 ^{o}C and moisture was increased to 15% (desolvetising). The meal was then cooled and dried to approximately 11 % moisture content. After cooling, the meal containing less than 2% oil was then stored. The stored samples were ground using a grinder (Polymix PX-MFC 90 D model) to pass through 1 mm sieve and then subjected to chemical analysis in triplicate as described in section Chapter 3 under section 3.26 and 3.27.

3.2.3 Preparation of the house

Before the arrival of experimental birds, the poultry house was cleaned. All dust was removed exposing all surfaces to a detergent. Equipment was washed with virkon (virucidal disinfectant). A mixture of formalin and salt was applied to all metabolic cages to ensure that all pathogens were killed. Thereafter, clean containers were placed underneath the metabolic cages to collect faecal samples.

3.2.4 Dietary treatments

The experimental diets (Table 3.1 and Table 3.2) were formulated to meet the nutritional requirements for broiler chicken grower phase according to Opti Feeds (Pty, Ltd) (Optifeeds (PTY) LTD, Lichtenburg, South Africa) recommendations. Five diets were formulated by replacing SBM in a commercial grower broiler diet (Optifeeds (PTY) LTD, Lichtenburg, South Africa) with graded levels of canola meal (CM) on a crude protein basis. The isonitrogenous and iso-energetic experimental diets were formulated by replacing the SBM component with CM as follows: control = (Broiler grower diet with no canola meal inclusion, CM37.5 = 37.5 g canola meal/kg soybean meal, CM62.5 = 62.5 g canola meal/kg soybean meal, CM87.5 = 87.5 g canola meal /kg soybean meal, CM175 = 175 g canola meal/kg soybean meal.

3.2.5 Nutrient metabolism trial

Twenty-five Potchefstroom Koekoek cockerels of 13 weeks old were randomly selected and placed individually in metabolism cages measuring 0.51 m x 0.49 m x 0.36 m for the measurement of nutrient digestibility. A completely randomised designed (CRD) with five dietary treatment, replicated 5 times was used. The cages were fitted with feed troughs, water nipples and perches. Feed was offered at *ad-libitum*. Feed and water were changed between 0700h and 0800h every day. The trial lasted for six days where the first three days were considered as an adaptation period and the last three days as the experimental period during which samples and measurements were taken (Bashar *et al*., 2010).

Table 3.1 Ingredient composition of experimental diets fed to Potchefstroom Koekoek cockerels from 5 to 18 weeks of age on air dry basis (g/kg)

	Diets[1]				
Ingredients	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**
Canola oilcake	0	37.6	62.7	87.7	175.0
Yellow maize	699.0	678.2	661.6	638.1	595.0
Prime gluten 60	11.8	11.7	11.8	44.0	24.0
Full fat soya	51.0	88.0	117.7	12.00	174.0
Soybean oil cake	197.0	149.3	112.0	77.0	0
Limestone powder	14.5	14.0	13.6	01.3	12.2
Mono calcium	7.2	6.7	6.7	6.5	05.6
Fine salt	3.2	3.3	3.3	3.2	3.2
Sodium-bicarbonate	1.7	1.6	1.6	1.7	1.6
Choline powder	0.8	0.8	0.8	0.8	0.8
Lysine	2.8	2.8	2.8	3.0	2.7
L-Threonine	4.1	4.0	0.4	0	0
Methionine	1.9	1.8	1.7	0.1	1.8
Phytase	1.7	1.7	1.7	0.7	1.7
Olaquindox	0.5	4.0	4.0	0	0.4
Coxistac	0.4	0.5	0.5	0.5	0.5
Total	100.00	100.00	100.00	100.00	100.00

SBM= soybean meal; CM =canola meal; [1]Diets: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM.

Table 3.2 Nutrient composition of experimental diets provided to Potchefstroom Koekoek cockerels from 5 to 18 weeks of age on air dry basis (%)

	Diets[1]				
Nutrients	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**
Moisture	11.34	11.24	11.13	11.03	10.93
ME(MJ/kg)	12.09	12.09	13.09	12.10	11.90
Protein	18.00	18.02	19.09	19.03	18.93
Crude fat	4.16	5.16	5.60	5.60	6.24
Crude fibre	2.32	2.72	3.03	3.20	4.21
Calcium	0.85	0.85	0.85	0.85	0.85
Sodium	0.18	0.18	0.18	0.18	0.18
Potassium	0.80	0.76	0.76	0.72	0.72
Phosphorus	0.50	0.51	0.52	0.53	0.53
Chlorine	0.30	0.30	0.30	0.30	0.30
Available phosphorus	3.80	3.80	3.80	3.73	3.73
Lysine	1.07	1.08	1.09	1.90	1.11
Arginine	1.10	1.10	1.10	1.10	1.10
Tryptophan	019	0.19	0.19	0.19	0.20
Methionine	0.45	0.43	0.47	0.45	0.52
Threonine	0.71	0.71	0.71	0.71	0.73
Histidine	0.43	1.65	0.48	0	0.53
Leucine	0.34	0.85	1.65	1.92	1.91
Valine	0.84	0.85	0.86	0.92	0.91

SBM= soybean meal; CM =canola meal; [1]Diets: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM; ME = metabolisable energy; MJ/kg = mega joules per kilogram

Excreta voided were collected twice daily from each of the replicates during the last three days on polythene sheets. Feed and excreta were weighed and stored (-15°C) in the freezer pending chemical analyses.

Apparent digestibility of crude fibre and minerals was calculated as described by (Bashar *et al*., 2010) using the equation:

$$Apparent\ nutrient\ digestibility = \frac{Nutrient\ \text{int}ake - Faecal\ nutrient}{Nutrient\ \text{int}ake} \times 100$$

3.2.6 Proximate analysis

Prior to chemical analysis, all samples (CM, SBM, formulated diets, refusals and faeces) were dried in a forced air oven at 55°C for 72hrs, followed by fine grinding using a hammer mill to pass through a 1 mm sieve. Moisture content was determined by weighing sample in crucible and drying in an oven at 105°C, until constant weight was obtained. Ash content was determined by ashing at 550°C for about 6 hours (AOAC, 2005, method number 924.05). Nitrogen was determined using Kjeldahl method (AOAC, 2005; method number 984.13). Crude protein was calculated by multiplying the percentage of nitrogen by 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by refluxing 0.45 g of samples with neutral detergent and acid detergent solutions respectively, for 1 hour using the ANKOM^{2000} fibre analyser (ANKOM Technology, NY, USA).

3.2.7 Determination of mineral content

The calcium (Ca), magnesium (Mg), potassium (K), sodium (N), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) content were analysed using ICP mass spectrophotometer from Perkin Elmer supplier in Animal Health Center Laboratory (North-West University, Mafikeng. South

Africa). Phosphorus was determined calorimetrically using sodium phenol and ammonium molybdate plus ascorbic acid as described by AOAC, 2012, method no. 976.06.

3.2.8 Statistical analysis

Chemical analyses for CM, SBM and formulated diets were done using three subsamples (pseudo replicates) and, therefore, the data was simply reported as mean of the three subsamples without any statistical analysis. However, nutrient utilisation data were analysed using PROC GLM of SAS (2010) according to the following linear model.

$$Y_{ij} = \mu + d_i + E_{ij}$$

Where Y_{ij} = response variable (nutrient utilisation), μ = general mean, d_i = the fixed effects of canola levels. E_{ij} = random error associated with observation, *ij*= assumed to be normally and independently distributed. When the analysis of variance revealed significant effect, probability of difference (PDIFF) option in the LSMEANs statement of the GLM procedure of SAS (2010) was used to separate means. The level of significance was set at $p<0.05$.

3.3 Results

3.3.1 Proximate and mineral composition

The average chemical composition obtained for the proximate and mineral composition of CM and SBM is shown in Table 3.3. Canola meal had numerically higher concentration of ash, neutral detergent fibre (NDF) and acid detergent fibre (ADF) while dry matter, organic matter and crude protein were higher in SBM.

Table 3.3 Proximate and mineral composition of soybean and canola meals

Proximate composition	**Soybean meal**	**Canola meal**
Dry matter ($g.kg^{-1}$)	909.0	930.4
Organic matter (%)	0.92	0.86
Ash (%)	0.06	0.07
Neutral detergent fibre (%)	23.43	31.25
Acid detergent fibre (%)	8.74	23.83
Crude protein ($g.kg^{-1}$)	385.3	344.9
Mineral content (µg/ml)		
Calcium (%)	31.95	15.95
Phosphorus (%)	89.21	46.08
Magnesium (%)	86.64	45.37
Sodium (%)	8.47	3.30
Sulphur (%)	26.37	8.46
Copper (%)	0.00	0.01
Zinc (%)	0.01	0.01
Manganese (%)	0.26	0.37
Iron (%)	0.26	0.31

3.3.2 Digestibility of dry matter, crude protein and fibre

The results on the effects of replacing SBM with graded levels of CM in Potchefstroom Koekoek cockerel diets on apparent digestibility of dry matter and crude protein and fibre are presented in Table 3.4. Diet had a significant effect ($P<0.05$) on apparent digestibility of crude protein. The Potchefstroom Koekoek fed diet CM175 had the lowest ($P<0.05$) crude protein digestibility while those fed the control diet had the highest ($P<0.05$). No significant differences were observed on apparent digestibility of crude protein of cockerels fed diets CM37.5, CM62.5 and CM87.5. There were no statistical differences observed on dry matter and crude fibre digestibility in PK fed on treatment diets. The range of apparent digestibility of dry matter was from 78.61 to 81.81% while that of crude fibre was from 11.56 to 20.79 %.

3.3.3 In vivo bioavailability of minerals

The effects of dietary inclusion of graded levels of CM on mineral *in vivo* bioavailability (%) in Potchefstroom Koekoek cockerels are shown in Table 3.5. The diet had a significant effect on the apparent digestibility of potassium only. The Potchefstroom Koekoek fed diet CM62.5 had the lowest ($P>0.05$) potassium digestibility, while those fed diet CM37.5 had the highest ($P>0.05$). For all other minerals, digestibility values were similar across dietary treatments.

Table 3.4 Effects of replacing soybean meal (SBM) with canola meal (CM) in Potchefstroom Koekoek cockerel diets on apparent digestibility (%) of dry matter, crude protein and crude fibre (mean ± SE)

	Diets[1]				
Parameter	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**
DMD	80.34 ± 7.14	75.03 ± 6.77	78.61 ± 6.77	80.79±6.77	81.81 ± 6.55
Crude protein	78.33 ± 1.57^{b}	76.34 ± 1.57^{ab}	74.67 ± 1.57^{ab}	75.09±1.57^{ab}	72.42 ± 1.57^{a}
Cruder fibre	15.85 ± 2.24	20.79 ± 2.24	11.56 ± 2.24	16.56 ± 2.24	17.29 ± 2.39

^{abc}Means within a row that do not share a common superscript differ significantly (P<0.05).

SBM= soybean bean meal; CM= canola meal; [1]Diets: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM; DMD = dry matter digestibility.

Table 3.5 Effects of dietary inclusion of canola meal on in vivo bioavailability (%) of minerals in Potchefstroom Koekoek cockerels

	Diets[1]					
Minerals	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**	**SE**
Macro minerals						
Calcium	48.73	50.93	50.7	30.92	37.78	8.83
Phosphorus	63.92	55.37	55.37	47.71	48.10	9.10
Magnesium	66.58	59.63	63.96	50.67	50.00	6.67
Potassium	50.42^{abc}	71.36^{c}	40.7^{a}	63.26^{bc}	64.86^{bc}	7.46
Sodium	46.06	45.39	41.90	41.15	31.78	5.53
Trace minerals						
Copper	49.07	48.81	50.39	31.68	53.45	8.44
Iron	50.38	49.43	33.30	42.38	49.79	7.82
Manganese	51.80	50.43	36.54	53.98	56.87	7.77
Zinc	58.80	64.00	61.95	61.32	58.56	8.30

^{abc}Means within a row that do not share a common superscript differ significantly ($P<0.05$).

SBM= soybean bean meal; CM= canola meal; [1]Diets: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM. SBM= soybean meal; CM= canola meal

3.4 Discussion

3.4.1 Proximate and mineral content.

Proximate and minerals composition of CM is affected by factors such as cultivar, environmental conditions during growth and harvesting periods and processing conditions (Khajali & Slominski, 2012). The higher neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentration in CM compared to SBM in the present study may be due to higher lignin, which is also associated with high tannin contents (Mansoori *et al*., 2015). Literature shows that higher lignin negatively affects energy digestibility. The current results compared well with studies by Khajali & Slominski (2012) and Chen *et al*. (2015). These authors also found higher ash, ADF and NDF in CM compared to SBM. Higher ADF and NDF limits the quantities of CM that can be included in poultry diets as a protein source.

Literature reported the CP contents of CM and SBM to be in the range of 34 to 41.1% (Chapter 2, Table 2.3) and 38.5 to 48% for SBM (Eekeren *et al*., 2006). Different CM and different SBM may differ in CP contents due to variations in concentrations of nutrients in the seeds, environment in which seed are grown and differences in oil extraction procedures (Newkirk, 2011). However, these ranges are higher than 34.4 % (CM) and 38.9% (SBM) reported in this study. Meanwhile, a similar value of 38% CP content of SBM was reported in Ethiopia (Ashnie, 2015) while Riyazi *et al*., (2008) reported close value of 35 % CP content of CM in Iran. The results show that CM is rich in protein and hence could be a potential substitute for SBM in poultry diets. The higher Ca, P, S and Mg concentration observed in SBM compared to CM indicate that the incorporation of CM in the diets for PK cockerels affect the concentration of

these minerals. These current results contradict the finding of Khajali & Slominski (2012) and Chen *et al*. (2015) who reported higher Ca, P, S and Mg content in CM compared to SBM.

3.4.3 Dry matter, crude protein and fibre digestibility

The observation that the replacement of SBM with CM in PK diets had no effects on apparent digestibility of dry matter and crude fibre compares well with a study by Paric *et al*. (2015). These workers found no statistical differences in dry matter and cruder fibre digestibility when up to 15% rapeseed meal was included in broiler finisher diets. In contrast, Gopinger *et al*. (2013), showed that dry matter digestibility decreased with the increasing levels of CM inclusion when 0, 10, 20, 30 and 40 inclusion levels were used from 0 to 35 days in broiler chicken diet. The authors further explained the decrease in dry matter digestibility coefficient as caused by increase in crude fibre content with incremental dietary CM. The difference in reports of Gopinger *et al*. (2013) and current results may be ascribing to the different strain of chickens used in the study. This implies that PK cockerels have better tolerance to fibre and anti-nutritional factors in CM when CM was added even up to 17.5%. The digestive tract of PK cockerels appeared to be adapted to feed with high fibre as elaborated in Chapter 5 section 5.4.1

Thacker & Petric (2011) studied the effects of incorporating 0, 3, 6, 9, 12 and 15% of canola protein concentrate in broiler chicken diets and found an increase in digestibility coefficient of dry matter with an increase in levels of canola protein concentrate in diets. The difference in reports of Thacker & Petric (2011) (Broiler) and current study (Indigenous) may be ascribe to the differences strain of chicken used in the study. It is implied that PK cockerels were not affected by the fibre and anti-nutritional factors present in the CM when it was included up to 17.5 %.

Similarly, Leeson *et al*. (1987) showed that CM can replace 100% of the SBM in broiler diets from 0 to 21 d of age without any significant effect on feed intake, weight gain or feed efficiency as well as protein, fat, calcium, phosphorus or magnesium retention and energy utilisation.

Crude protein digestibility in the PK cockerels fed diet CM175 was lower compared to PK cockerels fed the control diet. The current results are in line with the published reports of Landero *et al*. (2012). These workers found that inclusion of CM at 20% in the diets of weaning piglets reduced the CP digestibility and associated this reduction with the high crude fibre content. Gopinger *et al.* (2014) reported a quadratic response in terms of CP digestibility in response to CM inclusion levels up to 40% in the broiler chicken. Vast literature contradicts the current results (Thacker & Petric, 2011; Perić *et al.*, 2015; Hossain *et al*., 2013). The workers found no statistically difference on CP digestibility when CM was included in the diets of the broilers. The low protein digestibility in the current study can be explained by deleterious factors and crude fibre (Hossain *et al*., 2011). Various literature reported the presence of polyphenolic compounds (Mailer, 2004), protein inhibitors (Guillamon *et al*., 2008) and glucosinolates (Wickramasuriya *et al*., 2015) in CM has adverse effects on protein digestibility and absorption. Thacker & Petric. (2011) showed that dietary fibre reduces nutrient digestibility by increasing the rate of passage. Increased rate of passage limits the amount of time available for enzymatic digestion and absorption of nutrients.

3.4.2 Mineral digestibility

The Potchefstroom Koekoek chickens fed on CM62.5 had lower K digestibility coefficients compared to the other treatment diets. The reason for this is unknown. The lack of a significant

difference in Ca, P, Mg and Na concentration between CM diets and the control is consistent with research findings reported by Leeson *et al*. (1987). The authors found that replacement of SBM with CM at 0, 25, 50 and 100 % on broiler chicks and laying hens diets did not affect Ca, P, Mg and Na digestibility. Thacker & Petric (2011) reported increasing levels of P retention when feeding broiler chicken at inclusion levels of 0, 3, 6, 9, 12 and 15% of canola protein concentrate while Ca was not affected by any of the inclusion levels. Trace mineral digestibility was not affected (P >0.0.5) by the diet. The observed results indicate that digestibility of minerals in control (SBM) and CM-based diets was similar implying that indigenous chickens have the capacity to extract minerals from CM.

3.5 Conclusions

From the current study it can be concluded that there is little difference in terms of ash and crude protein content of SBM and canola meals. The results also confirm that CM tends to contain higher levels of NDF and ADF than SBM. Soybean meal contained greater Ca, P, Mg, Na and S than CM but the opposite was true for Mn and Fe content. Inclusion of CM in diets lowered CP digestibility but did not negatively affect dry matter and crude fibre digestibility parameters implying that CM can be used to replace SBM up to 175 g/kg without compromising the ability of PK chickens to digest the resultant diet. However, other factors such as essential amino acid profile, whose impact cannot be evaluated in a digestibility trial, may still affect the ability of the CM-based diets to support optimal growth performance of the PK chickens. In addition, the presence of anti-nutritional factors in CM may have a negative effect on the physiological processes of the chickens when fed over a longer period of time. These negative effects would not have been immediately apparent in a 6-day metabolism trial. It is, therefore, necessary to

assess the effects of these CM-based diets on growth performance and haemo-biochemical parameters in PK chickens.

3.6 References

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CHAPTER 4 - GROWTH PERFORMANCE, PROTEIN UTILISATION EFFICIENCY AND BLOOD METABOLITES IN POTCHEFSTROOM KOEKOEK COCKERELS FED ON GRADED LEVELS OF CANOLA MEAL

Abstract

The study was conducted to evaluate the effects of replacing soybean meal (SBM) with graded levels of canola meal (CM) on growth performance, protein utilisation efficiency and haemo-biochemical parameters of the Potchefstroom Koekoek (PK) cockerels. Five isonitrogenous and iso-energetic experimental diets were formulated by replacing the SBM component with CM as protein source as follows: Control = diet with no canola meal inclusion, CM37.5 = 37.5 g canola meal/kg soybean meal, CM62.5 = 62.5 g canola meal/kg soybean meal, CM87.5 = 87.5 g canola meal /kg soybean meal, CM175 = 175 g canola meal/kg soybean meal. A hundred and seventy-five, 36-day old PK cockerels were randomly allocated to the dietary treatments. Each treatment had 5 replicate pens holding 7 cockerels. The 36-day weight was used as the initial weight, with the cockerels being weighed weekly for 12 weeks. Average daily feed intake (AWFI), average weight gain (AWG), feed conversion ratio (FCR), protein consumed (PC) and protein efficiency ratio (PER) were measured and calculated on a weekly basis. Blood was collected in the 11th week for serum and haematological analysis. Diet significantly affected growth performance parameters, protein utilisation efficiency and serum biochemistry of the PK cockerels. Cockerels fed the control, CM3.75, CM6.25 and CM17.5 diets had higher ($P<0.05$) leucocyte counts compared to those fed diet CM87.5. Cockerels fed diets CM175 and CM62.5 had the highest ($P<0.05$) neutrophil and lymphocyte counts, respectively. However, cockerels fed diets CM37.5, CM87.5 and CM175 had the highest ($P<0.05$) normoblast counts, whilst those fed control and CM37.5 diets had the lowest normoblast ($P<0.05$) counts. Overall, the results from the study

suggest that replacement of the SBM with CM in poultry diet up to a level of 175.0 g/kg can be effectively used without any adverse effect on the PK chicken performance and health.

Keywords: canola meal, Potchefstroom Koekoek cockerels, protein efficiency ratio, haematology, serum biochemistry.

4.1 Introduction

Poultry will account for more than 40% of the global increase in demand for meat in 2020 (Rosegrant *et al*., 2001). These chicken production is one of the most important poultry production enterprises in low income communities (Saina, 2005). They are favoured because they can convert low quality feeds scavenged around homesteads into highly sought-after animal protein (Alders *et al*., 2009). Indigenous chicken production account for 80 % of poultry production in rural areas (Guéye, 2003). However, their productivity is due to suboptimal nutritional and other management factors (Ng'ambi, 2013). These chickens primarily depend on scavenged feed resources, which often supply inadequate nutrients (Alders *et al*., 2009; Okitoi *et al*., 2009; Kingori *et al*., 2010). Potchefstroom Koekoek is one the indigenous chicken strains reared in South Africa. It is characterised as a heavy breed, with high egg production potential and is highly adapted for household production. Its average adult body weight varies from 3 - 4 kg for cocks and 2.5 - 3.5 kg for hens and can reach sexual maturity in 130 days. However, improved nutrition may allow the PK to reach genetically potential (Tadelle *et al*., 2003; Kingori *et al*., 2007) in terms of productivity.

Poor quality protein has been reported to lead to growth depression (Sibbald, 1986; Naseem *et al*., 2006) and impaired immune function (Lie *et al*., 2007) in chickens. Provision of high-quality protein is, therefore, essential to increase nutrient intake for optimum production. Tadelle *et al*. (2003) and Kingori *et al*. (2007) revealed that indigenous chickens subjected to improved nutrition and management conditions showed a positive response in terms of their growth performance. Currently, there is no certified diet that suits the optimum production of indigenous chickens in South Africa. To supplement, most farmers currently use expensive commercial feed

formulated for exotic breeds, which may exceed the nutrient requirements of relatively slow-growing chickens (Kingori *et al*., 2010). High-quality protein, commonly supplied by soybean meal, is one of the most expensive ingredients in practical poultry diets (Rose, 1997). This is mainly due to the fact that both humans and livestock depend on SBM for protein and hence competition exists between the two. This has led to an increase in cost of the soybean on the world market. As such, there is a need to look for alternative, locally available and relatively cheaper sources of protein.

One such alternative dietary protein source for feeds is canola meal (CM), a relatively inexpensive by-product of oil extraction from rapeseed (Naseem *et al*., 2006). This is mostly because there is no direct competition between animals and humans for CM. Canola meal contains high concentration of protein and a well-balanced amino acid profile (Newkirk *et al*., 2003) as well as sulphur-containing amino acids, mineral and vitamins (Canola Council of Canada., 2015) compared to SBM. However, its utilisation in poultry diets could be limited due to the higher levels of fibre and anti-nutritional factors in its meal. Nevertheless, the development of low erucic acid and glucosinolates cultivar of canola has led to its increased usage in animal diets (Naseem *et al*., 2006; Riyazi *et al*., 2009; Gheisari *et al*., 2011). However, most research on CM has been carried out in exotic and improved poultry strains, which are considered to be of greater economic importance. As a result, there is lack of information on the possibility of incorporating CM in diets of indigenous chickens. Therefore, the objective of the current study was to determine the growth performance, protein utilisation efficiency and haemo-biochemical parameters of Potchefstroom Koekoek (PK) cockerels fed graded levels of CM as partial replacements for SBM. It was hypothesised that there was no significant difference in growth

performance, protein utilisation efficiency and haemo-biochemical parameters of Potchefstroom Koekoek (PK) cockerels fed graded levels of CM as partial replacements for SBM.

4.2 Material and methods

4.2.1 Study site

The study was carried out at the North-West University Research and Teaching Farm at Molelwane, whose location and climatic conditions are described in detail in Chapter 3, Section 3.2.3.

4.2.2 Experimental chickens and their management

A total of 175, thirty-six-day old Potchefstroom Koekoek (PK) cockerels weighing an average 342. 6 ± 165.2 g body weight were purchased from Grootfontein Fisheries Farm, Zeerust, North-West province, South Africa. The chicks were fed on broiler starter crumbs from Tau veevoere Company, Lichtenburg, North-West province, South Africa for the first five weeks. The experimental broiler grower diet was fed from six to 18 weeks of age. Two weeks before their arrival, the poultry house was cleaned by washing all equipment with biogel (detergent) purchased from Noordwes Koöperasie (Mafikeng, North-West province, South Africa). The ceiling, walls and floors were disinfected with verocid. Lastly, a mixture of formalin and salt was applied to floors, wall-junctions and around base posts. The water fonts were cleaned daily. Tubular feeders, with a capacity of 20 kg, were used and the feed was weighed and changed daily till the end of experiment. Feed and water were provided *ad libitum*. The management and care of the chickens were in accordance with Animal Research Ethics Committee, Mafikeng Campus (AREC-MC) accepted standards for the welfare and ethics of chickens. The study was

approved by the North -West University Institution Research Ethics Regulatory Committee (Ethics Number NWU 00517-16-A9).

4.2.3 Diet formulation

The five isonitrogenous and iso-energetic experimental diets used in this study were formulated as described in Chapter 3, Section 3.4.4.

4.2.4 Growth performance

Feed offered to the birds and refusals were weighed daily using an Adam 6010 model electronic balance (Adam, Gauteng Province, South Africa). Average weekly feed intake (AWFI) was calculated by subtracting refusals from feed offered to the birds. The birds were weighed at the beginning of the experiment and subsequently on a weekly basis. Average weekly body weight gain (AWG) was determined as interim weight (difference between the weight at end of previous week and average live weight at end of current week) divided by the feeding period in days as follows:

$$AWG = \frac{Average\ weight\ \ at\ end\ \ of\ \ the\ \ previous\ week\ \ -\ \ at\ the\ end\ \ of\ \ the\ current\ week}{Number\ of\ \ days\ between\ the\ two\ weight\ \ weight}$$

Weekly feed conversion ratio (FCR) was calculated by dividing feed consumption with body weight gain (AWG) per week as follows:

$$FCR = \frac{AWFI}{AWG}$$

4.2.5 Experimental design

The PK cockerels were individually weighed and randomly allocated to 25 pens each measuring (0.131 × 0.128 × 0.98 m) to which the five experimental diets were randomly allocated. Thus,

each dietary treatment had five replicate pens with each pen holding seven PK cockerels. The study was arranged in a completely randomised design (CRD) with the pen as the experimental unit. Rearing was done under natural lightning until slaughter at 13 weeks of age.

4.2.6 Protein utilisation efficiency

Weekly protein consumed (PC, g/bird) was calculated by multiplying the concentration of crude protein (CP_d) in the diet (g/kg DM consumed) by weekly feed intake (FI kg/bird)

$$PC = CP_d \; X \; FI$$

The protein efficiency ratio (PER, g/g) was calculated by dividing mean body weight gain (AWG) by the mean protein consumed (PC) as follows:

$$PER = {}^{AWG}\!/_{PC}$$

4.2.7 Haematology

Blood samples were collected at 11 weeks of age from three randomly selected birds in each replicate/pen for the determination of haematological indices. About 2ml of blood from wing vein of each chicken was aspirated with the use of 21-gauge needles fixed into purple top 4 ml ethylene diamine tetra-acetic acid (EDTA)-coated vacutainer tubes for determination of haematological parameters. Haematological indices (total erythrocytes count (TEC), haemoglobin (Hb), Haematocrit (HCT) and different leukocytes counts) were determined using the Sahli method and the values were recorded in g/100 ml (WHO, 1980) and MCV were analysed using automated Idexx Laser Cyte Haematology analyser (IDEXX Laboratories, Inc.) in the Animal Health Laboratory at the Centre for Animal Health Studies.

4.2.8 Serum metabolites

Two millilitres of blood from the wing vein of each chicken was collected with the use of 21-gauge needles into 4 ml vacutainer tubes. The blood was stored for 10 minutes at room temperature. After centrifugation (20 minutes, 1500 rpm), the serum was collected into 0.5 ml centrifuge tube and stored at -20^{o}C pending determination of serum metabolites. Serum metabolites (bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), urea, creatinine, triglycerides (TG), serum sodium (Na), potassium (K), chloride (Cl), calcium (Ca) and magnesium (Mg) were assayed using automated Idexx Vet Test Chemistry Analyser (Gilford Impact, 404lE, Ciba Coming Diagnostic Corp., Gilford Systems, Oberlin, OH 44774) in the Animal Health Laboratory at the Centre for Animal Health Studies.

4.2.9 Statistical analyses

Weekly feed intake, growth rate and feed conversion ratio data were analysed using the repeated measures procedures of SAS (SAS, 2010). The overall haematology and serum biochemistry data were analysed using one-way analysis of variance using SAS (SAS, 2010) according to the following general linear model.

$$Y_{ij} = \mu + d_i + E_{ij}$$

Where Y_{ijk} = response variable (feed intake, weight gain, growth rate, feed conversion ratio, haematology and serum biochemistry), μ = general mean, d_i = the fixed effects of diets, E_{ijk} = random error associated with observation ijk = assumed to be normally and independently distributed. When the analysis of variance revealed the existence of significant difference among treatment means, the probability of difference (PDIFF) option in the LSMEANs statement of the

GLM procedure of SAS (2007) was used to separate means. The level of significant was set at $p<0.05$.

4.3 Results

4.3.1 Growth performance

No mortalities were recorded throughout the experimental period. There were no significant dietary effects on average weekly feed intake (AWFI) of Potchefstroom Koekoek cockerels (Figure 4.1). In week 3 and 4, there was rapid increase in AWFI across all dietary treatments. Although, not significant, AWFI was lower in cockerels fed diet CM175 at weeks 4, 5, 6, 7, 8 and 11 compared to other treatments. The effect of diets on the average weekly gain (AWG) of PK cockerels is illustrated in Figure 4.2. Diet had no effect on AWG of PK cockerels. However, an expected linear increase in AWG was observed throughout the experiment for cockerels fed all diets. The rate of increase in cockerel fed control diet on AWG was lower than those fed other diets.

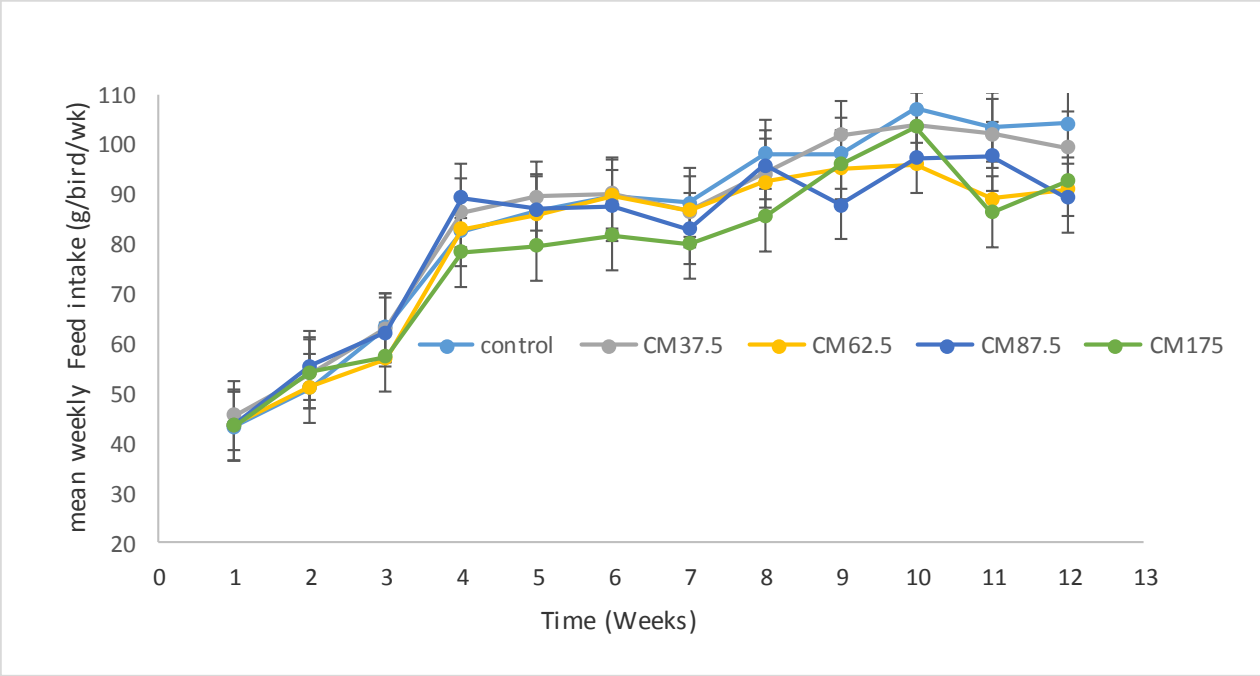

Figure 4.1 Average weekly feed intake of Potchefstroom Koekoek cockerels fed diets containing graded levels of canola meal as a substitute for soybean meal

SBM= soybean bean meal: CM= canola meal; Diets[1]: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM; g/birds/wk = gram /bird/week

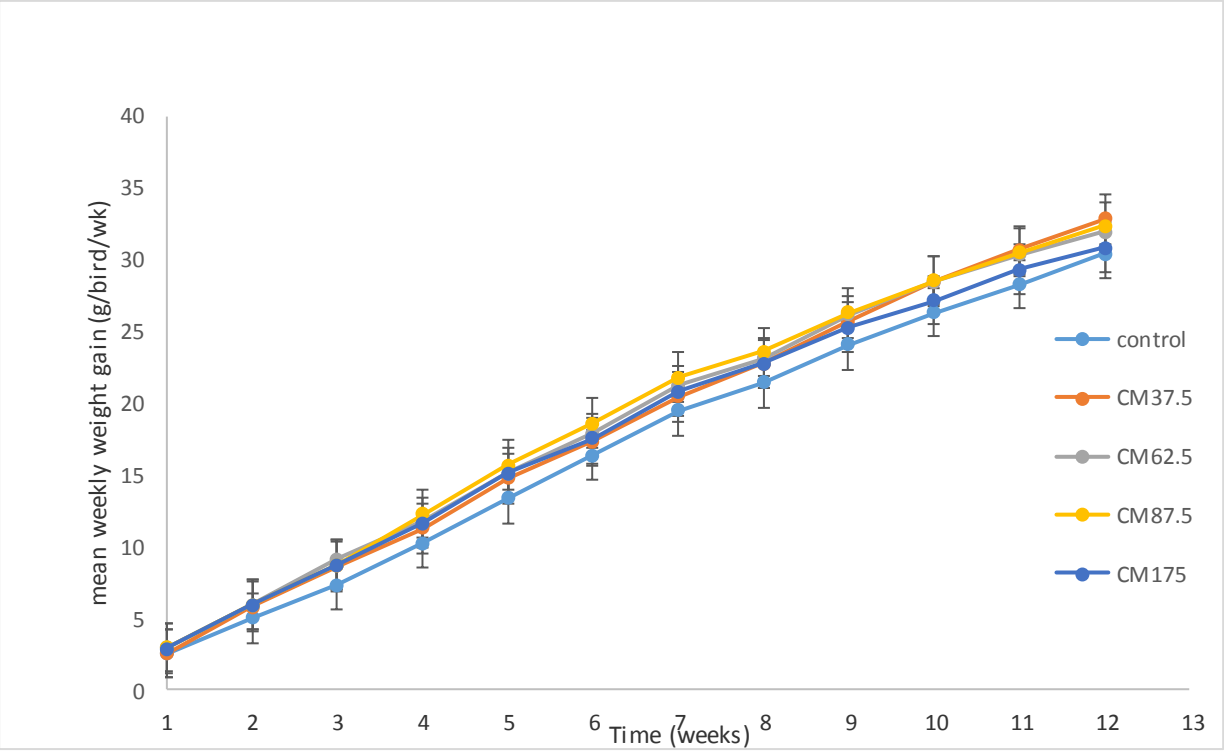

Figure 4.2 Mean weekly weight gain (g) of Potchefstroom Koekoek cockerels fed diets containing graded levels of canola meal as a substitute for soybean meal

SBM= soybean bean meal: CM= canola meal; Diets[1]: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM; g/birds/wk = gram /bird/week

Diets affect ($P<0.05$) weekly feed conversion ratio (FCR) of PK cockerels (Table 4.1). In week 2 and 3, the cockerels fed control diet had the highest ($P<0.05$) FCR.

4.3.2 Protein utilisation efficiency

The pattern of protein intake was exactly the same as that of feed intake. Diets affected protein consumption in weeks 3 and 4 of the experimental period (Figure 4.3). Higher protein level was consumed in cockerels fed diets CM87.5 compared to those fed other treamet diets. From week 1 onwards, PK cockerels slowly increased the consumption of protein until week 12. Results on protein utilisation efficiency showed that diets affected PER (Table 4.2). Significant effects of diet on PER were observed in weeks 2, 3 and 10. In weeks 2 and 3, cockerels fed control and CM87.5 diets had the lowest PER while in week 10 the lowest PER was observed in CM175 cockerels and the highest PER in CM37.5 cockerels.

Table 4.1 Feed conversion ratio of Potchefstroom Koekoek cockerels fed diets containing graded levels of canola meal as of substitute for soybean meal (mean ± SE)

	Time (weeks)											
Diets[1]	1	2	3	4	5	6	7	8	9	10	11	12
Control	2.49 ± 0.19	3.00 ± 0.15[b]	3.90 ± 0.22[c]	4.14 ± 0.55	3.97± 0.25	4.62 ± 0.55	4.08 ± 0.28	7.34 ± 0.65	6.07 ± 0.71	7.01 ± 1.02	9.38 ± 1.27	7.51 ± 1.16
CM37.5	2.76 ± 0.19	2.41 ± 0.15[a]	3.24 ± 0.22[b]	4.69 ± 0.54	3.70 ± 0.24	5.10 ± 0.55	4.13 ± 0.28	6.12 ± 0.65	5.24 ± 0.71	5.40 ± 1.01	6.91 ± 1.26	6.79 ± 1.15
CM62.5	2.19 ± 0.19	2.44 ± 0.15[a]	2.58 ± 0.22[a]	4.89 ± 0.54	3.83 ± 0.25	4.56 ± 0.55	3.80 ± 0.28	7.00 ± 0.65	4.50 ± 0.71	6.08 ± 1.01	7.23 ± 1.26	11.18 ± 1.15
CM87.5	2.16 ± 0.21	2.71 ± 0.16[ab]	3.26 ± 0.24[cb]	3.63 ± 0.61	3.67 ± 2.77	4.32 ± 0.61	3.67 ± 0.31	7.22 ± 0.73	5.22 ± 0.80	6.71 ± 1.13	7.52 ± 1.26	7.20 ±1.29
CM175	2.21 ± 0.71	2.54 ± 0.13[a]	2.97 ± 0.20[ab]	3.87 ± 0.49	3.29 ± 0.23	5.10 ± 0.50	3.52 ± 0.25	6.50 ± 0.60	5.70 ± 0.64	8.65 ± 0.92	6.01 ± 1.15	8.68 ±1.05

[abc]Means within a row that do not share a common superscripts differ significantly (P<0.05).SBM= soybean bean meal ; CM= canola meal; [1]Diets: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM.

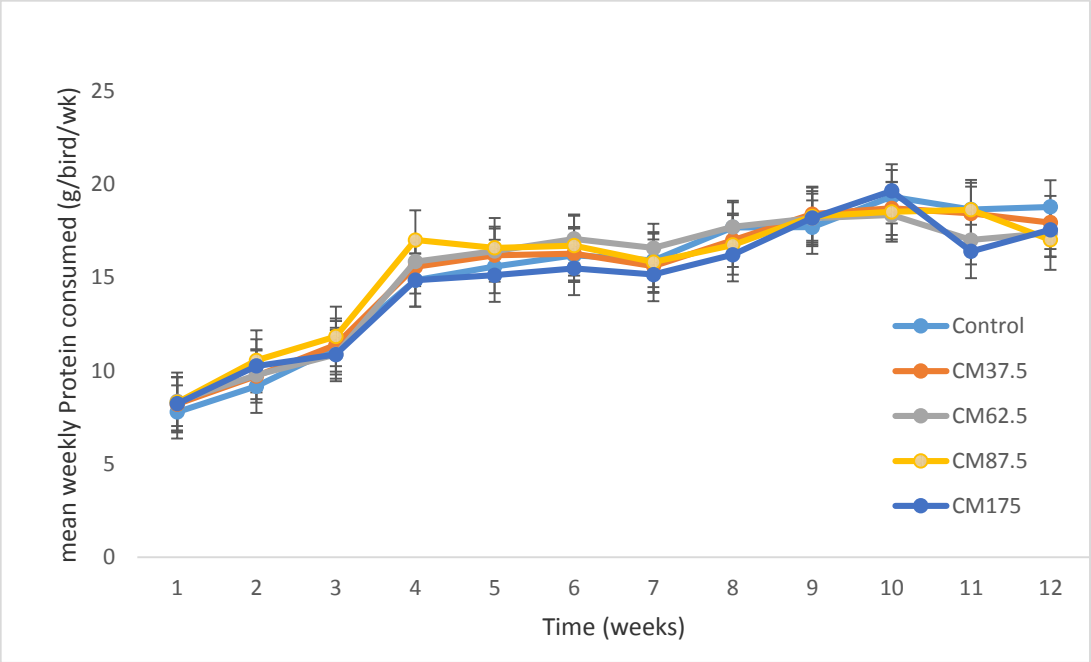

Figure 4.3 Weekly mean protein consumed (WMPC) by Potchefstroom Koekoek cockerels fed diets containing graded levels of canola meal as partial replacement for soybean meal

SBM= soybean bean meal: CM= canola meal; Diets[1]: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM; g/birds/wk = gram /bird/week

Table 4.2 Protein efficiency ratio of Potchefstroom Koekoek cockerels fed diets containing graded levels of canola meal as partial replacements for soybean meal

	Time (weeks)											
Diets[1]	1	2	3	4	5	6	7	8	9	10	11	12
Control	0.32	0.27^{a}	0.21^{a}	0.20	0.20	0.20	0.20	0.11	0.15	0.12^{ab}	0.11	0.11
CM37.5	0.30	0.34^{b}	0.25^{ab}	0.17	0.22	0.16	0.20	0.14	0.16	0.15^{b}	0.12	0.12
CM62.5	0.34	0.31^{ab}	0.30^{c}	0.18	0.20	0.16	0.20	0.11	0.17	0.13^{ab}	0.11	0.09
CM87.5	0.35	0.28^{a}	0.24^{a}	0.24	0.21	0.21	0.21	0.18	0.21	0.12^{ab}	0.11	0.11
CM175	0.34	0.30^{ab}	0.21^{bc}	0.23	0.23	0.16	0.22	0.12	0.14	0.10^{a}	0.13	0.09
SE	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.01

^{abc}Means within a row that do not share a common superscript differ significantly (P<0.05).

SBM= soybean bean meal; CM= canola meal; [1]Diets: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM.

4.3.3 Haematology

Diet significantly affected (P<0.05) all haematological parameters except erythrocyte counts, haemoglobin, haematocrit, monocytes, eosinophils and basophils (Table 4.3). Cockerels fed control (22.7 x 10^9/L), CM37.5 (16.8 x 10^9/L), CM62.5 (19.7x10^9/L) and CM175 (16.7 x 10^9/L) diets had higher (P<0.05) leucocyte counts compared to PK cockerels fed diet CM87.5 (14.3 x 10^9/L). Cockerels fed diet CM175 had the highest (P<0.05) neutrophil counts. On the other hand, the highest (P<0.05) lymphocyte counts were observed in cockerels fed diet CM62.5 (90.40%). However, cockerels fed diets CM37.5 (5263.3), CM87.5 (5934.5 /100WBC) and CM175 (4868.6/100WBC) had the highest (P<0.05) normoblast counts, whilst those fed control (3537.1/100WBC) and CM37.5 (4032.0/100WBC) diet had the lowest (P<0.05). No differences (P>0.05) were observed in erythrocyte counts, haemoglobin, haematocrit, monocytes, eosinophils and basophils of cockerels fed all treatment diets.

4.3.4 Serum biochemistry

The results showed that inclusion of CM in Potchefstroom Koekoek diets had no effect on serum bilirubin, alanine transaminase (ALT), aspartate transaminase (AST) and serum total protein, sodium, potassium, albumin, chloride, urea, creatinine, calcium total, magnesium and triglyceride concentration (Table 4.4).

Table 4.3 Haematological parameters in 18-week old Potchefstroom Koekoek cockerels fed graded levels of canola meals as partial replacements for soybean meal (mean ± SE)

	Diets[1]				
Parameter	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**
Erythrocytes count (x 10^{12}/L)	2.67 ±0.18	2.61 ±0.26	2.97 ±0.26	2.93 ±0.18	3.06 ±0.21
Haemoglobin (g/dL)	10.11 ± 0.43	10.38 ±0.46	10.70 ± 0.51	10.93 ±0.41	10.78 ±0.43
Haematocrit (L/L)	0.39 ±0.01	0.38 ± 0.01	0.39 ± 0.02	0.39 ±0.02	0.39 ± 0.02
Leucocytes count (10^9/L)	22.65 ±2.33^{b}	16.81 ±2.52^{ab}	19.67 ±2.77^{ab}	14.33 ± 2.19^{a}	16.73 ±2.33^{ab}
Neutrophils (%)	5.71±2.23^{a}	9.33 ±2.42^{ab}	2.40 ±2.65^{a}	5.00 ± 2.10^{a}	13.70±2.24^{b}
Lymphocytes (%)	84.57 ±2.78^{ab}	78.67 ±3.01^{a}	90.40 ±3.27^{b}	86.50 ± 2.60^{a}	78.29±2.78^{a}
Monocytes (%)	7.42 ±1.86	9.33±2.00	4.80±2.20	5.00±1.74	5.71±1.90
Eosinophils (%)	0.5 ±0.70	1.33±0.80	0.80±0.82	1.00±0.82	1.14±0.70
Basophils (%)	1.71±1.05	1.33±1.14	1.60±1.24	2.50±0.98	1.14±1.05
Normablasts (/100 /WBC)	3537.14±610.65^{a}	5263.33±659.58^{ab}	4032.00±722.53^{a}	5934.50±571.21^{b}	4868.57±610.65^{ab}

^{abc}Means within a row that do not share a common superscript differ significantly (P<0.05)

SBM= soybean bean meal; CM= canola meal; Diets[1]: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM; g/dL=gram/decilitre; L= litre; WBC=white blood cell.

Table 4.4 Serum biochemistry parameters in 18-week old Potchefstroom Koekoek cockerels fed graded levels of canola meal as partial replacements for soybean meal

	Diets[1]					
Parameter	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**	**SE**
Bilirubin (umol/L)	0.71	0.63	0.60	0.60	0.67	0.16
ALT (IU/L)	0.40	0.60	0.40	0.40	0.40	0.28
AST (IU/L)	182.8	196.8	193.4	187.8	199.8	7.60
Total protein (g/L)	40.80	43.20	43.60	39.60	43.80	1.85
Sodium (mmol/L)	153.6	152.2	154.0	152.8	153.2	1.12
Potassium (mmol/L)	4.57	4.93	3.85	4.40	4.20	0.49
Albumin (g/L)	15.80	15.60	17.20	15.80	16.20	0.67
Chloride (mmol/L)	114.0	113.8	114.4	113.6	114.0	0.93
Urea (mmol/L)	0.40	0.29	0.33	0.41	0.27	0.06
Creatinine (umol/L)	18.00	18.00	18.00	18.00	18.00	0.00
Calcium total (mmol/L)	2.93	2.84	2.79	2.75	2.79	0.69
Magnesium (mmol/L)	1.12	1.11	1.20	1.13	1.09	0.04
Triglycerides (mmol/L)	0.97	0.75	0.73	0.72	0.67	0.10

[abc]Means within a row that do not share a common superscript differ significantly (P<0.05).

SBM= soybean bean meal ; CM= canola meal; Diets[1]: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM; AST = aspartate transaminase; ALT = Alanine Transaminase; IU/L = International units/litre; mmol/l = millimoles /litre; umol/L = Micromole/litre; g/L = gram/litre

4.4 Discussion

4.4.1 Growth performance

Although many studies have been conducted to evaluate the effect of dietary CM on growth performance of chickens, the results have not been conclusive. Moreover, it appears that no studies have been done on the inclusion of CM in diets for indigenous chickens. The observation that the inclusion of CM in PK cockerels' diets had no effects on AWFI, AWG and FCR compares well with published reports (Thacker & Petri, 2011; McNaughton *et al*., 2014; Gopinger *et al*., 2014; An *et al*., 2016). These workers found no statistical difference in AWFI, AWG and FCR of broilers when up to 20% CM was included as protein source in broiler finisher diets. This results imply that 17.5% CM can substitute SBM as protein source in diets for indigenous chicken.

Given that PK chickens are better adapted to non-conventional feed resources with relatively high levels of fibre than broilers used in previous studies; it comes as no surprise that their performance was not negatively affected by CM inclusion. Literatures also reported no significant difference in feed intake observed when up to 20% CM was included in broiler diets (Ahmed *et al*., 2015; Naseem *et al*., 2006). Nevertheless, the lack of differences in growth parameters in the current study contrasts with findings by Borcea *et al*. (1996) who found that addition of 7 and 10% CM negatively affected broiler daily weight gain and FCR, resulting in a significant body weight depression. The difference in the present study's findings on AWFI and AWG to these reports could be due to strain and age differences in the chickens used.

Feed intake is directly proportional to the age of the birds (Sohail *et al*., 2013). Ferket *et al*. (2006) observed that chicken increased their feed intake as the amount of limiting nutrients in the feed decreased in an attempt to obtain more of these nutrients to satisfy their requirements. The increase in AWG with age of chickens in all treatment diets could be attributed to age-related increase in nutrient requirements as well as the improved ability of PK cockerels to utilise more nutrients in the CM-based diets despite the high fibre and glucosinolates, which are regarded as growth suppressants.

4.4.2 Protein utilisation efficiency

The utilisation efficiency of dietary protein in the treatment diets, as indicated by PER in the present study is lower than the values reported by Ngworgu & Fasogbon (2007). However, the observation that PER decreased with increasing weeks is in line with the findings of Kamran *et al*. (2008), who reported a linear reduction in PER during the grower to finisher stages. The decline of PER with age is explained by the increase in feed intake. The other reason could be that as chicken grow (ages) less protein is needed to build the body resulting in high protein being wasted through faeces hence low PER. Protein efficiency ratio is affected by the levels of protein, digestibility and the level of essential amino acids (first limiting amino acids) (Maina, *et al*., 2007).

Across the CM inclusion levels, dietary protein content did not differ between diets and the digestibility of protein in control (SBM) and CM-based diets was similar. Therefore, similar PER for PK cockerels across all treatment diets likely resulted from similar amino acids levels (lysine and methionine) between CM-based diets and the control (SBM). Highly digestibly protein and

low protein diets maximise PER value (Widyaratne & Drew, 2011). In this case, it is clear that CM can be used to replace SBM in indigenous chicken diets at levels up 175 g/kg without any detrimental effects on PER, if diets are formulated to be equal in metabolisable energy and digestible amino acids.

4.4.3 Haematology

Haematological parameters are good indicators of the physiological status of animal and their variation is used to assess the response to various physiological situations (Ologhobo *et al*., 2013). Khan & Zafar. (2005) highlighted that haematological parameters can also be used to determine the response of animals to the diet they are fed. The normal ranges of haematological parameters vary with animal *spp* (Etim *et al*., 2014). The normal ranges of the haematological parameters of poultry are as follows: erythrocytes 2.5-3.5 $x10^{12}$/L, haemoglobin 7-13 g/dL, Haematocrit 0.22 - 0.35 L/L, leucocytes count 12 -30 $x10^{9}$/L, neutrophils 0-4%, lymphocytes 54-73%, monocytes 5-10%, eosinophils 1.5-6.0 % and basophils 1-5% (Jain, 1993). All the values obtained in this study fell within these normal ranges. Therefore, it can be reveals that CM provided sufficient quality dietary protein which resulted in optimum concentration of haematological parameters hence it can be used to replace SBM without any negative effects on health status of the chickens.

The result of this study showed no significant differences for most of the parameters measured. This is consistent with findings by Ramesh *et al*. (2006) and Woyengo *et al*. (2011). The results indicated that most of parameters measured fell within the normal reference range for healthy chickens (Mitruka & Rawsley, 1977; Jain, 1986). However, the erythrocytes count and Hb

linearly increased as the CM inclusion levels increased in the diets. This suggest that diets with CM stimulates blood formation. The high weekly body weight gain observed in cockerels fed CM-based diets may be a sign of high digestibility of protein as well as the role of plant bioactive compounds with nutraceutical properties which protect the cockerels against infectious disease (Cencic & Chingwaru, 2010). Amino acids regulate the activation of T and B lymphocytes (Li *et al.*, 2007) hence improve immune response of the chickens. Indeed, Das *et al*., 2012 confirmed that nutraceutical include isolated nutrients like vitamins, minerals, amino acids and fatty acids.

The increase in red blood cell count implies an increase in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs. Hacbeth *et al*., (1983) reported that higher RBC values are associated with high-quality protein in the diets and disease-free animals. Leucocytes in the chicken body provide a quick response to the area where there is serious infection and inflammation and provide against de rapid and potent defence against infectious disease. In the present study leucocytes counts (14.33-22.65 x 10^9/L) fell within the normal physiological ranges of 12-30 x 10^9/L (Bounous & Stedman, 2000; Kececi & Col, 2011)

4.4.4 Serum biochemistry

Diets in the present study did not significantly affect serum biochemical parameters. The values of ALT obtained in the present study were within the range of 0-10 IU/L, which is considered to be normal for chickens (Schmidt *et al*., 2007). The elevation of ALT implies cell damage (nonspecific) and it is present in most tissues but higher in RBC than in plasma. The AST, which

is found more in liver cells and less in kidneys, was numerically higher in CM-based diets compared to control but fell within the normal range of 70-220 U/L (Meluzzi *et al*., 1992). These results are in line with reported AST and ALT values in quails fed diets containing up to 30% of major source as CM (Saki *et al*., 2017**).** Therefore, the results indicated that inclusion of CM up to 17.5% had no toxic effects within the liver of the cockerels hence CM has a potential to be used as an alternative protein source to replace SBM.

Elevation of liver enzymes may indicate liver cell damage (Ghasemi *et al*., 2013**)**. Serum AST activity begins to rise during the pre-haemolytic periods (Thompson & Todd, 1974). Indeed, the consumption of CM, which contains glucosinolates, can lead to increased metabolic activity in the liver and kidney, leading to hyperplasia, hypertrophy and necrosis of cells in these organs (Tripathi & Mishra, 2007). On the other hand, Djuricic *et al*. (2011) highlighted that high activities of ALT and AST occur as a result of accelerated muscular tissue turnover. The present result signified that the health of PK cockerels was not negatively affected by inclusion of CM in the diets.

Albumin transports protein and regulates osmotic pressure in birds. The values of total protein content obtained in the current study fell within the normal range of 30 – 50 g/L for the majority of birds' species (Lloyd & Gibson, 2006; Nazifi *et al*., 2012). A higher concentration of albumin and total protein is associated with dehydration whereas lower concentrations are associated with malnutrition and infections (Esubonteng *et al*., 2011). Iyayi (1998) also noted that the levels of total protein and creatinine depend on the quality and quantity of dietary protein ingested. Thus,

the similar total protein and albumin concentration observed in this study could be an indication of similar quality and quantity of protein in the test diets as indicated by similar PER.

The values for serum triglycerides were 0.97, 0.75, 0.73, 0.72 and 0.67 mmol/L for cockerels fed control, CM37.5, CM62.5, CM87.5 and CM175 diets, respectively. Despite the lack of significant differences (P>0.05) on treatment diets in serum triglycerides concentration, cockerels fed on control diets had a numerically higher level of triglycerides compared to other treatment diets. Richard & Forgos (2011) reported triglycerides as major source of energy for tissue functioning because of its long chain of high-energy fatty acid. Similar to current findings, Payvastegan *et al*. (2013) did not find significant variation in broiler serum triglycerides when CM was included in diets fed to at 10% and 20% levels. Kamran Azad *et al*. (2009) reported similar results when canola seed was included at 15% in broiler diets.

The PK cockerels fed diets with CM had the same levels of creatinine, which indicates equal renal functioning and protein muscular metabolism. Same levels of creatinine were reported by Szymeczko *et al*. (2010) when 18% of rapeseed was included in broiler grower diets. The values of serum sodium, potassium, calcium, magnesium and chloride in the present study (Table 5.2) were similar between PK cockerels fed CM-based diets and those on the control diet. This is in agreement with several published reports (Bowers *et al*., 1989; Szymeczko *et al*., 2010; Payvastegan *et al*., 2012; Payvastegan, *et al*., 2013; Saki *et al*., 2017). These researchers reported similar range of blood minerals in different poultry species. This reveals that both CM and SBM are rich source of these minerals. Indeed, it was documented that CM is relatively good source of essential minerals (CCC, 2009).

4.5 Conclusions

Potchefstroom Koekoek cockerels fed on CM-based diets containing up to 175 g/kg inclusion level performed equally well compared to PK cockerels fed on control diets throughout the entire experimental period. No significant negative effects of graded levels of CM were observed on feed intake, weekly body weight gain, FCR, PC and PER of PK cockerels. Inclusion of CM in diets had no effect on haematology and serum biochemistry parameters. These findings indicate that CM can be used as an alternative dietary protein source and in commercial grower diets for indigenous cockerels with no negative effects on production and health. The effects of CM inclusion in diets on growth performance can also translate to the effects on the final product, which is meat. It is, therefore, necessary to assess the effect of CM inclusion in diets on carcass characteristics and meat quality of PK chickens to ensure that no negative effects are imparted to the final product.

4.6 References

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CHAPTER 5 - REPLACEMENT OF SOYBEAN MEAL WITH CANOLA MEAL IN POTCHEFSTROOM KOEKOEK COCKEREL DIETS: CARCASS CHARACTERISTICS, VISCERA MACROMORPHOMETRY AND MEAT QUALITY

Abstract

This study was conducted to evaluate the effects of a partial replacement of the soybean meal (SBM) with graded levels of canola meal (CM) on carcass characteristics, viscera macromorphometry and meat quality of the Potchefstroom Koekoek (PK) cockerels. A total of 175, 5-week old PK cockerels were randomly allotted to the following isocaloric and iso-nitrogenous treatment diets; Control = diet with no canola meal inclusion, CM37.5 = 37.5 g canola meal/kg soybean meal, CM62.5 = 62.5 g canola meal/kg soybean meal, CM87.5 = 87.5 g canola meal /kg soybean meal, CM175 = 175 g canola meal/kg soybean meal. Completely randomise design (CRD) was used and data were analysed using the SAS (SAS, 2007). Treatment means were separated using probability of difference (PDIFF. The experimental diets were initial fed from week six until seventeen of age after which the PK cockerels were humanely slaughtered. Meat portions, internal organs and meat quality parameters were measured. Cockerels fed diet CM37.5 had the highest ($P<0.05$) hot carcass weights**.** The Potchefstroom Koekoek cockerels fed control diet had the lowest ($P<0.05$) breast muscle, wing, drumstick and vertebrae weight. The PK cockerel fed control diet had the lowest ($P<0.05$) heart (22.20g) and liver weights (7.9 g). Higher ($P<0.05$) small intestine weights were observed on the PK cockerels fed diet CM175. The L* value (51.40) and b* value (11.00) of the breast muscle from the PK cockerels fed diet CM37.5 were significantly higher than those fed the control diet (50.93 (L*) and 9.50 (b*)). The redness (a* value) of the breast muscle increased significantly with the inclusion levels of CM. The pH_u (Ultimate pH) values of the meat from PK cockerels

fed diet CM37.5 (5.97) were significantly lower ($P<0.05$) than that of the cockerels fed control diet (6.18). However, the PK cockerels fed the control and CM37.5 diets had the lowest shear force values. Breast muscle of cockerels fed the control diet had the highest ($P<0.05$) concentration of Ca, Mg, P, Na and K. From the results, it can be concluded that inclusion of CM in the diet increase the carcass and meat portion weights but raised the shear force value. Hence, the SBM in the PK diets can be substituted with up to 175 g/kg CM without negatively affecting the carcass characteristics, viscera macromorphometry and most meat quality parameters except meat texture.

Keywords: Canola Meal, Potchefstroom Koekoek cockerels, colour, drip loss, cooking loss.

5.1 Introduction

The main reason for rearing indigenous chicken is to produce birds that provide a ready source of protein mainly for rural consumers and thus contributing to food security (Neggese & Melesse, 2009). The meat from indigenous chickens is lean with low intramuscular fat (therefore healthier) and is highly accepted by consumers compared with meat from commercial broilers. Additionally, the meat is perceived to be tastier and juicier with great flavour (Amsalu, 2003; Roberts, 1999). However, there are many factors that may affect quality of the meat among others nutrition, breed, age and processing (Onyimonyi & Ernest 2009). Teye *et al*. (2011) demonstrated that the type of feed given to an animal has significant effects on the carcass and organoleptic qualities of meat. It is, therefore, expected that dietary composition may influence muscle growth and ultimately meat quality.

Poultry diets are formulated from a mixture of ingredients, including cereal grains, cereal by-products, fats, plant derived protein sources, vitamin and mineral supplements, crystalline amino acids and feed additives. The aforementioned ingredients affect the quality of the meat from the birds. Feeding diets with a high energy and protein was reported to result in an improved carcass yield and it also decreased lipid content (Hess, 2004). Waldroup *et al*. (2001) indicated that increasing dietary crude protein increased the content of protein and amino acid in the broiler carcasses. Soybean meal (SBM) has been extensively used as the conventional protein source when compounding poultry feeds. However, SBM is becoming expensive mainly because of the competition between humans and livestock. This calls for exploration of alternative protein sources such as canola meal.

In Chapter 4, it was observed that dietary had improved growth performance, protein utilisation and had no negative effects on the general health of chickens. It can, therefore, be postulated that CM inclusion in diets may affect the carcass characteristics and meat quality of PK cockerels. Currently, there is little information on the influence of canola meal inclusion in indigenous chicken diets on meat quality. The present study was, therefore, conducted to investigate the effect of partially replacing SBM with graded levels of canola meal in PK cockerel diets on carcass characteristics, size of internal organs and meat quality. It was hypothesised that partially replacing SBM with graded levels of canola meal in PK cockerel diets does not have any effect on carcass characteristics, size of viscera macromorphometry and meat quality

5.2 Materials and methods

5.2.1 Study site, management of chickens, diet formulation and experimental design

The study was carried out at the North-West University Research and Teaching Farm at Molelwane, whose location and climatic conditions are described in detail in Chapter 3, section 3.2.3. The management of chickens, diet formulation and experimental design were as described in detail in Chapter 4, under section 4.2.2, 4.2.3 and 4.2.4, respectively.

5.2.2 Slaughtering protocol

After the 12-week feeding trial (Chapter 4), the PK cockerels were slaughtered to assess the carcass characteristics, size of internal organs and meat quality. Feed was withdrawn on the night before the day of slaughter to empty the digestive tract. The body weights of the chickens were measured before slaughtered to obtain the slaughter weight (SLW). The birds were placed in the crates and transported to Rooigrond slaughter house (Rooigrond, Mafikeng, South Africa) where

there were slaughtered. The birds were slaughtered humanely by electrical stunning and then killing by cutting jugular vein for bleeding.

5.2.3 Determination of carcass characteristics

Immediately after slaughter, the feathers were plucked and gastro intestinal tract (GIT) were removed. The carcasses were then weighed to obtain the hot carcass weight (HCW) of dressed birds. Hot carcass yield was calculated by dividing hot carcass weight by pre-slaughter weight. The carcasses were then chilled at 4 ºC for 24 hours and the cold carcass weights (CCW) were recorded. The carcasses were packed in polythene bags and stored in a freezer ($\pm 4^0$C) pending meat quality evaluation.

$$Hot\ carcass\ yield\ (\%) = \frac{Hot\ carcass\ weight}{slaughter\ weight} \times 100$$

$$Dressing\ percentage\ = \frac{Carcass\ weight}{Body\ weight} \times\ 100$$

Three birds per replicate were randomly selected for determination of carcass characteristics and meat quality. For the measurement of carcass cuts, head and shanks were removed close to the scull and at hock joint, respectively and were the heads and shanks weighed. Wings were removed by cutting at the humeoscapular joint, the cuts were made through the rib head to the shoulder girdle and the vertebrae was then removed intact by pulling interiorly (Alikwe *et al*., 2011). The breast muscle, wings, shank, thighs and vertebrae (back) were each weighed separately. The weights of the GIT and other viscera including the liver, gizzard and heart were recorded.

Breast muscle and drumstick ratio were computed using the following equation:

$$Breast\ muscle\ percent = \quad \frac{Breast\ muscle\ (g)}{Hot\ Carcass\ weight\ (g)} \times\ 100$$

$$Drumstick\ percent = \frac{Drumstick\ (g)}{Hot\ Carcass\ weight\ (g)} \times 100$$

Ten breasts were randomly selected from each treatment group (total of 50 samples each) for meat quality evaluation...

5.2.4 Meat colour and pH measurement

The meat colour was measured immediately after slaughter and 24 hours post slaughter. The colour (L* = luminosity, a* = Red colour intensity and b* = Yellow colour intensity) was measured on the breast muscle (BM) using a spectrophometer (CM 2500c model, Konica Minolta, Inc. Japan). The three distinct points of the breast muscle were measured by rotating the spectrophometer between each measurement to obtain a mean value of the colour. A portable digital pH meter (CRISON pH25, CRISON Instruments SA, Spain) with a piercing electrode was used to measure the pH of breast muscle (BM) of each individual bird at 45 minutes for initial pH (pH_i) and 24 hours post slaughter to obtain the ultimate pH (pH_u) (Sanka & Mbaga, 2014).

5.2.5 Water holding capacity

The pressure method as described by Delezie.*et al*., (2002) was used to determine the water holding capacity (WHC). About 100 g meat sample from pectoral major (PM) was cut and weighed to obtain the initial weight using a digital scale sensitive up to 0.01 g. The meat sample was then placed in between 2 filter papers, placed on a flat surface and approximately 60 kg weight was applied on the sample for 5 min. Thereafter, the meat sample was re-weighed. The WHC was calculated as the ratio of the amount of water retained over the initial sample weight.

5.2.6 Drip loss

Approximately 30g meat strips were sampled from the breast muscle parallel to the fibre direction then weighed to get the wet sample using a digital scale sensitive to 0.01 g. The samples were suspended inside a plastic container and sealed under atmospheric pressure. The samples were then held at 2^0C for 72 hours after which they removed from the container. The samples were blotted with paper towels to remove excess surface moisture and were then re-weighed. The drip loss was then calculated by subtracting the blotted sample weight from the initial sample weight. The drip loss was expressed as a percentage of the initial sample weight (Honikel & Hamm, 1994).

5.2.7 Cooking loss measurement

Raw meat cubes were cut from the breast muscle, weighed *in natura*, then placed in a plastic bag and cooked in a water bath at 75 °C for 45 minutes (Rizz *et al*. 2007). The samples were then cooled in running water for 15 min, dried with soft tissue and weighed (Sanka & Mbaga, 2014). Cooking loss was calculated as percentage loss of weight during cooking relative to the weight of raw muscle (Gopinger *et al.,* 2014) according to the following formula:

$$Cooking\quad loss\,(\%) \quad = \quad \frac{Weight\quad before\quad cooking\quad -\quad Weight\quad after\quad cooking}{Weight\quad before\quad cooking} \times 100$$

5.2.8 Tenderness

Breast muscles were wrapped in aluminium foil and baked to reach an internal temperature of 85°C, which temperature was then maintained for 30 minutes. Smaller samples were then cut parallel to the muscle fibres with the aid of a Meullenet - Owens Razor Shear Blade (A/MORS)

with a diameter of 1.2 cm. The shear force was recorded using the Texture analyser (TA XT plus).

5.2.9 Mineral content of meat

The calcium (Ca), magnesium (Mg), potassium (K), sodium (N), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) content was analysed using ICP mass spectrometer from Perkin Elmer supplier in animal health centre laboratory (North-West University, Mafikeng. South Africa). Phosphorus was determined calorimetrically using sodium phenol and ammonium molybdate plus ascorbic acid method (AOAC, 2012, method number. 976.06)

5.2.10 Statistical analyses

Data on carcass characteristics, internal organs and meat quality parameters were analysed using one-way analysis of variance as contained in PROC GLM of SAS (2010) according to the following general linear model:

$$Y_{ijk} = \mu + d_i + E_{ijk}$$

Where Y_{ijk} = response variable, μ = general mean, d_i the fixed effects of canola levels. E_{ij} = random error associated with observation ijk = assumed to be normally and independently distributed. When the analysis of variance revealed the existence of significant difference among treatment means, the probability of difference (PDIFF) option in the LSMEANS statement of the GLM procedure of SAS (2007) was used to separate means. The level of significance was set at $p<0.05$.

5.3 Results

5.3.1 Carcass traits and weight of external components

The effects of feeding graded levels of CM as partial replacement for SBM on PK cockerel's carcass traits and external organ weights are presented in Table 5.1. Cockerels fed diet CM37.5 had the highest ($P<0.05$) hot carcass weights. Higher ($P<0.05$) hot carcass yield (HCY) were observed on carcasses of PK fed diet CM175 compared to those fed the control and other treatment diets. Lower ($P<0.05$) shank weight and length were observed in cockerels fed control diet compared to other diets. Potchefstroom Koekoek (PK) cockerels fed control diets had the lowest ($P<0.05$) breast muscle, wing, drumstick and vertebrae weights. There was an increase in weights of the cuts as the inclusion level of CM increased. Similarly, breast muscle ratio and drumstick muscle ratio increased ($P<0.05$) with an increase dietary CM. Diet had no significant effects ($P>0.05$) on dressing percentage and cold carcass weight (CCW) of the carcass from the PK cockerels.

5.3.2 Viscera macromorphometry

Diet significantly affected viscera macromorphometry of the PK cockerels (Table 5.2). The PK cockerels fed the control diet had the heaviest ($P<0.05$) heart (7.9 g). There was a linear increase in heart weight of cockerels as inclusion levels of CM increased. Cockerels fed diet CM175 had the heaviest ($P<0.05$) livers. Significantly higher ($P<0.05$) small intestine weights were realised from PK cockerels fed CM175 diet (136.6 g) compared to those fed control diet (124.5 g). Dietary had no effects on the weight of the spleen, proventriculus, gizzard, caeca and the length of small intestine.

Table 5.1 Effects of substitution of soybean meal with graded levels of canola meal on carcass traits and weight of external organ in 18-week old Potchefstroom Koekoek cockerels (mean ± SE)

	Diets[1]				
Carcass traits	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**
Slaughter weight (g)	1788.98 ± 49.85	1931.08 ± 49.85	1892.97 ± 49.85	1882.00 ± 49.85	1882.68 ± 49.85
Hot carcass weight (g)	1236.61 ± 31.04^{a}	1297.17 ± 29.03^{b}	1245. 44± 28.45^{a}	1237.56 ± 27.37^{a}	1248.75 ± 29.00^{a}
Cold carcass weight (g)	1201.86 ± 26.96	1263.54 ± 25.22	1233.52 ± 24.72	1213.74 ± 23.78	1236.58 ± 25.22
Hot carcass yield (%)	67.17 ± 1.65^{a}	67.15 ± 1.55^{a}	65.16 ± 1.52^{a}	65.75 ± 1.46^{a}	69.80 ± 1.55^{b}
Cold carcass yield (%)	64.18 ± 1.43	65. 40 ± 1.33	65.32 ± 1.31	64.49 ± 1.21	65.68 ± 1.34
Shank length (cm)	9.60 ± 0.22^{a}	10.04±0.22^{ab}	10.06 ±0.22^{a}	10.30 ±0.22^{b}	10.55 ± 0.22^{b}
Shank weight (g)	81.60 ± 2.70^{a}	89.30 ±2.70^{b}	81.80 ± 2.70^{ab}	84.90 ± 2.70^{ab}	82.60 ± 2.70^{ab}
Wing weight (g)	75.80 ± 2.94^{a}	93.60 ± 2.94^{b}	93.50 ±2.94^{b}	87.30 ± 2.94^{b}	92.70 ± 2.94^{b}
Breast muscle weight (g)	155.10 ± 13.94^{a}	218.10 ± 13.94^{b}	203.10 ±13.94^{b}	178.50 ± 13.94^{ab}	194.90 ±13.94^{b}
Breast muscle ratio	12.42 ± 1.00^{a}	16.81 ± 1.00^{b}	16. 42 ± 1.00^{b}	14.42 ± 1.00^{ab}	17.01 ± 1.00^{b}
Drumstick weight (g)	207.20 ± 6.4^{a}	241.60 ± 6.41^{b}	238.90 ± 6.41^{b}	240.80 ± 6.41^{b}	239.00 ±6.41^{b}
Drumstick ratio	16.59 ± 0.51^{a}	18.63 ± 0.51^{b}	19.32 ± 0.51^{b}	17.03 ± 0.51^{a}	19.19 ± 0.51^{b}
Vertebrae weight (g)	149.00 ±9.51^{a}	184.90 ± 9.51^{b}	186.30 ± 9.51^{b}	167.90 ±9.51^{ab}	185.30 ±9.51^{b}

^{abc}Means within a row that do not share a common superscript differ significantly (P<0.05).

SBM= soybean bean meal; CM= canola meal; Diets[1]: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM.

Table 5.2 Effects of substitution of soybean meal with graded levels of canola meal on visceral weight and length in 18-week old Potchefstroom Koekoek cockerels

	Diets[1]					
Internal organ	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**	**SE**
Heart (g)	7.90[a]	9.40[bc]	10.00[c]	8.60[ab]	9.20[bc]	0.44
Liver (g)	22.20[a]	24.60[ab]	24.70[ab]	22.90[a]	26.20[b]	0.62
Spleen (g)	3.90	4.20	4.20	3.90	3.60	0.34
Proventriculus (g)	8.70	9.70	9.40	8.50	8.20	0.72
Gizzard (g)	32.20	36.00	31.00	30.50	33.90	2.26
Caeca weight (g)	8.10	7.50	8.70	11.30	11.00	1.38
Caeca length (cm)	16.75	17.00	17.10	17.20	30.50	5.90
Small intestine weight (g)	124.45[a]	134.00[b]	130.10[ab]	132.35[ab]	136.60[b]	3.30
Small intestine length (cm)	32.40	32.90	32.75	32.45	34.10	1.66

[abc]Means within a row that do not share a common superscript differ significantly (P<0.05).

SBM= soybean bean meal; CM= canola meal; Diets[1]: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM.

5.3.3 Meat quality Traits

The effect of CM inclusion on meat colour, pH, water holding capacity, drip loss, cooking loss and shear force values of PK cockerel breast muscle is shown in Table 5.3. The lightness (L* value) of the breast muscle from PK cockerels fed the CM37.5 diet (51.40) was significantly higher than that for PK cockerels fed the control diet (50.93). There were no significant differences in the lightness (L* value) of breast muscle from cockerels fed control, CM62.5, CM87.5 and CM175 diets. The redness (a* value) of breast muscle from PK cockerels increased significantly with an increase in dietary CM such that the cockerels fed on the diet with the highest inclusion level (CM175) resulted in the breast meat having the highest value of redness (5.15). The yellowness (b* value) of the breast muscle from PK cockerels fed diet CM37.5 was significantly higher than those of PK cockerels fed the other diets.

The meat pH decline was more pronounced in the breast muscle of cockerels fed diet CM37.5, which had the lowest breast muscle ultimate pH (pH_u). Chickens fed diets CM62.5 and CM175 had similar ($P > 0.05$) shear force values, which were the highest ($P < 0.05$. There was an increase in drip loss with the increase in CM such that meat from cockerels reared on the control diets had the lowest drip loss (5.36 ± 2.83%) while those reared on diet CM175 had highest value (7.65 ± 2.83%). Across dietary treatment there were no significant difference in the WHC and cooking loss of the meat.

Table 5.3 Effects of substitution of soybean meal with graded levels of canola meal on meat quality of 18-week old Potchefstroom Koekoek cockerels (mean ± SE)

	Diets[1]				
Instrumental measurements	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**
Meat colour					
L*(lightness)	50.93 ± 1.00[a]	51.40 ± 1.00[b]	48.86 ± 1.00[a]	49.10 ± 1.00[ab]	48.46 ± 1.00[a]
a*(redness)	3.59 ± 0.33[a]	5.09 ± 0.33[b]	4.66 ± 0.33[ab]	4.52 ± 0.33[ab]	5.15 ± 0.33[b]
b* (yellowness)	9.50 ± 0.55[a]	11.00 ± 0.55[ab]	10.70 ± 0.55[a]	10.86 ± 0.55[a]	12.00 ± 0.55[b]
pH_0	7.10 ± 0.04[a]	7.10 ± 0.04 [a]	7.14 ± 0.04[a]	7.20 ± 0.04 [ab]	7.25 ± 0.04 [b]
pH_u	6.18 ± 0.05[b]	5.97 ± 0.05[a]	6.07 ± 0.05[ab]	6.11 ± 0.05[b]	6.19 ± 0.05[b]
Water Holding Capacity (%)	20.17 ± 2.15	23.10 ± 2.15	20.10 ± 2.15	20.92 ± 2.15	18.72 ± 2.15
Drip loss (%)	5.36 ± 2.83	5.70 ± 2.83	6.61± 2.83	6.78 ± 2.83	7.65 ± 2.83
Cooking loss (%)	22.72 ± 11.7	21.20 ± 10.32	16.19 ± 10.94	17.79 ± 12.64	24.98 ± 12.64
Shear force (N)	13.50 ± 1.56[a]	13.93 ± 1.37[a]	18.33 ± 1.46[b]	15.11 ± 1.69[ab]	19.89 ±1.85[b]

[abc]Means within a row that do not share a common superscript differ significantly (P<0.05).

SBM= soybean bean meal; CM= canola meal; Diets[1]: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM.

5.3.4 Macro minerals content of meat

Diet had a significant effect on the macro mineral content of breast muscle of PK cockerels (Table 5.4). The breast muscle of cockerels fed control diet had the highest ($P<0.05$) concentration of Ca followed by the breast muscle of cockerels fed diets CM175, CM37.5, CM62.5 and CM87.5. Cockerels fed diet CM175 had the highest ($P<0.05$) concentration of Mg in their breast muscle followed by the breast muscle of cockerels fed the control, CM37.5, CM62.5 and CM87.5 diets. Higher concentration ($P<0.05$) of P and Na was observed in the breast muscle of cockerels fed diet CM175 and the lowest in the breast muscle of cockerels fed diets CM37.5, CM62.5 and CM87.5. Cockerels fed diet CM175 had the highest ($P<0.05$) K concentration in their breast muscle followed by those fed diets CM37.5, CM62.5 and CM87.5.

5.3.5 Trace minerals content of meat

Diet also had a significant effect on trace mineral contents of breast muscle of PK cockerels (Table. 5.5). Cockerels fed diet CM37.5 (2.91 µg/mL) had the highest ($P<0.05$) concentrations of breast meat Fe followed by cockerels fed diets CM175 (2.30 µg/mL), control (1.78 µg/mL), CM87.5 (1.05 µg/mL) and CM62.5 (0.72 µg/mL) being the lowest ($P<0.05$). Higher concentration ($P<0.05$) of breast muscle Mn was observed in cockerels fed diet CM175 (0.04 µg/mL) and lowest in cockerels fed diet CM62.5 (0.006 µg/mL). The highest ($P <0.05$) Cu concentration (0.18 µg/mL) was observed in breast muscle of cockerels fed control diet while those fed diets CM37.5 (0.03 µg/mL) and CM62.5 (0.02 µg/mL) had the lowest ($P<0.05$) concentrations. The highest ($P<0.05$) Zn concentration was observed in the breast meat of cockerels fed diet CM175 (0.14 µg/mL) followed by those fed control diet (0.09 µg/mL) while

the lowest concentrations were observed in cockerels fed diets CM37.5 (0.03 µg/mL) and CM62.5 (0.02 µg/mL).

Table 5.4 Effects of substitution of soybean meal with graded levels of canola meal on macro minerals contents (µg/ml) of meat of 18-week old Potchefstroom Koekoek cockerels

	Diets[1]					
Macro elements	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**	**SE**
Calcium	45.01^{d}	16.59^{b}	12.05^{a}	10.11^{a}	26.81^{c}	0.96
Phosphorus	34.23^{b}	21.94^{a}	20.64^{a}	20.93^{a}	38.95^{c}	0.79
Magnesium	11.53^{c}	10.83^{b}	10.50^{b}	7.73^{a}	13.34^{d}	2.56
Sodium	44.16^{b}	33.98^{a}	31.48^{a}	33.28^{a}	49.99^{c}	0.10
Potassium	98.24^{d}	71.38^{b}	61.44^{c}	75.34^{a}	131.09^{e}	2.39

^{abc}Means within a row that do not share a common superscript differ significantly (P<0.05).

SBM= soybean bean meal; CM= canola meal[1]; Diets[1]: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM.

Table 5.5 Effects of substitution of soybean meal with graded levels of canola meal on trace minerals contents (µg/ml) of meat of 18-week old Potchefstroom Koekoek cockerels

	Diets[1]					
Trace elements	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**	**SE**
Iron	1.78[c]	2.91[e]	0.72[a]	1.05[b]	2.30[d]	0.11
Manganese	0.02[c]	0.02[c]	0.006[a]	0.013[b]	0.04[d]	0.0006
Copper	0.18[c]	0.03[a]	0.02[a]	0.12[b]	0.16[b]	0.004
Zinc	0.09[c]	0.03[a]	0.02[a]	0.07[b]	0.14[d]	0.003

[abc]Means within a row that do not share a common superscript differ significantly (P<0.05).

SBM= soybean bean meal; CM= canola meal; Diets[1]: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM.

5.4 Discussion

5.4.1 Carcass traits and cut measurements

The observation that hot carcass weights of PK cockerels fed diet CM37.5 were significantly higher than those fed the control diet could be due to higher slaughter weight. These results agree with published reports on quails (Karayagız & Bulbul, 2015) and in broilers (Naseem *et al*., 2006 and Ahmed *et al*., 2015). These published reports found higher hot carcass weight when CM was used in the diets as protein source. The digestive tract of indigenous chickens is adapted to feed with high fibre content compared to exotic breeds. Indeed, Mcainsh *et al*. (2004) has reported that indigenous chickens, by nature, are foragers with an ability to extract a considerable amount of nutrients from the high fibre diets (forages).

The lack of difference observed on cold carcass weight and yield on the PK cockerels agreed with previous reports on broiler chickens by Ahmed *et al*. (2015); Mushtaq *et al*. (2007); Kocher *et al*. (2001); Sarıçiçek *et al*. (2005); and Naseem *et al*. (2006). The reason for lack of difference in CCW while significant differences were observed in HCW could be explained by the differences in drip loss. Cold carcass yield has been found to be a good indicator of total edible meat after storage. Therefore, the results indicated that CM inclusion in grower diet did not reduce any edible meat of PK cockerels**.**

Lower shank weight and length in cockerels fed control diet compared to those fed the other diets were observed in the current study. These could be associated with the low slaughter weight of cockerels fed the control diet. Similar observations were reported by Okeudo *et al*. (2006) when palm kernel cake was used to replace SBM in diets of broiler chickens. However, this

observation contradicts previous findings by Gopienger *et al*. (2014), who noticed that the addition of CM had no adverse effects on shank weight. The discrepancy between the present study and previous findings may be explained by the type of birds (indigenous vs commercial broiler chickens). Indigenous chickens have a greater digestive capacity than broilers and hence may digest CM more efficiently thus enhancing mineral bioavailability.

Potchefstroom Koekoek (PK) cockerels fed the control diet had the lightest breast muscle, wing, drumstick and vertebrae weights. These could be associated with the low carcass weight of cockerels fed the control diet. Other studies also report no significant differences in breast weight percentage of broilers fed diets containing up to 20% CM (Thanaseelaan *et al*., 2008; Naseem *et al*., 2006). The low drumstick weight in PK cockerels fed control diet contradicts the findings of other studies (Laudadio & Tufarelli, 2010; Tadelle *et al*., 2003) in broiler chickens. The types of birds (indigenous vs. broiler chicken) used in the various studies might contribute to the variation in the results reported.

5.4.2 Visceral macromorphometry

The lack of a difference in weights of spleen, proventriculus, gizzard and caeca as well as small intestine length of cockerel fed diet with low levels of CM and those fed control diet (Table 5.2) is consistent with reports in literature (Karayagız & Bulbul, 2015; Naseem *et al*., 2006; Saricicek *et al*., 2005). Despite the fibre and glucosinolates present in CM-based diets, PK cockerels utilised these diets efficiently and to the same extent as the control diets. These indicates that PK cockerels can efficiently utilise the canola based diets irrespective of the high fibre contents in the diets.

The linear increased in the sizes of caeca (length and weight) and small intestines (length and width) of cockerels fed diets with the inclusion levels of CM can be attributed the need for chickens to increase their organs as an adaption mechanism to dietary fibre. Jorgensen *et al*. (1996) and Ahamed & Olorede (2003) reported that fibre in monogastric diets has mechanical effects on small intestine and cause the gastro-intestinal tract to enlarge and thicken. This adaptation mechanism to high fibre diets ensures that the digesta has a longer residence time in the GIT resulting in improved digestion. This agree with a study published by Ugwu & Onyimonyi. (2008) who found an increase in small intestine weight when Bambara nut sievate was used as a protein source for broiler chicken diets. However, Okeudo *et al*. (2006) contradict these findings reporting no statistical differences in small intestine weight when palm kernel cake (protein source) was used in finisher broiler diets. Differences in type of birds and diet composition might have contributed to this divergent report.

The increased heart and liver weight observed on PK cockerels fed diets containing CM may be related to the observed high slaughter weight and hence large heart and liver is needed to support large body mass. The other reason might be related to the liver damage and its hypertrophy caused by toxic effects of the hydrolytic products of glucosinolates (Wickramasuriya *et al*., 2015).

5.4.3 Meat quality Traits

Meat colour is one of the most important meat quality attributes and plays an important role on the acceptance of meat and consumer perception of the freshness of the products. Colour is influenced by genotype, feeding and age of the birds (Fletcher, 1999). In this study, the L*values

of the breast muscle were in the normal range of 48.46 to 50.93 and the meat would not be considered to be excessively pale (Laudadio & Tufarelli, 2010; Motsepe, 2016; Jaturasitha *et al*. 2008). Diet did not have any effects on lightness of the meat as observed in other studies (Gopinger *et al*., 2014; An *et al*., 2016). The L* value indicates the paleness of meat, with a high L* associated with poor meat quality and can be related to increasing moisture, glycogen, iron, ash and certain fatty acid ratios (Wattanachant *et al*., 2004). However, inclusion of CM at 17.5% resulted in increased moisture, glycogen, iron, ash and certain fatty acid ratios. The redness (a*) values ranged from 3.57 to 5.15 in the present study and were very much lower than 10.98 value obtained for PK males reported by Motsepe (2016). The yellowness (b*) values in the present study ranged between 9.50 and 12.00 and were lower than the 17.05 reported by Motsepe (2016) on PK male chickens.

The strong relationship between pH_0 and pH_u post-mortem is a result of the decline in pH from the time of slaughter to when there is post-mortem aging (24hrs post-mortem). The range is determined by how much glycogen is in breast muscle prior to slaughter and how rapidly the remaining glycogen is converted to lactic acid after slaughter (Dyubele *et al*., 2010). However, pH_{24} (pH_u) of the carcasses of PK cockerels were higher than the value (5.51) reported by Motsepe (2016) and Jaturasitha *et al*. (2008) on the meat of Black boned (5.88), Thai (5.77), Bresse (5.88) and Rhode (5.68) indigenous chicken of Thailand. Development of rigor mortis, pH and osmotic pressure and sarcomere length influences water holding capacity (WHC) by changing the cellular and extracellular components (Northcutts *et al*., 1994) and leads to improved muscle tenderness and appearance. Lopez *et al*. (2011) noted that a high WHC results in lower cooking loss and vice versa. Lack of significant difference effects in WHC and cooking

loss of the breast meat agrees with published reports (Gopinger *et al*., 2014; Moraes *et al*., 2016; Mikulski *et al*., 2012). This indicates that CM did not affect WHC and cooking loss of the meat hence more water was held between myofibrils and would be highly acceptable to the consumers.

Current results show that shear force values increased with CM inclusion meaning that the meat becomes tougher with increasing dietary CM. The significantly higher shear force value in chickens fed CM-based diets could be explained by the effects of diets on muscle deposition that cause an increase in muscle fibre and reduction of fat content in meat. Generally, meat tenderness is affected by marbling (Tang *et al*., 2007). Gopienger *et al*. (2014) noted that inclusion of CM up to 400 g/kg increased meat tenderness. Mikulski *et al*. (2012) reported a decrease in shear force values when 180 g/kg of rapeseed meal was added in grower turkey diets. However, Tuunainen *et al*. (2016) found no significant differences in tenderness when broilers were fed rapeseed meal. Results from the current investigation reveal that CM inclusion increases shear forces hence the meat might be less acceptable to the consumer.

5.4.4 Macro minerals content of meat

Diet has been reported to be an important factor affecting the mineral content of animal tissue (Lin *et al*. 1989). This study showed a significant effect of diet on the Ca, P, Mg, K and Na concentration of PK cockerel breast meat. As expected birds fed the control diet had significantly higher breast Ca and Mg. Zapata *et al*. (1998) found no significant difference in breast Ca and Mg when broilers were fed commercial diets with or without mineral supplementation. Manyeula *et al*. (2013) found no difference ($P<0.05$) in breast Ca in Tswana hens fed *Imbrasia*

belina (Westwood) or *Vigna subterranea* (l) Verde or *Tylosema esculentum* (Burchell) Schreiber as sources of protein. The observation that PK cockerels fed diet CM175 had higher breast P, Na and K concentration could be due to higher concentration of these minerals in the diet and ability of the birds to absorb and assimilate these minerals from CM-based diets.

5.4.5 Trace minerals content of meat.

The composition of zinc and manganese contents in the breast meat were higher in the meat of PK cockerels fed diet CM175 compared to those fed on other treatment diets. Zinc deficiency can lead to loss of appetite, growth retardation, skin changes and immunological abnormalities in humans (Wei *et al*., 2016). The zinc values reported in present study were lower than those reported in literature (Stef & Gergen, 2012). Manganese values in PK cockerel breast muscle in the current study were lower ($P<0.05$) than the value reported by Wei *et al*. (2016) in free-range broilers and Stef & Gergen (2012) in broilers.

Potchefstroom Koekoek cockerels fed diet CM3.75 had higher iron in the meat while PK cockerels fed diet CM6.75 had the lowest ($P<0.05$). Adequate iron in a diet is very important for decreasing the incidence of anaemia (Ghaedi *et al*., 2006). The Fe values reported in the current study were lower than those previously reported in free-range chickens (Wei, *et al*., 2016). The current results imply that breast meat from cockerels fed diet CM175 had higher breast Zn, Mn and Fe muscle contents. Cockerels fed diets CM62.5 and CM87.5 had significantly lower meat copper content compared to meat of those fed other treatment diets. In the current study copper content of meat was found to be lower than the one reported by Ferreira *et al*. (2005). In contrast, a higher value (2.96 μg/g) for meat Cu content was reported by Stef & Gergen (2012) in broiler

chickens fed commercial diets. The lower concentration of these trace mineral in breast meat of PK cockerels could be due to inadequate supply of these minerals in feed, or from their insufficient absorption and assimilation from the digestive tract due to anti-nutritional factors.

5.5 Conclusions

The results from the current study showed that carcass traits, external organs weight, viscera macromorphometry and meat quality from carcasses of PK cockerels fed CM-based diets compared well to those from the carcasses from the PK cockerels fed the control diet. It can be concluded that CM can be used to substitute SBM up to 175.0 g/kg without affecting carcass characteristics, viscera macromorphometry and quality of the meat from PK cockerels. Growth and muscle development are always linked to bone development. It is, therefore, necessary to assess the influence of CM inclusion in diets on bone development of PK cockerels given the variation in mineral availability induced by the presence of CM in the diets.

5.6 References

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CHAPTER 6 - BONE BREAKING STRENGTH, DENSITY AND MINERAL COMPOSITION IN POTCHEFSTROOM KOEKOEK COCKERELS FED ON GRADED LEVELS OF CANOLA MEAL

Abstract

This study was conducted to evaluate the effects of partially replacing soybean meal (SBM) with graded levels of dietary canola meal (CM) in Potchefstroom Koekoek (PK) cockerel diets on bone traits and mineralisation. Five dietary treatments were formulated by partially replacing SBM in a chicken grower diet with CM at the following levels: control = diet with no canola meal inclusion, CM37.5 = 37.5 g canola meal/kg soybean meal, CM62.5 = 62.5 g canola meal/kg soybean meal, CM87.5 = 87.5 g canola meal /kg soybean meal, CM175 = 175 g canola meal/kg soybean meal. A hundred and seventy-five, 36-day old PK cockerels were randomly allocated to the dietary treatments. Each treatment had 5 replicate pens holding 7 cockerels. At the end of the experimental period (13 weeks), PK cockerels were humanely slaughtered. Right tibiae were removed for the bone mineral composition analysis and characterisation. There were no significant dietary effects on tibia length, weight, width, density, diameter proximal end, diameter distal end, breaking strength and ash percentage. Diets, however, had a significant effect on macro and trace mineral concentrations of the tibia. Tibia from cockerels fed diet CM175 had the lowest ($P<0.05$) Ca and P content. Lower ($P<0.05$) tibia Mg and Na concentration were observed in the cockerels fed diets CM87.5 and CM175 compared to tibia of those cockerels fed on the other diets. Feeding of the CM had no effects on bone breaking strength, density and mineral composition but a better bone mineralisation was noted in bones from cockerels fed diets CM37.5 and CM62.5.

Keywords: mineralisation, bone quality, tibia, soybeans meal, canola meal.

6.1. Introduction

Physiologically bone development is an indication of the status of minerals in the diets. Bone ash content and breaking strength are used to assess the bioavailability of calcium and phosphorus in chicken diets (Shaw *et al*., 2010). Poor bone mineralisation increases the incidence of leg and bone weakness, lameness and other bone abnormalities resulting in high production losses and having a negative effect on birds' welfare (Dibner *et al*., 2007; Onyango *et al*., 2003).

Bone consists of living cells and intercellular matrix that is filled with minerals salts. Approximately 70% minerals, 20% organic matrix and about 10% water. Bone quality can be measured by determine ash content, mineral composition, shear strength and Seedor index. The Seedor index is a calculation that considers bone volume and the result can indicate bone mineral density (Paz *et al*., 2008). The mineral matrix is contributing 60 to 70% and is mainly composed of Ca and P (Rath *et al*., 2000). Several response criteria can be used to evaluate dietary mineral utilisation including digestibility, growth performance and bone mineralisation. These aforementioned parameters are influenced by many factors including dietary factors such as dietary minerals. Deformity of bone is one of the basic symptoms of mineral deficiency in the birds' diet (El-Husseiny *et al*., 2012). Minerals are essential for maintenance and normal functioning of the poultry, therefore, their adequate supply should be ensured through feed because deficiencies lead to reduced growth whereas oversupply can harm production efficiency and will impact on the environment negatively.

While a lot of studies have been conducted on bone development, bone defects and mineralisation in broilers, very little work has been done to assess nutritional effects on bone

development and mineralisation in indigenous chickens. In previous chapters (Chapter 4 & 5), it was observed that inclusion of CM in diets desirably affected growth, carcass and meat quality characteristics of PK cockerels. It can also be expected that CM inclusion in diet can also influence bone development. The present research, therefore, assesses the influence of dietary CM in diets on bone development in PK development. Apart from the high protein levels, Canola meal contains considerable amounts of minerals and secondary plant metabolites that can influence the dynamics of mineral digestion and assimilation that may also influence bone development in chickens (Hossain *et al*., 2016). Therefore, the objective of the study was to determine the influence of dietary inclusion of CM on bone characteristics and mineralisation in indigenous PK chickens. It was hypothesised that inclusion of CM in PK grower diets has no influence on bone breaking strength, density and mineralisation.

6.2 Materials and methods

6.2.1 Study site, preparation of the house, experimental birds and their management

The study site, preparation of the house, experimental birds and their management, diet formulation and treatments and experiment design of the feeding trial are described in section 3.2.1, 3.2.3 and 4.2.2, respectively.

6.2.2 Diet formulation, treatments and experiment design

The diet formulation, treatments and experiment design of the feeding trial are described in section 3.4.4 and 4.2.4 respectively.

6.2.3 Birds and sample collection

The experimental birds described in Chapter 5 (section 5.2.2) were used to investigate the effects of dietary CM on bone breaking strength, density and bone mineral composition. As described in Chapter 4, at the end of the experimental period five (5) cockerels from each replicate were randomly selected for bone development and mineralisation analysis. The birds were slaughtered as described in section 5.2.2 and the right tibia from each cockerel was removed for bone biomechanics and mineralisation analysis.

6.2.4 Bone biomechanics

At 13 weeks of age, five cockerels were selected from each replicate (i.e. 25 cockerels per treatment) after humane slaughter as described in Chapter 5. The right leg from each of the birds was excised at femorotibia articulation and defleshed. The right tibia was then weighed and tibia length (TL) and width (TW) measured by Vanier calliper with an accuracy of 0.001 cm (Zhang & Coon 1997). Bone breaking strength (BBS) was determined by placing the bone horizontally between brackets set at 10 mm apart and the breaking strength was measured by applying pressure using Texture analyser (TA XT plus; Stable Micro System , Surrey, Uk). The tibia density (TD) was calculated according to Moraes *et al*. (2017). The tibiae were individually sealed in plastic bags to minimise moisture loss and stored in a freezer at -18^{o}C for later analysis (Zhang & Coon 1997). Tibia density (TD) was calculated by Seedox index (Seedox *et al*., 1991), which is the value obtained by dividing the tibia weight by tibia length as follows:

$$Tibia\ density = \frac{Tibia\ weight\ (g)}{Tibia\ length\ (mm)}$$

Ash content was determined by ashing at 550°C for about 6 hours (AOAC, 2005, method number 924.05). Ash percentage (AP) was calculated of as follows:

$$Ash\ percentage = \frac{Total\ ash\ (mg)}{Total\ weight\ (mg)}\ x\ 100$$

6.2.5 Tibia mineral composition

Bone calcium (Ca), magnesium (Mg), potassium (K), sodium (N), iron (Fe), zinc (Zn), manganese (Mn), chlorine (Cl) and copper (Cu) were analysed using an ICP Mass Spectrometer (Perkin-Elmer, 1982, NexION 300Q) in Animal Health Center Laboratory (North-West University, Mafikeng, South Africa) Phosphorus was determined calorimetrically using sodium phenol and ammonium molybdate plus ascorbic acid as described by AOAC, 2012, method no. 976.06.

6.2.6 Statistical analyses

Tibia bone characteristics and mineral composition data were analysed using PROC GLM of SAS (2008) as a completely randomised design according to the following linear model:

$$Y_{ijk} = \mu + d_i + E_{ijk}$$

Where Y_{ijk} = response variable, μ = general mean, d_i = the fixed effects of canola levels. E_{ijk} = random error. When the analysis of variance revealed the existence of significant difference among treatment means, Probability of difference (PDIFF) option in the LSMEANs statement of the GLM procedure of SAS (2007) as used to locate treatments that were significantly different from one another. The level of significant was set at $p<0.05$.

6.3 Results

6.3.1 Tibia characteristics

The results on tibia characteristics showed that inclusion of CM in diets had no effect on tibia length, weight, width, density, diameter proximal end (TDPE), diameter distal end (TDPE), breaking strength and ash percentage (Table 6.1).

6.3.2 Macro minerals content for the tibia

Diets had a significant effect on macro minerals content of tibia bone in PK cockerels (Table. 6.2). Cockerels fed diet CM175 had the lowest ($P<0.05$) bone Ca and P contents. Higher ($P<0.05$) Mg levels were observed from the tibia of cockerels fed the control (51.0 ug/mL) and CM375 (53.3 ug/mL) diets compared to those fed other diets. Potchefstroom cockerels fed diets CM87.5 (62.5 ug/mL) and CM175 (62.1 ug/mL) had the lowest ($P<0.05$) tibia Na contents. Cockerels fed the control (191.3 ug/mL) and CM37.5 (191.3 ug/mL) diets had the high ($P<0.05$) tibia K. Cockerels fed the control (52.5 ug/mL) diet had the lowest ($P<0.05$) tibia S content.

6.3.3 Trace minerals content of the tibia

Diets had a significant effect on trace elements content of tibia in PK cockerels (Table 6.3). Cockerels fed diet CM175 (0.044ug/mL) had higher ($P<0.05$) tibia Mn content and cockerels fed diet CM87.5 (0.0021 µg/mL) had the lowest. There was no significant difference in tibia Mn content of cockerels fed the control (0.0034 µg/mL), CM37.5 (0.0032 µg/mL) and CM62.5 (0.0038µg/mL) diets

Table 6.1 Effects of substitution of soybean meal with graded levels of canola meal on right tibia characteristics of 18-week old Potchefstroom Koekoek cockerels (mean ± SE)

	Diets[1]				
Tibia characteristics[2]	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**
Length (mm)	148.3 ± 0.35	144.3 ± 0.32	149.7 ± 0.32	145.7 ± 0.32	147.4 ± 0.32
Weight (g)	10.87 ± 0.65	9.47 ± 0.59	10.67 ± 0.59	10.62 ± 0.59	9.85 ± 0.59
Width (mm)	8.76 ± 0.46	8.97 ± 0.42	8.81 ± 0.42	9.10± 0.42	9.14 ± 0.67
Density (gmm^{-1})	0.73 ± 0.03	0.65 ± 0.03	0.71 ± 0.03	0.72 ± 0.03	0.67 ± 0.03
TDDE (mm)	131.5 ± 0.03	137.3 ± 0.31	133.0 ± 0.31	139.9 ± 0.31	135.7 ± 0.31
TDPE (mm)	232.8 ± 2.45	239.8 ± 2.23	283.7 ± 2.24	239.4 ± 2.24	227.2 ± 2.24
Breaking strength (N)	19.51 ± 1.39	21.84 ± 1.39	21.22 ± 1.39	23.16 ± 1.39	23.52 ± 1.39
Ash percentage	88.72 ±1.86	86.00 ± 1.86	86.02 ± 1.86	87.65 ± 1.86	82.77 ± 1.86

[abc]Means within a row that do not share a common superscript differ significantly ($P<0.05$).

SBM= soybean bean meal; CM= canola meal; Diets[1]: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM.

[2]Tibia characteristics: TDPE = Diameter Proximal End; TDDE = Diameter Distal End.

Table 6.2 Effects of substitution of soybean meal with graded levels of canola meal on macro mineral content of tibia of 18-week old Potchefstroom Koekoek

	Diets[1]					
Minerals (µg/ml)	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**	**SE**
Calcium	4314.3[b]	4243.0[b]	4204.2[b]	3607.1[ab]	3268.2[a]	294.6
Phosphorus	464.1[ab]	445.1[ab]	480.4[b]	437.4[ab]	385.2[a]	28.9
Magnesium	51.0[b]	53.3[b]	46.1[a]	41.1[a]	47.2[a]	3.9
Sodium	103.6[c]	67.3[a]	83.6[b]	62.5[a]	62.1[a]	5.0
Potassium	191.3[d]	191.3[d]	156.6[c]	106.4[b]	82.9[a]	9.8
Su1phur	52.5[a]	71.8[b]	76.4[b]	77.6[b]	83.2[b]	5.7

[abc]Means within a row that do not share a common superscript differ significantly (P<0.05).

SBM= soybean bean meal; CM= canola meal; Diets1: Contro[l] = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM.

Table 6.3 Effects of substitution of soybean meal with graded levels of canola meal on trace mineral content of tibia of 18-week old Potchefstroom Koekoek

	Diets[1]					
Trace elements (µg/ml)	**Control**	**CM37.5**	**CM62.5**	**CM87.5**	**CM175**	**SE**
Iron	19.4	20.5	21.6	17.5	22.9	2.000
Manganese	0.0034^{b}	0.0032^{b}	0.0038^{b}	0.0021^{a}	0.044^{c}	0.002
Copper	0.15^{d}	0.13^{c}	0.11^{b}	0.09^{a}	0.08^{a}	0.007
Zinc	0.59^{b}	0.59^{b}	0.63^{b}	0.49^{a}	0.55^{ab}	0.040
Chlorine	6667.9^{b}	6448.7^{ab}	8199.9^{c}	5638.3^{a}	5534.6^{a}	292.8

[abc]Means within a row that do not share a common superscript differ significantly (P<0.05).

SBM= soybean bean meal; CM= canola meal; Diets[1]: Control = Broiler grower diet with only SBM as a major protein source; CM37.5 = Broiler grower diet where 37.5 g/kg of SBM is substituted with CM; CM62.5 = Broiler grower diet where 62.5 g/kg of SBM is substituted with CM; CM87.5 = Broiler grower diet where 87.5 g/kg of SBM is substituted with CM; CM175 = Broiler grower diet where 175.0 g/kg of SBM is substituted with CM.

Higher (P<0.05) Cu concentration was observed in the tibia of cockerels fed the control (0.15 µg/mL) diets followed by the tibia of cockerel fed diet CM37.5 (0.13 µg/mL) while the lowest concentrations were on the tibia of cockerel fed diets CM87.5 (0.09 µg/mL) and CM175 (0.08 µg/mL). Higher concentration (P<0.05) of tibia Cl was observed in cockerel fed diet CM62.5 (8199.9 µg/mL) and lowest was in the tibia of cockerel fed diets CM87.5 (5638.3 µg/mL) and CM175 (5534.6 µg/mL). Cockerels fed diets CM87.5 and CM175 (5534.6 µg/mL) had the lowest (P<0.05) content of Zn in the tibia.

6.4 Discussion

6.4.1 Macro mineral content of the tibia

Calcium and P are the two most abundant minerals in bone. The distribution of these two minerals affects the formation and mineralisation of bone **(**Szymeczko *et al*., 2010). In the GIT, mineral interaction, intestinal pH and interaction of dietary protein with fat and carbohydrates affects Ca and P absorption (Waldenstedt *et al*., 2006). The observation that tibia Ca and P contents in PK cockerels fed diet CM175 were significantly lower than those fed the control diet and other diets could be due to lower availability of these minerals to the birds due to the present of anti-nutritional factors (glucosinolates, phytate and oxalates) present in canola meal. These anti-nutrients bind minerals resulting in a reduction in absorption hence unavailability (Woyengo & Nyachoti, 2011; Khajali & Slominski, 2012**)** for bone formation.

It has been reported that high dietary fat reduces Ca absorption leading to lower Ca and P in bone irrespective of Ca source (Ryssen *et al*., 2014). However, Hossain *et al*. (2013) and Leeson (1987) found no significant difference in tibia Ca and P content in the bone of broilers fed diets containing predominantly CM. This lack of concordance in findings could be due to

differences in ingredients used to formulate diets in these studies. Kahindi *et al*. (2016) state that dietary ingredients type affects efficiency of P utilisation. The lower ($P<0.05$) tibia Mg, Na and K contents of the cockerels fed diets CM87.5 and CM175 compared to other tibia of cockerel fed other diets observed in the current study could be due to poor mineral digestibility. From Chapter 3, section 3.6.2, Table 3.5, it is clear that inclusion of CM at 87.5 and 175 g/kg numerically reduce mineral digestibility which is again reflected in bone mineral content from the same dietary treatment in this chapter. Magnesium promote bone formation by activating osteoclasts (Toba *et al*., 2000) and it is also the Ca antagonist, which improves Ca, Mg and P ratios and reduces the excess Ca in relation to P (Skřivan *et al*., 2016). Potassium is the third most abundant mineral in the animal body which combine with Na and Cl for the formation of bone (NRC, 1994). It participates in the processes that are essential to the body homeostasis (Oliveir *et al*., 2005). Results from this study show that up to 87.5 g/kg of CM could be used to replace SBM in PK grower diets.

6.4.2 Trace mineral contents of the tibia

The low tibia Mn, Cu, Zn and Cl contents in cockerel fed diets CM87.5 and CM175 in the current study could be due to the presence of anti-nutritional factors. This observation contradicts the previous findings of Hossain *et al*. (2015) who noticed that the addition of CM had no adverse effects on tibia Mn, Zn and Cu. The low Mn, Zn and Cu tibia contents in the current study indicated the low contents of these minerals in CM87.5 and CM175 diets. The difference between the findings in the current study and that by Hossain *et al*. (2015) could be the genetic make-up of chicken (strain). Broilers and indigenous chicken utilise minerals differently. Similarly, in the studies conducted by other authors (Kwiecień *et al*., 2016; Mohanna & Nys, 1999), the content of Zn in the tibia was reported to increase along with the increasing levels of the element in the diet.

Trace minerals are only required in small amounts and may contribute less to bone development (Hossain *et al*., 2015). Zinc stimulates the synthesis of DNA in osteoblasts and increases bone weight and the concentration of Ca ions (Ma & Yamaguchi, 2000). The higher contents of Mn, Zn and Cu in tibia of cockerels fed the control diet may stimulate bone growth and increase bone strength, as these trace minerals are linked to use of the macro minerals in bone development (Kwiecień *et al*., 2016; Medeiros *et al*., 1997). It is not known why, in the present study, tibia Mn concentration in the CM175 chickens was elevated. However, these two minerals are only required in small amounts and may contribute less to bone development than Ca and P.

6.5 Conclusions

The findings that CM inclusion in diets did not negatively affect bone development suggest that CM can be used as a protein source with comparable effects on general performance of chicken as SBM. Tibia parameters can be indicative of bone development and bone conditions e.g. lameness associated with deficiencies or excesses of certain minerals. From the study, CM inclusions in diets resulted in tibia parameters that compared well with those of the Control (SBM) diet. It can, therefore, be concluded that inclusion of CM up to 175 g/kg is not detrimental to bone growth and development and thus performance of the PK chickens.

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CHAPTER 7
GENERAL DISCUSSION, IMPLICATIONS AND RECOMMENDATIONS

7.1 General discussion

The research was carried out to determine the physiological responses and meat quality properties of the Potchefstroom Koekoek cockerels offered with canola meal as an alternative to soybean meal. Chemical characterisation of the canola and soybean meal alongside with the nutrient digestibility of the canola meals-based diets offered to the Potchefstroom Koekoek cockerels were determined.

Growth performance, protein utilisation efficiency and blood metabolites of the Potchefstroom Koekoek cockerels fed with the graded levels of canola meal were determined. The effect of graded levels of canola meal on carcass characteristics, viscera macromorphometry and meat quality of the Potchefstroom Koekoek were also determined. Bone breaking strength, density and mineral composition in the Potchefstroom Koekoek cockerels fed with the graded levels of canola meal were investigated. The purpose of this chapter is to summarise findings from the four studies in relation to the hypothesis and how the results can be used to improve small scale farmers. Research gaps and future research direction in relation to solving the problem faced by small scale farmers are suggested. Firstly, it is important to determine the chemical composition and the nutrients digestibility of tested protein source in order to formulate tested diets.

Chapter 3 of the study hypothesised that there were no differences in dry matter, crude protein, ash, mineral content of SBM and CM. The study fails to reject the hypothesis and confirm that there are no major differences in most proximate and minerals content with the exception of NDF and ADF, representing the fibre fraction. It was also hypothesised that

partial replacement of the SBM with the CM in poultry diets would not affect apparent digestibility of nutrients in Potchefstroom Koekoek cockerels. The study rejects the hypothesis and confirms that formulated diets had similar apparent digestibility values for minerals, dry matter and fibre but high inclusion of CM reduced ($P < 0.05$) CP digestibility. However, anti-nutritive factors like glucosinolates, sinapine, phytic acid, polyphenolic compounds, erucic acid, protein inhibitors and indigestible non-starch polysaccharides are present. It is, therefore, essential to assess the effects of inclusion levels of CM as a replacement for SBM on growth performance and haemo-biochemical parameters of indigenous chickens as well as to determine its optimum inclusion levels.

The hypotheses tested was that there was no significant difference in growth performance, protein utilisation efficiency and haemo-biochemical parameters of Potchefstroom Koekoek (PK) cockerels fed graded levels of CM as partial replacements for SBM. It was demonstrated in Chapter 4 that diets had no effects on AWFI, AWG and FCR. These findings indicate that CM can be used as an alternative protein source and incorporated in commercial grower diets and used to feed indigenous chickens with no negative production. Diets had no effects on protein consumed and PER implying that dietary protein content did not differ between diets and the digestibility of protein in control (SBM) and CM-based diets was similar. All the values obtained in this study for haematology and serum biochemistry fell within these normal ranges of chicken. The present result signified that the health of PK cockerels was not negatively affected by inclusion of CM in the diets. The hypothesis was, therefore, not rejected. However, the effects of CM inclusion in diets on growth performance can also translate to the effects on the meat. It is, therefore, necessary to assess the effect of CM inclusion in diets on the most preferred and consumed chicken parts and edible offals of PK chickens to ensure that no negative effects are imparted to the meat.

In Chapter 5, the hot carcass weight was high in PK cockerel fed diet CM37.5. Shank weight and lengths, breast muscle, wings, drumstick and vertebrae weight were lower in PK cockerels fed control diet and imply that CM can be used to substitute SBM up to 175.0 g/kg without hampering carcass traits and weight of external organs. These findings agree with proposed hypothesis that there was significant difference in carcass traits and weight of external between control and CM based diets. No significant differences were observed in spleen, proventriculus, gizzard, Caeca, small intestine length. This signified that the inclusion of CM in the diets did not affect size of internal organs. However, these findings reject the proposed alternative hypothesis that there are significant differences in spleen, proventriculus, gizzard, Caeca and small intestine length between PK cockerels fed controls and CM based diets.

The lightness (L* value), redness (a* value), yellowness (b* value) of the breast muscle from PK cockerels fed the CM based diet was significantly higher than that for PK cockerels fed the control diet. This implies that inclusion of CM in the diet of chickens alter the colour of the meat of chicken fed the diet. These findings agree with proposed hypothesis that there are significant differences in lightness (L* value), redness (a* value), yellowness (b* value) between control and CM based diets. The meat pH decline was more pronounced in CM3.75 which had the lowest ultimate pH (pH_u). Cockerels fed on CM6.25 and CM17.5 had the similar highest ($P<0.05$) shear force values. These findings agree with the proposed hypothesis that there are significant differences in meat pH and shear force values between control and CM based diets. No variation ($P<0.05$) were observed in water holding capacity (WHC), cooking loss and drip loss in all treatments diets implying that inclusion of either CM or SBM did not make any difference in parameters measured. However, these findings

reject the proposed alternative hypothesis that there are significant differences in water holding capacity (WHC), cooking loss and drip loss between PK cockerels fed controls and CM based diets.

Higher ($P<0.05$) meat Ca and P contents was observed on PK cockerels fed on control diets while for P, Na and K meat contents the highest were observed in PK cockerels fed CM175 diet. These findings agree with the proposed hypothesis that there are significant differences in Ca, P, Mg and S bone contents between control and CM based diets. This imply that inclusion of CM in diet to fed PK cockerels lower the Ca and P meat contents and promote the P, Na and K meat contents. For the trace minerals, higher inclusion levels of CM had higher ($P<0.05$) Mn and Zn meat contents compared to the control diets while Cu meat contents was higher ($P<0.05$) in control diet. This shows that dietary CM increased the contents of P, Na and K on the meat. It is, therefore, necessary to assess the influence of CM inclusion in diets on bone breaking strength, density and mineral composition of PK cockerels given the variation in mineral availability induced by the presence of CM in the diets.

The results in bone mineralisation study (Chapter 6) showed that cockerels fed CM175 had the lowest ($P<0.05$) bone Ca and P contents while higher ($P<0.05$) bone Mg and K were observed in cockerels fed CM37.5 as compared to control diets. Cockerel fed CM87.5 and CM17.5 had the highest ($P<0.05$) bone concentrations of Na. This implies that inclusion of CM lower bone Ca and P contents and promotes Mg and K contents.

Higher ($P<0.05$) bone Cu, Zn and Cl concentrations observed in CM87.5 and CM175 diets compared to other treatment diets imply that inclusion of CM promotes bone trace mineral contents. These findings agree with proposed hypothesis that there was significant difference

in Mn, Cu, Cl and Zn bone contents between control and CM based diets. No significant differences were observed in Fe bone contents. These findings reject the proposed alternative hypothesis that there are significant differences in Fe contents between PK cockerels fed controls and CM based diets.

Lastly the whole study establishes that the diet of indigenous chickens can be formulated using CM as protein source. However, CM as protein source did not had any difference in minerals retention, dry matter and crude fibre digestibility, Growth performance, protein utilisation efficiency, blood metabolites, carcass characteristics, internal organs, meat quality, bone development and mineralisation as compared to control diet.

7.2 Recommendations

Canola meal is a good source of protein important that can be used to replace an expensive soybean meal. Canola meal can be bought in South Africa as by-product at lower price compared with SBM used to fed indigenous chickens to improve the growth performance, health, digestibility, carcass characteristic, meat quality and bone development of the chicken. It is suggested that canola meal 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 improper inclusion of this alternative protein source based on its recommended inclusion level and growth capacity. It would be advisable for the small farmer to use CM to replace SBM as it will reduce the cost and it is clearly seen from the current results that CM did not have affect effects in all measured parameter on indigenous chickens.

7.3 Further research recommendation

1. The effect of CM inclusion on day old chicks be researched till they reach maturity age to have a comprehensive understanding of how CM is utilised.
2. Nutrient utilisation, growth performance, bone mineralisation and meat quality in Potchefstroom Koekoek cockerels fed phytase-treated canola-based diets
3. Research on the growth and economic performance of broiler chickens fed on graded levels of canola meal with or without multi-enzyme supplementation should be conducted
4. It is important to study the effects of canola based diets concentration in the skin and liver of the CM fed chickens.